LIQUID CHROMATOGRAPHIC DETERMINATION OF ATRAZINE AND ITS DEGRADATION PRODUCTS IN WATER

By Thomas R. Steinheimer, U.S. Geological Survey, and
Martin G. Ondrus, University of Wisconsin - Stout

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Dallas L. Peck, Director

For additional information write to:
Chief, National Water Quality Laboratory
U.S. Geological Survey
Box 25046, Mail Stop 407
Federal Center
Denver, CO 80225-0046

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CONVERSION FACTORS

Metric units (International System) in this report may be converted to inch-pound units by using the following conversion factors:

Multiply	*By*	*To obtain*
kilopascal (kPa)	1.32×10^{3}	inch of mercury (Hg)
liter (L)	0.264	gallon
microgram (µg)	2.20×10^{-9}	pound
microgram per liter (µg/L)	8.35×10^{-5}	pound per gallon
microgram per milliliter (µg/mL)	8.35×10^{-2}	pound per gallon
microliter (µL)	2.64×10^{-7}	gallon
micron (µ)	3.93×10^{-5}	inch
micrometer (µm)	3.93×10^{-5}	inch
milligram per liter (mg/L)	8.35×10^{-2}	pound per gallon
milligram (mg)	2.20×10^{-6}	pound
milligram per square meter (mg/m^2)	8.91×10^{-3}	pound per acre
milliliter (mL)	2.64×10^{-4}	gallon
milliliter per minute (mL/min)	1.58×10^{-2}	gallon per hour
nanogram (ng)	2.20×10^{-12}	pound
nanogram per liter (ng/L)	8.35×10^{-8}	pound per gallon
nanogram per microliter (ng/µL)	8.35×10^{-2}	pound per gallon
nanometer (nm)	3.93×10^{-8}	inch

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By THOMAS R. STEINHEIMER, U.S. GEOLOGICAL SURVEY

and

MARTIN G. ONDRUS, UNIVERSITY of WISCONSIN - STOUT

ABSTRACT

A method for the quantitative determination of atrazine and its principal degradation products in water is described. Bonded-phase extraction is used to isolate three principal metabolites of atrazine from both surface- and ground-water samples. Analytes are extracted from water by passage of the sample across a sorbent matrix consisting of a reversed-phase silica packing that has been chemically modified to incorporate cyclohexyl groups at the surface. High-performance liquid chromatography employing a methanol-water gradient is used for separation. Analytes are detected by using a combination of filter photometers together with a photodiode-array system. Identification of analytes is done by retention-time and absorption-spectra matching against authentic reference standards. Terbuthylazine is used for internal-standard quantitation.

Method validation involved determination of recovery percentage for all analytes from spiked water. Test matrices chosen for this evaluation included laboratory-distilled water, Mississippi River water (from a dam near Winona, Minn.), and ground water from several shallow aquifers in Wisconsin in areas of known atrazine contamination. Spike levels ranged from 0.2 to 20 micrograms per liter. All determinations were run in quadruplicate. Nominal limit of detection, based upon a 0.5-liter test sample, is approximately 0.4 microgram per liter.

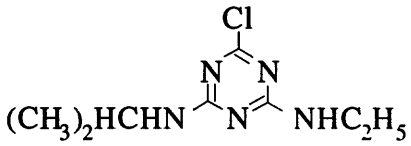
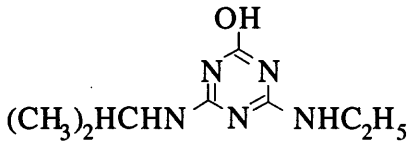
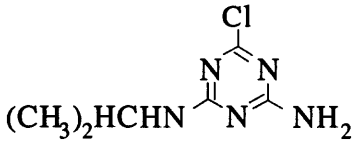
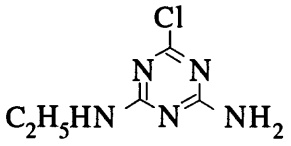
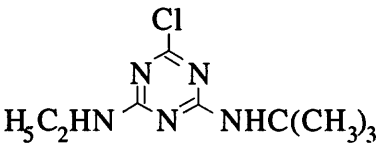
INTRODUCTION

