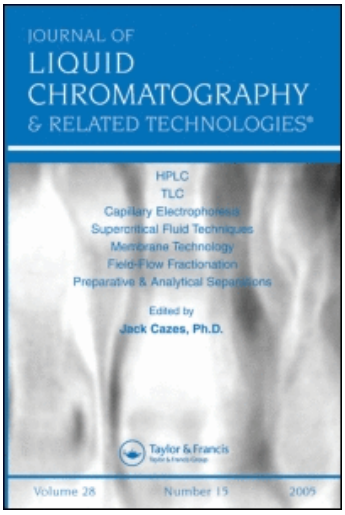

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Atrazine and Hydroxyatrazine Residues in Soil by Ion-Pair, Reversed-Phase High Performance Liquid Chromatography

Qian Wenheng[a]; Nancy A. Schultz[a]; James D. Stuart[b]; James C. Hogan Jr.[c]; Antoinette S. Mason[c]
[a] The Institute of Water Resources, U-18, the University of Connecticut Storrs, Connecticut [b] Department of Chemistry, U-60 the University of Connecticut Storrs, Connecticut [c] Connecticut Department of Health Services, Bureau of Laboratories, Hartford, Connecticut

To cite this Article Wenheng, Qian , Schultz, Nancy A. , Stuart, James D. , Hogan Jr., James C. and Mason, Antoinette S.(1991) 'Determination of Atrazine and Hydroxyatrazine Residues in Soil by Ion-Pair, Reversed-Phase High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 14: 7, 1367 — 1392
To link to this Article: DOI: 10.1080/01483919108049326
URL: http://dx.doi.org/10.1080/01483919108049326

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(7), 1367-1392 (1991)

DETERMINATION OF ATRAZINE AND HYDROXYATRAZINE RESIDUES IN SOIL BY ION-PAIR, REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

QIAN WENHENG[1], NANCY A. SCHULTZ[1], JAMES D. STUART[2]*, JAMES C. HOGAN, JR.[3], AND ANTOINETTE S. MASON[3]

[1]*The Institute of Water Resources, U-18*
[2]*Department of Chemistry, U-60*
The University of Connecticut
Storrs, Connecticut 06269-3060
and
[3]*Connecticut Department of Health Services*
Bureau of Laboratories
10 Clinton Street
Hartford, Connecticut 06106

ABSTRACT

An improved method is presented for the determination of atrazine and hydroxyatrazine in soils. The method involves performing an initial extraction of the soil sample with a mixture (90:10 v/v) acetonitrile-aqueous 0.1 M hydrochloric acid. After centrifugation, the extract is concentrated onto a disposable strong cationic exchange, sulfonic acid-bonded, solid phase extraction (SPE) cartridge, and interfering soil substances are removed by rinsing. The atrazine and hydroxyatrazine are eluted with a 50:50 (v/v) acetonitrile-aqueous 0.1 M K2HPO4 solution. The

* Author to whom correspondence should be addressed

analysis was performed using a reversed-phase, high performance liquid chromatographic column of C18-bonded silica with an ion-pairing solvent system (50:50 v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid and 0.10 M phosphoric acid. This isocratic solvent system provided an excellent separation of not only atrazine, simazine and propazine but also of hydroxyatrazine, hydroxysimazine and various dealkylated products of atrazine. An ultraviolet diode array detector provided linear calibration plots in the 0.1-10 ppm range, with lower limits of detection of 0.01 ppm for atrazine and propazine. This improved extraction and HPLC analysis method was used to monitor the persistence of atrazine in the soil of three local corn fields. Over the 130-day growing season of the corn, the levels of atrazine in the upper 2-3 cm of the soil were found to drop from 2.0 to about 0.2 ppm. No significant levels of hydroxyatrazine were found in the farm soils sampled.

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine AT), with trade names of Aatrex, Gesaprim or Griffex, is an s-triazine herbicide used world-wide for its effectiveness in controlling broadleaf weeds on cropped lands. Stemming from this widespread, annual application to farm soils, residues of AT have been found in 4,551 of 8,804 surface water samples and 366 of 2,750 ground water samples in the U.S.A. (1). In a study of State of Connecticut drinking water samples, AT was the most frequently detected pesticide (2). Huang et al. have recently reported finding AT, simazine (SI), alachlor and metolachlor throughout the soil profile down to the water table, using sensitive isotope-dilution gas chromatographic/mass spectrometric procedures. These researchers caution that such residues could be leached into the groundwater for years to come (3).

In soil, AT is slowly degraded over periods of weeks-to-months through hydrolytic reactions and microbiological transformations (4). The principal reactions are hydrolysis at the 2-position to yield hydroxyatrazine (HYAT) and dealkylations at either or both the 4- and 6-positions to yield the de-ethyl atrazine (DEAT), de-isopropyl atrazine (DIAT), and de-ethyl,de-isopropyl atrazine (DEDIAT) (4,5). Erickson and Lee have provided a recent review

article on the degradation of the various s-triazine herbicides (6).

Determination of s-trazine herbicides and their derivatives usually entails an initial solvent extraction from their source material, followed (when necessary) by derivatization of the residues and analysis by gas chromatography (GC) with a nitrogen specific detector. However, due to lack of volatilization and thermal instability, HYAT cannot be directly analyzed by GC. Muir and Baker reported developing a semi-quantitative determination of HYAT from soil using a lengthy clean-up procedure, followed by reacting the HYAT with diazomethane to form a volatile ether derivative and by using packed column gas chromatography with a Hall conductivity detector. Average recoveries of 68 per cent, s.d. of 2.7 per cent for n=18, were obtained using soil spiked with HYAT at the 0.79 ppm level (7).

In 1979, Ramsteiner and Hörmann reported using normal-phase HPLC with an ultraviolet detector set at 240 nm to monitor the hydroxy-metabolites of four s-triazine herbicides: (AT), propazine (PR), simazine (SI) and terbutylazine extracted from soil and plant material. Comparative studies between different extraction methods showed that a heated (Soxhlet) extraction provided the highest recoveries for the hydroxy-s-triazines studied and that phosphoric acid increased the solubility of the hydroxy-s-trazines. Their methanol extracts were passed through a strong cation exchange resin column with the hydroxy-s-triazines eluting with methanol. After evaporation of the methanol, the extracts were analyzed using a 10-μm silica HPLC column (Lichrospher Si-60) with a mobile phase mixture of chloroform-methanol-water-87% H_3PO_4 acid (70:30:60:0.1 v/v/v/v). Detection limits for HYAT of 0.05 mg/1000 g of plant material were reported (8). In a later modification, it was reported that soil samples were Soxhlet extracted overnight with a mixture of methanol-water (80:20 v/v); additional hydrochloric acid was then added. Hydroxyatrazine and hydroxysimazine were concentrated onto a XAD-4 resin column and eluted with methanol. The resulting fraction was reduced in volume

and then injected onto a 5-μm reversed-phase, HPLC column (Altex Ultrasphere ODS, 250 mm x 4.0 mm i.d.) using a mobile phase (60:40 v/v) methanol-water containing 0.1 M citric acid and 0.020 M n-octanesulfonic acid in order to form an ion-pair with the hydroxy-s-triazine molecules. A limit of detection of 5 ng for HYAT was reported using 240 nm excitation on the 0.04 AUFS range. Recoveries of HYAT from three different soil types spiked at levels of 0.10 ppm to 1.0 ppm were reported to range from 75 - 102 per cent with an average of 86, s.d. of 9 for n=20 (9).

In 1981, Beilstein et al. reported using reversed-phase HPLC with a 250 mm x 4.6 mm i.d. column packed with 5-μm LiChrosorb RP-18 to separate seventeen s-triazine herbicides and various of their hydroxylated and de-alkylated products. A linear 20-min. gradient from aqueous 0.10 M K3PO4, pH of 6.7, to 70:30 (v/v) methanol:aqueous 0.10M K3PO4 was used with the column kept a 2 °C. A linear calibration range from 90 pmol-480 μmol for AT with detection at 220 nm and from 40 pmol-60 μmol for HYAT with detection at 240 nm was reported (10).

In 1982, Vermeulen et al. showed that reversed-phase HPLC with an octyl Ultrasphere (250 x 4.0 mm i.d.) column and an isocratic solvent system of 40:60 (v/v) methanol-aqueous 0.05 M ammonium acetate, pH adjusted to 7.4, was suitable to monitor the concentrations of atrazine and its degradation products from soil extracts. Average recoveries for AT of 78.4 per cent, with a s.d. of 4.2 per cent, and for HYAT of 72.5 per cent, with a s.d. of 4.8 per cent, were reported from 5 ppm spiked additions to a sandy loamy soil using an extracting solution of acetonitrile-water (90:10 v/v). It was noted that recoveries varied with soil type. In agreement with earlier reports, a pK_a of 1.71 for AT and 5.15 for HYAT were determined spectrometrically (11).

Hydroxy-s-triazines have been reported by Russell et al. to undergo a series of equilibrations in aqueous solutions, as depicted by Figure 1. On the basis of the observation of a strong infrared absorption at 1740 cm^{-1} assigned to a carbonyl group, a rapid enol-keto isomerization resulting in a 1 to 4 ratio of the

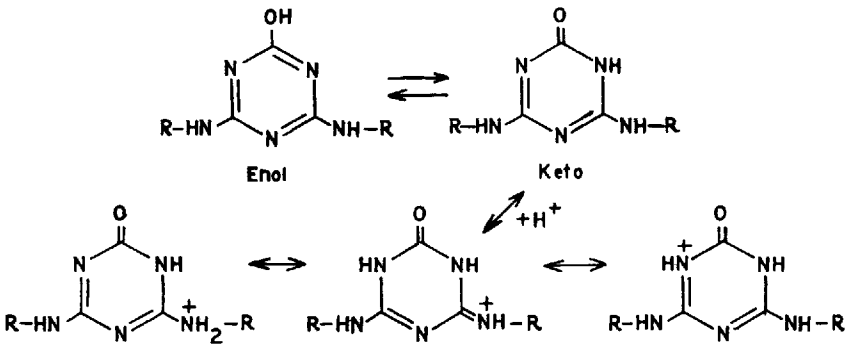

Figure 1. Tautomeric and various possible protonated resonance structures for hydroxy-s-triazines. (According to Jordan et al., (18). Permission to reproduce this figure given by the publisher Springer-Verlag Heidelberg).

enol-form to the keto-form for the hydroxyatrazine and hydrozypropazine was proposed (12). From nuclear magnetic resonance studies, it was proposed that the keto-form undergoes various resonance changes involving protonation at one of the three ring nitrogens to form positively charged structures. (Part of the intent of this work was to use a negatively-charged, sulfonic acid counter-ion in the HPLC mobile phase to form ion pairs with the protonated forms of the hydroxy-s-triazines depicted in Figure 1).

In 1984, Wachob reported using disposable solid phase extraction (SPE) cartridges to improve the clean-up step and to obtain high recoveries of AT from soil samples. An average recovery for AT of 100.2 per cent with a s.d. of 0.8 for n=5 from a 2 ppm spiked soil and an average recovery of 97.0 per cent with a s.d. of 3.4 for n=5 from a 0.5 ppm spiked soil were obtained. The soil type was not specified; however, it was reported that interferences commonly associated with solvent extraction of soil samples were effectively removed by using the SPE cartridge (13). (These same SPE cartridges were used in the present study).

In 1988, Pacakova et al. reported using a 5 μm reversed-phase HPLC column (Separon SIC 18, 150 mm x 4.5 mm I.D.) to separate eighteen different s-triazine herbicides, their derivatives or by-products in various mobile phase ratios of methanol-water and NaH2PO4. The variation in each compound's capacity factors, k', with the methanol content, pH and ionic strength of the mobile phase were reported. For an optimum separation of the hydroxy-products from the parent s-triazines, an isochratic mobile phase of 40:60 (v/v) methanol-aqueous 0.010 M NaH2PO4, with pH adjustment to 6.8, was suggested. The authors used these conditions to investigate the effect of substituent groups and the solution's pH on the photolysis rate of the various s-triazines (14).