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 others, 1985). Atrazine is extensively used for pre- and post-emergent weed control on corn, wheat, soybean, maize, and other crops throughout the Midwest and elsewhere in the Country. Typical application rates for atrazine on corn are 200 to 400 mg/m^2 of active ingredient depending upon soil properties, nature of crop, atmospheric conditions, and (or) irrigation program (Crop Protection Chemicals Reference, 1988). This large usage of a single weed control agent has resulted in great concern over the ultimate disposition of atrazine residues in surface and ground waters. An estimated 3 percent of all field-applied atrazine is lost to freshwater streams from contiguous land (Hormann and others, 1979; Muir and Baker, 1978). Other particulate-phase components of hydrologic systems also may capture and retain atrazine derivatives. Residues of atrazine (in combination with other agrochemicals) resulting from field application to cultivated land have been measured in shallow aquifers throughout the Midwest and Southeast as well as identified in major river drainage basins (Younos and Weigman, 1988; Wauchope, 1978). Given the possibility for potentially massive contamination of natural-water resources, most States have no enforcement regulations to deal with atrazine residues in water supplies drawn for irrigation or potable supply. Many States, some with major agriculture, do not require rigorous record-keeping practices on sales or usage of pesticides (Gianessi, 1987). An exception, the State of Wisconsin, Department of Natural Resources, has proposed a not-to-exceed limit for atrazine in ground water of 0.35 µg/L (Nyer, 1988).

The environmental-fate chemistry of atrazine is not well understood and probably involves competitive and very complex processes. Field-applied atrazine is known to undergo both N-desalkylation and deamination reactions that are brought about by fungi present in the soil (Kaufman and Blake, 1970; Kaufman and Kearney, 1970) and by bacteria present in the soil (Behki and Khan, 1986; Giardina and others, 1980). Desalkylation reactions occur on either one or both secondary amino-nitrogen atoms. Two- and three-carbon fragments apparently are lost directly. These conversions produce desethyl- and desisopropylatrazine. Each of these congeners of atrazine retains phytotoxicity in

Table 1.--*Structures and names of atrazine congeners and internal standard**

[IUPAC, International Union of Pure and Applied Chemistry; CAS, Chemical Abstracts Service]

Structure	IUPAC name Common name (abbr.)	CAS registry number
	2-Chloro-4-ethylamino-6-(1-methyl-ethyl)amino-sym-1,3,5,-triazine atrazine (A)	1912-24-9
	2-Hydroxy-4-ethylamino-6-(1-methyl-ethyl)amino-sym-1,3,5,-triazine hydroxyatrazine (HA)	2163-68-0
	2-Chloro-4-amino-6-(1-methylethyl)-sym-1,3,5,-triazine desethylatrazine (DEA)	6190-65-4
	2-Chloro-4-amino-6-ethylamino-sym-1,3,5-triazine desisopropylatrazine (DIA)	1007-28-9
	2-Chloro-4-ethylamino-6-(2-methyl-2-propyl)amino-sym-1,3,5-triazine terbuthylazine (TBA)*	5915-41-3

the soil. Loss of alkyl substituents from both amino-nitrogen atoms produces desethyl, desisopropylatrazine. Atrazine is known to undergo abiotic hydrolysis reactions leading to dechlorination and the formation of hydroxyl derivatives, most of which are not phytotoxic. Table 1 shows the chemical structure, common chemical name and International Union of Pure and Applied Chemistry (IUPAC) system name, and Chemical Abstracts Service (CAS) registry number for each compound.

The desalkylation, deamination, and hydroxylation of atrazine probably occur simultaneously through chemical, photochemical, and microbiological transformations that are indistinguishable in the environment. These competing

pathways may provide alternate routes to indeterminate stages within an essentially degradative pathway. Accordingly, any water sample tested is likely to contain any or all of the degradation intermediates listed (along with others not listed) in table 1. Most of the presumed atrazine metabolites resulting from cleavage of the triazine ring are unreported in the literature.

During the past ten years, much emphasis has been 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 determinations in water. The NPD is especially appropriate for the determination of triazine herbicides that contain 5 gram-atoms of nitrogen per mole (35 percent nitrogen by weight). Most of these methods are for multiresidue determinations and are used for detecting many triazines in a single extract (Pressley and Longbottom, 1982; Bradway and Moseman, 1982; Popl and others, 1983; Steinheimer and Brooks, 1984; Lee and Stokker, 1986). Each of these methods uses extraction with an organic solvent in the liquid phase or on a solid sorbent. 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 and others, 1988). Solvent extraction followed by GC-MS employing an ion-trap detector has been applied to surface water samples from the lower Mississippi River for a series of herbicides including several triazine metabolites (Pereira and others, 1990). A novel approach utilizing pressure devices to extract atrazine and desalkylated hydroxyatrazine congeners from plant tissues also has been reported (Nelson and Khan, 1989).

High performance liquid chromatography (HPLC) also has been used successfully for separation of mixtures of triazine herbicides. Most commonly, detectors of choice are the spectrophotometer or the mass spectrometer. Most methods use reversed-phase mode of separation by using methanol-water or acetonitrile-water as mobile phase (Paschal and others, 1978; Dufek and Pacakova, 1979; Subach, 1981; Binner, 1981; Beilstein and others, 1981; Lawrence, 1981; Williamson and Evans, 1981; Parker and others, 1982). Separation of desalkylatrazines and hydroxyatrazine by reversed-phase HPLC has been reported (Vermeulen and others, 1982). Recently, the simultaneous determination of 22 pesticides in both surface water 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 a sample volume of 1 L. Identifications were based upon absorption maxima at 220, 230, and 245 nm, and the verification was supported by comparison of full-absorption spectra. Two liquid-liquid extraction methods have been compared in another HPLC method for atrazine and several metabolites using a photodiode-array detector to compare full ultraviolet spectra (Durand and Barcelo, 1989).