Due to the extensive, world-wide use of atrazine, considerable interest exists in its persistence in the soil and migration into the ground water and surface water. It was the intent of this present research to develop a viable extraction method for both AT and HYAT from farm land soils, high in carbon content, to which atrazine had been applied and to monitor the concentrations of AT and HYAT found in the upper 2-3 cm of the sprayed soil over the growing season of the corn. A reversed-phase, isocratic HPLC mobile phase of (50:50 v/v) acetonitrile-aqueous 0.10 M H3PO4, containing 0.020 M n-heptanesulfonic acid with the pH adjusted 2.8, was used to improve the solubility of the HYAT and to provide sufficient resolution between AT and HYAT. A diode array detector was used not only for its improved sensitivity but also to provide the capability of rapidly obtaining the ultraviolet spectrum of each eluting peak so as to aid in its qualitative identification.

MATERIALS

High Performance Liquid Chromatography

The HPLC consisted of a Perkin Elmer (Norwalk, CT) Model LC-4 pumping system with an ISS-100 autoinjector, a Rheodyne (Cotati,

CA) Model 7125 valve-and-loop injector fitted with a 6 μL loop and a Perkin Elmer Model LC-235 diode array detector. The absorption spectrum of each eluting peak was scanned over the range of 190-360 nm. The chromatograms were displayed on a Perkin Elmer Model LCI-100 recording integrator. The HPLC column, 300 mm in length and 4.6 mm in internal diameter, was packed with 5 μm spherical, C18-bonded silica (Ultremex C-18, Phenomenex Inc., Torrance, CA). A flow rate of 1.0 mL/min was used with the column kept at ambient temperatures. For extractions of AT and HYAT from the soil samples, a wrist-action shaker (Model 75, Burrell Corp., Pittsburgh, PA) and a centrifuge (Model FXD, International Equipment Co., Needham Heights, MA) were used.

Chemicals

Authentic samples of AT and various other s-triazines, HYAT, hydroxysimazine (HYSI), other AT by-products were of analytical standard quality and kindly donated by Ciba Geigy Corp. of Greensboro, N.C. A commercial mixture of Atrazine 4L (Universal Cooperatives, Inc., Minneapolis, WI) was applied at an application rate of about 570 g per 4047 M^2 (1.25 lb/acre) to the corn fields by workers of the University of Connecticut Agriculture Department. Other chemicals and solvents used in this research were of reagent or HPLC quality. Individual stock solutions of authentic s-triazine standards were prepared at the 100 ppm level in 50:50 (v/v) acetonitrile-aqueous 0.1 M H_3PO_4. The hydroxy-s-triazines were first solubilized in 0.1 M HCl to which the 50:50 (v/v) acetonitrile-aqueous 0.1 M H_3PO_4 was then added. When not in use, the concentrated standard solutions were refrigerated at 4 °C. Aromatic sulfonic acid, solid phase extraction (SPE) cartridges (Part No. 7090-03, J.T. Baker Chemical Co., Phillipsburgh, N.J.), 3-mL in size, were fitted into a Baker-10 vacuum manifold (7018-0, J.T. Baker Chemical Co.) and used as described by Wachob (13). A 75-mL reservoir was fitted above the extraction cartridge to facilitate the rinsings.

METHODS

Soil Sampling

Soil sampling plots, 1.8 m by 1.8 m, delineated by small piles of rocks, were located in each of the three corn fields belonging to The University of Connecticut. The plots were surveyed and were located 12.2 m from an U.S.A. Geological Survey marker or other identifying landmarks. During the actual soil sampling, the date, time, weather conditions and height of the corn were noted. A "bulb planter" was used to collect a surface core of about 6.4 cm in diameter and about 2-3 cm in height of the corn field soil Five cores, resulting in a total of about 250 grams of soil, were collected at random locations within the 1.8 m by 1.8 m plot, composited and stored in glass jars equipped with Teflon ® jar seals. The soil samples were kept in a cooler surrounded by ice packs, and upon return to the laboratory, stored at 4 °C until time of analysis. For the subsequent analyses, only those portions of the soil sample that passed through a 2.0 mm sieve were used. The soils of all of the three corn fields sampled were classified as an Agawam fine sandy loam (coarse-loamy, mixed, mesic Typic Dystrochrepts) according to the system used by the U.S. National Cooperative Soil Survey (15). The three fields sampled would be rated as being of prime farm land soils by the U.S. Department of Agriculture, Soil Conservation Service (16).

Soil Sample Preparation

A 25.00 g sample of the sieved soil was placed in a 250-mL Erlenmeyer flask to which 50.0-mL of 90:10 (v/v) of acetonitrile-aqueous 0.10 M HCl was added. This mixture was vigorously shaken using a wrist-action shaker for one hour; the extract was then centrifuged at 4000 rpm for 10 min. For soil spiking experiments, a concentrated standard solution, that would result after dilution to being a 2.0 ppm solution in AT and HYAT,

was placed in contact with the dry, sieved soil for one hour with periodic hand swirling of the flask. The previously described extraction step was then carried out. Then 5.00-mL of the centrifuged soil supernate was diluted with 25.0-mL of a 0.1 per cent acetic acid solution. This mixture was aspirated through the aromatic sulfonic acid solid phase extraction (SPE) cartridge at a rate of approximately 3 to 5 mL/min. This cartridge previously had been activated by rinsing with 20-mL of methanol, followed by 20-mL of deionized water, then by 2-mL of 0.1 per cent acetic acid. The sorbed soil extract on the SPE cartridge was then washed with about 1.0-mL of acetonitrile followed by about 2-mL of deionized water to remove interfering soil coextractants. The sorbed AT and HYAT were eluted using 2.00-mL of 50:50 (v/v) acetonitrile-aqueous 0.10 M K_2HPO_4 solution, having a pH of 8.2-8.5.

RESULTS AND DISCUSSION

Table 1 compares the per cent recoveries for AT and HYAT at the 2.0 ppm spiking level onto the same soil using various extraction methods and extracting solutions. (The earlier review chapter by Mattson *et al.* (17) on the extraction and quantitative determination of *s*-triazine herbicides from soils provided an excellent starting point for this work). The soil used for these extractions was a Lower Basal Till that was known to have a low-binding capacity for organic compounds and a low carbon content. High recoveries, 100 per cent for AT and 91 per cent for HYAT, were obtained by shaking vigorously 50 g of the spiked soil with a mechanical wrist-action shaker for 30 min. in an extracting solution of (90:10 v/v) acetonitrile-aqueous 0.10 M hydrochloric acid. Table 2 summarizes certain of the important chemical and physical characteristics of the various sand and soils studied.

Table 3 presents additional data for the per cent recoveries of AT and HYAT at a 2.0 ppm spiking level for the various soils having very different carbon contents. The left-hand portion of

TABLE 1

Comparison of the Per Cent Recoveries for Atrazine and Hydroxyatrazine. Using Different Extraction Methods at a 2.0 ppm Spiking Level from the Lower Basal Till Soil

Extraction Method	Solvent	Per Cent Recovery	
		Atrazine	Hydroxyatrazine
Soxhlet Extraction 10 g. of spiked soil for 8 hours	Methanol	99 (n=2)	49 (n=2)
Shaking vigorously 50 g. of spiked soil for 30 min.	Acetonitrile-water (90:10 v/v)	86 (n=2)	50 (n=2)
Shaking vigorously 50 g. of spiked soil for 30 min.	Acetonitrile-0.10 M H_3PO_4 (90:10 v/v)	71 (n=2)	34 (n=2)
Shaking vigorously 50 g. of spiked soil for 30 min.	Acetonitrile-0.020 M n-heptanesulfonic acid, 0.10 M H_3PO_4, (90:10 v/v)	91 (n=2)	69 (n=2)
Shaking vigorously 50 g. of spiked soil for 30 min.	Acetonitrile-0.10 M HCl (90:10 v/v)	100 (n=2)	91 (n=2)

TABLE 2

Certain Chemical and Physical Characteristics of the Sand and Soils Studied *

Soil Name	Texture of the Soil	pH	Per Cent Organic Matter	Per Cent Clay	Cationic Exchange Capacity, meq/100g
Ottawa Sand	sand	6.30	0.04	0.01	1.91
Lower Basal Till, Woodstock	fine sandy loam	5.40	1.07	4.31	4.32
Lower Spring Manor Farm	fine sandy loam	6.41	6.52	10.3	15.58
Lee Farm	fine sandy loam	5.99	5.17	13.5	10.21

* Soil classifications by H.D. Luce, Dept. of Agronomy, Univ. of Connecticut. Soil characterizations provided by members of the Nanjing Institute of Soil Science, Nanjing, P.R.C.

TABLE 3

Comparison of the Per Cent Recoveries of Atrazine and Hydroxyatrazine at the 2.0 ppm Spiking Level from the Various Soils Studied.

Soil Name	Per Cent Organic Matter	After Wrist-Action Shaking* Per Cent Recovery		After Use of SPE Cartridge* Per Cent Recovery	
		Atrazine	Hydroxyatrazine	Atrazine	Hydroxyatrazine
Ottawa Sand	0.04	91 (n=2)	96 (n=2)	- -	- -
Lower Basal Till, Woodstock	1.07	98 (n=4)	85 (n=4)	69 (n=6)	79 (n=6)
Lower Spring Manor Farm	6.52	90 (n=2)	83 (n=2)	78 (n=3)	50 (n=3)

* See the Experimental Section-Soil Sample Preparation for the experimental procedures.

Table 3 shows data from the wrist-action shaking and centrifugation steps alone, while the right-hand portion presents the per cent recoveries from the wrist action shaking/extraction coupled to the solid phase extraction (SPE) cartridge concentration and rinsing steps. It should be noted from Table 3 that the per cent recoveries were found to vary for the different soils. Similar variations in per cent recoveries of HYAT with soil types have been reported by Vermeulen et al. (11) and were noted in a recent review article by Erickson and Lee (6). The 50 per cent recoveries for HYAT are among the highest values reported from a farm land soil with approximately six per cent organic matter. The use of 0.10 M hydrochloric acid likely assisted in the protonation and solubilization of the basic s-triazine nitrogens and may aid in the conversion of HYAT to the keto form depicted in Figure 1.

Comparison of chromatograms of soil extracts before and after the use of the SPE cartridges demonstrated the effectiveness of these small cationic exchange SPE cartridges in removing the early eluting incipients from soil extracts. The left hand and middle parts of Figure 2 show chromatograms of the extract of a farm soil taken from an unsprayed area of Lee Farm found to have a 0.1 ppm residual of AT and to which 4.0 ppm HYAT had been added. Figure 2(a) shows the chromatogram of the soil extract before it was passed through the SPE cartridge while Figure 2(b) is the chromatogram of the same soil extract after it had been eluted from the SPE cartridge. Figure 2(c) shows a chromatogram of the extract of the Upper Spring Manor Farm Site-1 soil after elution from the SPE cartridge that was determined to have 12.3 ppm AT and 1.2 ppm HYAT. In Figure 2 (b) and (c), it should be noted that the peaks for HYAT and AT are well resolved from any early eluting peaks due to incipients that would be coextracted from the farm land soils. To investigate further the performance of the SPE cartridges, the elution distribution of 10 ppm solutions of AT and HYAT were individually measured from representative SPE cartridges. Figure 3 shows that 99.3 per cent of the recoverable

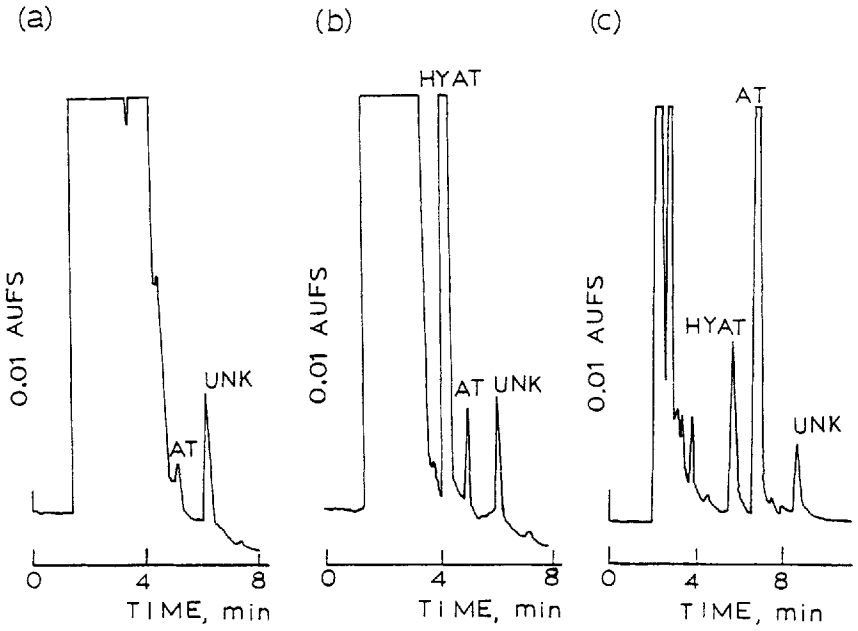

Figure 2. Chromatograms showing the effectiveness of the sample clean-up provided by the solid phase extraction (SPE) cartridges for the determination of atrazine (AT) and hydroxyatrazine (HYAT) from farm land soils. (a) Without passing through the SPE cartridge (b) The same soil extract after passing through the SPE cartridge. Figure 2 (a) and (b) represent the chromatograms of the extract of the soil from an unsprayed area of Lee Farm found to have a 0.1 ppm residual level of AT and to which 4.0 ppm of HYAT was added. The HPLC conditions were 70:30 (v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid and 0.10 M H_3PO_4, pH=2.8. Fig. 2 (c) was a soil extract from Upper Spring Manor Farm Site-1 determined to contain 12.3 ppm AT and 1.2 ppm HYAT. HPLC elution conditions were 50:50 (v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid, 0.10 M H_3PO_4, pH 2.8. UNK signifies the peak for an unknown compound which had an absorption maximum at 211 nm.

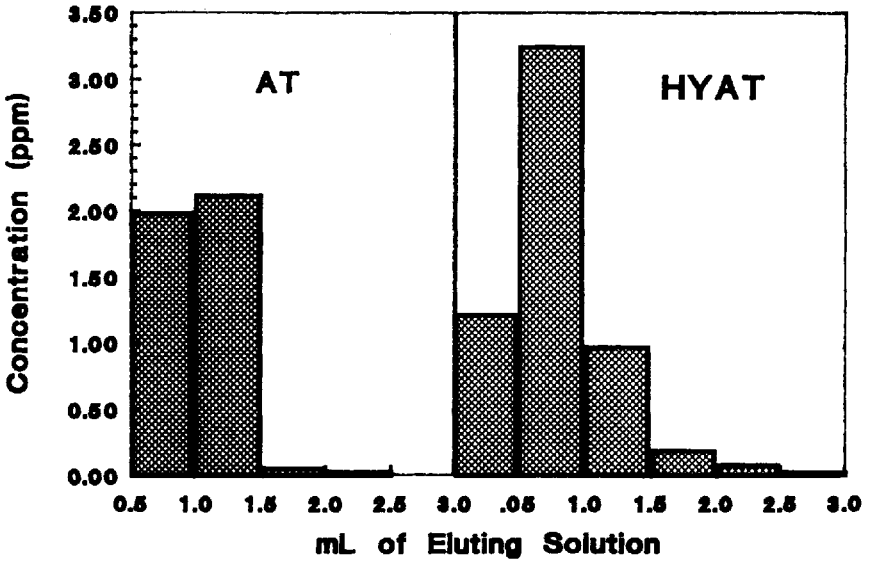

Figure 3. Elution distribution of AT and HYAT level, from the solid phase extraction (SPE) cartridge. Experimental Method described in the Soil Sample Preparation Section.

AT and 98.2 per cent of the recoverable HYAT were eluted from the SPE cartridge in the first 2.0 mL elution with 50:50 (v/v) acetonitrile-aqueous 0.1 M K2HPO4.

Table 4 summarizes certain of the preliminary chromatographic conditions used and the observations made when various mobile phases were compared during the developement of a separation of AT and HYAT on the 300 mm long, C18-bonded silica HPLC column used in this study. It became evident that enhanced sensitivity for both AT and HYAT in terms of increases in relative peak heights and shifts in absorption maxima occured when the acidic, ion-pairing mobile phase was used. Figure 4 presents a chromatogram showing the separation of atrazine (AT), simazine (SI), hydroxyatrazine (HYAT), hydroxysimazine (HYSI), the de-ethylAT (DEAT), the de-isopropylAT (DIAT), and the de-ethyl,de-isopropylAT (DEDIAT) in

TABLE 4

Elution and Spectral Response Behaviors of Atrazine and Hydroxyatrazine in Various Mobile Phases.

Mobile Phase	Elution Time Unretained Peak	Capacity Factor		Diode Array Response* pk. height (cm)		Absorption Maximum (nm)	
	min.	AT	HYAT	AT	HYAT	AT	HYAT
Methanol-water (70:30)	2.37	4.16	1.52	33.66	37.45	220	215
Acetonitrile-water, 0.01M K2HPO4, (50:50)	2.37	2.91	0.66	26.5	40.0	220	215
Acetonitrile-water, 0.1M H3PO4, (70:30)	2.36	3.89	ND	26.44	ND	220	ND
Acetonitrile-water, 0.1M H3PO4, 0.020M heptanesulfonic acid, (70:30)	2.34	1.21	0.76	90.68	50.1	220	240

* Detector sensitivity set on the 0.010 AUFS range, 12 ng of each compound injected. ND = not detected.

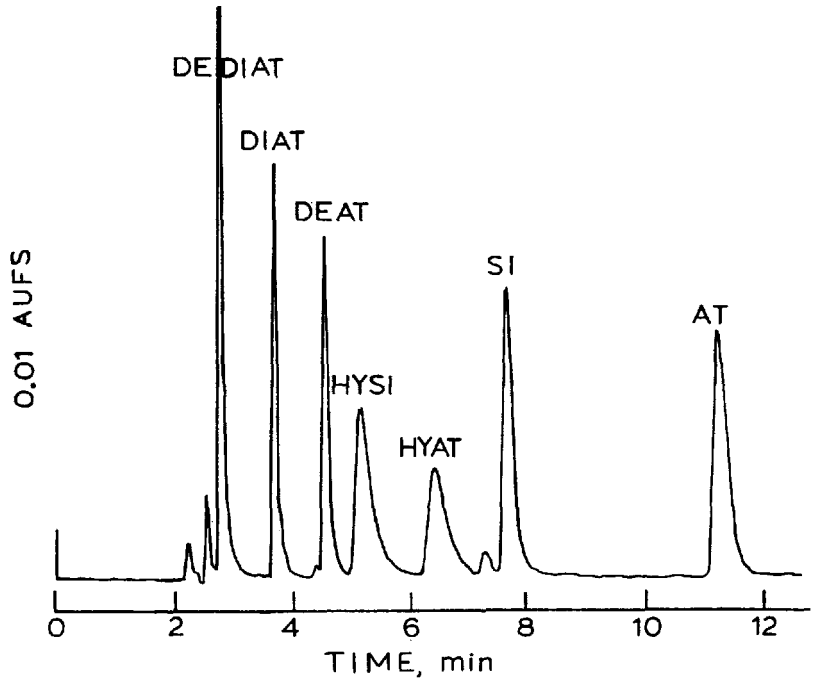

Figure 4. Reversed-phase, ion-pair HPLC separation of various s-triazines and certain of their degradation products. Column: 5-μm, C18-bonded, spherical silica, 300 mm long and 4.6 mm i.d., mobile phase: 50:50 (v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid and 0.10 M H3PO4, pH = 2.8, flow rate: 1.0 mL/min, sensitivity range of 0.01 AUFS on the photo diode array detector, about 6 nanog. of each compound injected. The peak identities are: DEDIAT = de-ethyl,de-isopropylatrazine, DIAT = de-isopropylatrazine, DEAT = de-ethylatrazine, HYSI = hydroxysimazine, HYAT = hydroxyatrazine, SI = simazine and AT = atrazine.

the isocratic solvent system of 50:50 (v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid and 0.10 M H3PO4 at a flow rate of 1.0 mL/min. The separation of these compounds was also investigated in the presence of two other ion-pair reagents, n-octanesulfonic acid and n-decylsulfonic acid. The capacity factors of the various compounds are summarized in Table 5. The

TABLE 5

Retention Behavior of s-Triazine Herbicides and Certain of Their Dealkylated and Hydroxylated By-Products in Mobile Phases of Acetonitrile-Aqueous 0.10 M H_3PO_4 (50:50, v/v) Containing Different Ion-Pairing Reagents*.

Compound:	n-heptanesulfonic acid	n-octanesulfonic acid	n-decylsulfonic acid
	k′	k′	k′
Unretained Peak**	0.00	0.00	0.00
De-ethyl,de-isopropyl-Atrazine	0.088	0.080	0.072
De-isopropyl-Atrazine	0.43	0.39	0.37
De-ethyl-Atrazine	0.76	0.69	0.65
Hydroxysimazine	0.98	1.08	1.41
Hydroxyatrazine	1.46	1.57	2.37
Simazine	1.93	1.70	1.57
Atrazine	3.30	2.84	2.59
Propazine	5.58	4.69	4.24

* Each ion-pair reagent was present at the 0.020 M concentation level. For various other experimental conditions refer to the Experimental section.

** Average elution times of 2.61, 2.61 and 2.63 min. were obtained respectively for the three mobile phases containing the different ion-pair reagents.

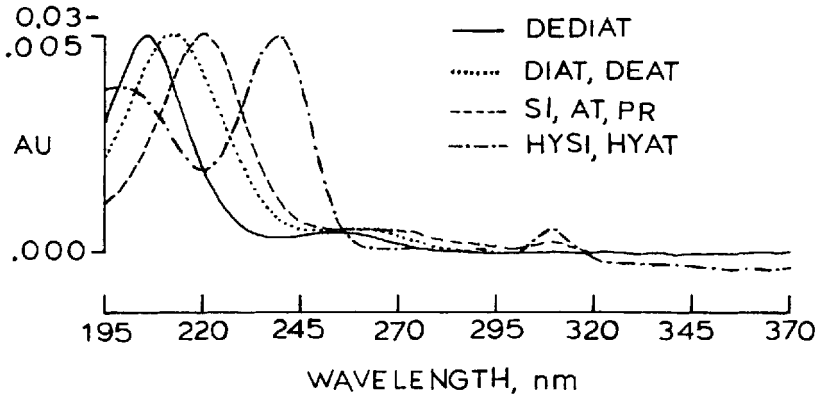

Figure 5. Composite of the ultraviolet spectra for the various s-triazines and certain of their degradation products as obtained by the diode array detector. Absorbances normalized to the 0.05 AUFS settings. Other conditions as noted in Figure 4.

n-heptanesulfonic acid was determined to be the ion-pair reagent of choice because it provided for the highest resolution between HYAT, SI and AT and gave the best peak shape for the compounds of interest. From Table 5, it is interesting to note that the capacity factors for AT, SI, Propazine (PR) and the de-alkylated products of AT decreased whereas those for HYAT and HYSI increased with increasing length of the alkyl group of the ion-pair reagent.

The scanning diode array detector used in this study provided a convenient method for qualitative identification of the eluting peaks based upon their ultraviolet spectra. Previously, UV absorption maxima were reported for the s-triazine herbicides at 220 nm, the hydroxy compounds in acid solution at 240 nm and the de-alkylated products, when detected, from 200-212 nm (8-11, 14). Figure 5 presents a composite of the UV absorption spectra of the compounds studied as obtained by the diode array detector in the mobile phase: (50:50 v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid, 0.10 M H3PO4, pH of 2.8. In the

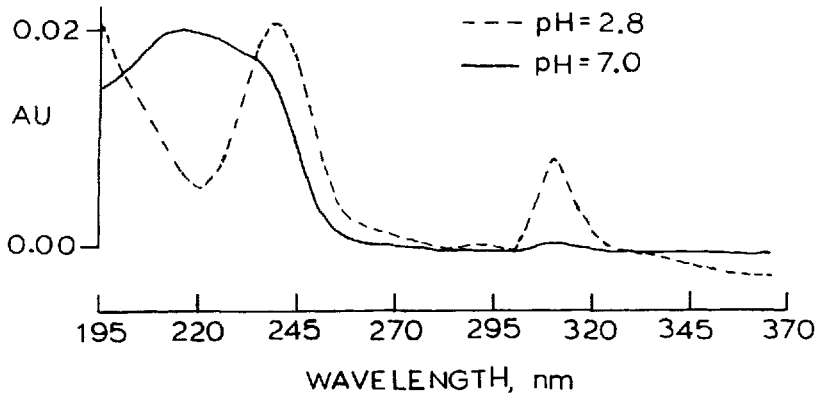

Figure 6. Ultraviolet absorption spectra of HYAT at pH 2.8 in the mobile phase 50:50 (v/v) acetonitrile-water containing 0.020 M n-heptanesulfonic acid with 0.10 M H3PO4, and at a pH of 7.0 in 70:30 (v/v) acetonitrile-water containing 0.010 M K2HPO4.

preliminary mobile phase development studies with methanol-water, an impurity peak with an absorption maximum of 206 nm often co-eluted with the AT peak. In neutral pH mobile phases, the peaks for HYAT and HYSI were found to be significantly broader with considerable tailing attributed to the enol-keto isomerization depicted in Figure 1. Figure 6 presents the UV absorption spectra obtained on the diode array detector for HYAT at pH values of 2.8 and 7.0.

Studies were performed to verify that there was a linear relationship between peak height and concentration of the compound injected. Table 6 presents a summary of the linear least squares regression equations for AT, HYAT and PR. In all cases, over the range from 0.4 to 10.0 ppm, the three compounds had Pearson's linear correlation coefficients of 0.999. Lower limits of detection were estimated to be 0.01 ppm for AT and PR and 0.04 ppm for HYAT. A maximum absorption wavelength of 220 nn was used for detection of AT and PR; 240 nm was used for the detection of HYAT.

TABLE 6

Linear Least Squares Regression Analyses of the Diode Array Detector Responses for Atrazine, Hydroxyatrazine and Propazine *.

Compound	Slope	Y-intercept	Pearson's Linear Correlation Coef.	Lower Limit of Detection**
Atrazine	41.1	0.563	0.9992	0.01 ppm
Hydroxyatrazine	24.5	-0.922	0.9995	0.04 ppm
Propazine	36.8	1.58	0.9999	0.01 ppm

* The linear regression equation: peak height = slope x Concentation + Y-intercept for the concentration levels of 0.4, 1.0, 2.0, 4.0, 8.0 and 10.0 ppm. Slope in units of cm of peak height/ppm, Y-intercept in units of cm of peak height.

** Lower limit of detection determined from the x-axis value when the detector signal of the compound corresponded to three times the standard deviation of the noise.

TABLE 7

Summary of the Analyses for Atrazine in the Upper 2-3 cm of the Soil from Three Corn Fields, Expressed in ppm, Micrograms of Atrazine per Gram of Soil.

Days after Spraying	Average Height of Corn, cm	Lee Farm Site-1	Lee Farm Site-2	Lower Spring Manor Farm	Upper Spring Manor Farm Site-1	Upper Spring Manor Farm Site-2
-3	--	0.00	--	0.00	0.02	--
0*	--	2.03	--	1.49	2.31	1.28
18	5	1.04	--	3.01**	12.3**	11.0**
39	50	0.73	0.50	0.66	2.43**	3.26**
60	150	0.17	0.26	0.46	0.62	0.95
81	305	0.18	0.15	0.37	1.03**	2.55**
102	335	0.19	0.11	0.30	0.49	0.70
130*	350	0.20	0.07	0.19	0.14	0.28

* Soil sampled after the application of the atrazine herbicide; corn harvested on days 130 and 131.

** Elevated values were confirmed by repeating or verifying the analyses. These elevated values were attributed to the heterogeneous nature of the sampling of the upper 2-3 cm of the soil.

Results and Discussion of the Field Studies

The developed method involving an acid solution extraction, concentration onto the solid phase extraction (SPE) cartridge, and then separation by reversed-phase HPLC analysis with an ion-pair reagent and a diode array detector, was used to monitor the degradation of AT in the soil from three local corn fields. Only the upper 2-3 cm of the soil was sampled at 21 day intervals throughout the growing season. The three corn fields had been initially sprayed over a three-day interval in mid-May with a commercial mixture of Atrazine 4L herbicide. (Using the developed HPLC method, the commercial Atrazine 4L was found to have 43.8 per cent atrazine, in comparison to a level specified on the manufacturer's label of 43.0 per cent as active ingredients. No hydroxyatrazine was found to be present in the Atrazine 4L prior to the spraying). The HPLC peaks assigned to AT or HYAT were identified by comparison of their retention times and by matching their UV absorption spectra to those of known standards as recorded by the diode array detector.

Table 7 summarizes the analyses for AT found in the farm soils sampled. In general, the levels of AT from the five sampling sites in the three corn fields dropped from about 2.0 ppm to 0.2 ppm over the 130 days. There were sporadically high values for AT, marked by an **, that were confirmed by repeated analyses of the soil samples or by careful review of the data. These high values were attributed to improper or non-homogeneous sampling of the upper 2-3 cm levels of the atrazine-sprayed soils. In only five of the thirty-six samples reported in Table 7 was a peak for HYAT detected. The levels of HYAT were found to be less than 0.6 ppm and a peak for HYAT was detected only when an elevated level, above 2.5 ppm, was detected for AT. (In a separate study, it was shown that AT was not converted to HYAT in the acid extraction and SPE cartridge clean-up step.)

Of potential interest, two samples of ground water from shallow wells located at the edge of two of the corn fields were

taken during the growing season of the corn. A ground water well located about 12.2 m from the sampling site at the Lower Spring Manor farm field, contained 0.1 ppb of AT 22 days after spraying. Whereas 42 days after spraying, the groundwater sampled from a well 12.2 m from the Lee Farm Site 1 soil sampling site contained a level of 3.8 ppb of AT. Implications for contamination of nearby ground water by repeated, yearly straying of corn fields with AT is being reviewed by State of Connecticut regulatory agencies.

ACKNOWLEDGMENTS

The permanent address of Qian Wenheng is Nanjing Institute of Soil Science, Chinese Academy of Sciences, P.O. Box 821, Nanjing, The Peoples' Republic of China. We thank Dr. H. LeBaron and R. K. Williams of the Agricultural Division of Ciba-Geigy Corp., Greensboro, North Carolina, U.S.A., for valuable and timely comments, for sending procedures and analytical standards of AT, SI, PR and their various degradation products. We thank Russel Spencer and Joseph Poland of the State of Connecticut, Department of Health Services, for their help and encouragement. We thank Mr. G. Zimmer, Farm Manager, Agriculture Department, University of Connecticut, for his cooperation. This research was funded in part by Grant No. 14-08-0001-G1552 from the U.S.A. Department of the Interior administered by The University of Connecticut's Institute of Water Resources. This article has not been subject to official Department of Interior review and no endorsement should be inferred. This paper was presented as Talk No. 739 at the Pittsbugh Conference on Analytical Chemistry and Applied Spectroscopy, New York City, New York, March 1990.

REFERENCES

1. STORET, U.S. Environmental Protection Agency, Data Base of the Results of Agricultural Water Pollution, "Incidence of the Detection of Atrazine", 1987.

2. "Study of Pesticide Contamination in Connecticut's Ground Water, Interim Report", State of Connecticut, Department of Environmental Protection, Hartford, 1988.

3. Huang, L.Q. and Frink, C.R., Distribution of Atrazine, Simazine, Alachlor, and Metolachlor in Soil Profiles in Connecticut, Bull. Environ. Contam. Toxicol., 43, 159, 1989.

4. Kaufman, D.D. and Kearney, P.C., Microbial Degradation of s-Triazine Herbicides, Residue Rev., 32, 235, 1970.

5. Sheets, T.J., Persistence of Triazine Herbicides in Soils, Residue Rev., 32, 287, 1970.

6. Erickson, L.E. and Lee, K.H., Degradation of Atrazine and Related s-Triazines, Crit. Rev. Environ. Control, 19, 1, 1989.

7. Muir, D.C.G. and Baker, B.E., A Method for the Routine, Semiquantitative Determination of Hydroxy-s-triazines in Soils, J. Agric. Food Chem., 26, 420, 1978.

8. Ramsteiner, K.A. and Hormann, W.D., High-Pressure Liquid Chromatographic Determination of Hydroxy-s-Triazine Residues in Plant Material, J. Agric. Food Chem., 27, 934, 1979.

9. LeBaron, H. and Williams, R.K., Agricultural Division, Ciba-Geigy Corp., Greensboro, N.C., Private Communications, 1989-1990.

10. Beilstein, P., Cook, A.M. and Hutter, R., Determination of Seventeen s-Triazine Herbicides and Derivatives by High-Pressure Liquid Chromatography, J. Agric. Food Chem., 29, 1132, 1981.

11. Vermeulen, N.M.J., Apostolides, Z., Potgieter, D.J.J., Nel, P.C. and Smit, N.S.H., Separation of Atrazine and Some of Its Degradation Products by High-Performance Liquid Chromatography, J. Chromatogr., 240, 247, 1982.

12. Russell, J.D., Cruz, M., White, J.L., Bailey, G.W., Payne, Jr., J.D. and Teasley, J.I., Mode of Chemical Degradation of s-Triazine by Montmorillonite, Science, 160, 1340, 1968.

13. Wachob, G.D., Solid-Phase Extraction of Triazine Herbicides from Soil Samples, LC Magazine, 2, 756, 1984.

14. Pacakova, V., Stulik, K. and Prihoda, M., High-Performance Liquid Chromatography of s-Triazines and Their Degradation Products Using Ultraviolet Photometric and Amperometric Detection, J. Chromatogr., 442, 147, 1988.

15. U.S. Department of Agriculture, Handbook No. 436, Soil Toxonomy- A Basic System of Soil Classification for Making and Interpreting Soil Surveys, U.S. Printing Office, Washington D.C., 20402, 1975, pp. 1-754.

16. U.S. Department of Agriculture, Important Farmlands of Tolland County, Soil Conservation Service, Storrs, CT 06268, 1979, pp. 1-10.

17. Mattson, A.M., Kahrs, R.A. and Murphy, R.T., Quantitative Determination of Triazine Herbicides in Soils by Chemical Analysis, Residue Rev., 32, 371, 1970.

18. Jordan, L.S., Farmer, W.J., Gooden, J.R. and Day, B.E., Nonbiological Detoxication of the s-Triazine Herbicides, Residue Rev., 32, 267, 1970.