HPLC also is applied to the determination of atrazine residues in soils. This method has been compared with a colorimetric method (Vickrey and others, 1980) and with a capillary-column gas-chromatographic method (Xu and others, 1986) for the parent herbicide and selected metabolites.

Rapid and inexpensive immunochemical assays for pesticide residues in environmental samples are a developing new technique for water-quality studies. However, due to the limitations of polyclonal antibodies used in the haptens, many of these suffer from an uncertainty associated with the analyte specificity of measurement. A recent report describes the first successful application of monoclonal-antibody based enzyme linked immunosorbent assays (ELISA) to the determination of hydroxyatrazine and atrazine in soil and water (Schlaeppi and others, 1989).

PURPOSE AND SCOPE

This report describes a rapid analytical method for simultaneous determination of atrazine, two desalkylatrazines, and hydroxyatrazine in surface-water and ground-water samples. It incorporates a relatively new technique in the field of trace organic analysis in environmental samples-specifically, bonded-phase extraction on reversed-phase sorbents. This method provides a new tool with which to detect and monitor herbicide residues in surface water and shallow aquifers. It is rapid compared to traditional solvent extraction procedures and is comparatively inexpensive to setup.

METHOD FOR DETERMINATION OF ATRAZINE AND ITS DEGRADATION PRODUCTS

Water samples are collected in clean narrow-mouth, flint-glass amber bottles previously heated overnight at 325 °C, and fitted with Teflon-lined metal screw caps. Samples are shipped to the laboratory and stored at 4 °C until extracted. Each sample is extracted by passage through a cyclohexyl bonded-phase extraction cartridge that is subsequently eluted with methanol. A measured volume of the final extract is analyzed by gradient-mode HPLC using a photodiode-array detector. Qualitative and quantitative determinations are carried out using an internal standard. Final concentrations are reported in micrograms per liter.

Application

This method has been validated for the determination of atrazine and its degradation products in surface water and ground water; however, it may be suitable for determination of other triazine herbicides and their degradation products. All water samples must be filtered through organic-binder-free glass-microfibre filter pads to remove suspended sediment. The filtrate is then analyzed according to this method, and results reported as dissolved-phase concentrations. Analytes that are determined include atrazine, hydroxyatrazine, desethylatrazine, and desisopropylatrazine. Terbuthylazine, a triazine herbicide not registered for agricultural use in the United States, was chosen as internal standard for quantitation. Nominal detection limit for this method, based upon a 0.50-L test sample, is 0.4 µg/L. The range of concentrations in a water sample for which the method can be used is:

atrazine - 0.4 to 40 µg/L
hydroxyatrazine - 0.4 to 40 µg/L
desethylatrazine - 0.4 to 40 µg/L
desisopropylatrazine - 1.0 to 100 µg/L

Interferences

Interferences may result from any co-extracted organic compounds found in natural waters that exhibit substantial absorbance at the chosen wavelengths. For some samples, a micro-column clean-up procedure prior to instrumental analysis may be required. One authentic test matrix used for recovery studies was the Mississippi River near Winona, Minn., and no substantial interferences were observed. Several other major classes of herbicides were examined for interference with instrumental determination, and no interference was observed. These classes included alpha-chloroacetanilide, o-dinitroaniline, and urea derivatives, and represent the kinds of chemicals that are likely to be co-applied with atrazine formulations.

A blank must be included with each sample set. This is done in order to insure no interference from solvents, reagents, or any other equipment or device in contact with the sample.

Filtration of both organic solvents and aqueous samples is required to remove particulates. All mobile-phase mixtures are degassed by vacuum filtration. Following equilibration of the instrumental system, a blank-gradient run with no injection is performed to determine the presence of interferences in the mixed-solvent mobile phase or in the instrumental hardware itself.

Apparatus

All glassware is washed in a warm detergent solution, rinsed with organic-free water followed by HPLC-grade methanol or 2-propanol, and heated overnight in an oven at 325 °C. Clean glassware is rinsed with solvent immediately prior to use. Apparatus used in this method are the following:

Adapter—Polypropylene, liquid-tight seal between cartridge barrel top and reservoir, Analytichem[1], or equivalent;

Back-flush vacuum trap—Vacuum flask, 2- to 4-L capacity, with assembled delivery stem and vacuum tubing;

Balance—Electronic microbalance, Cahn model 4100 electrobalance, or equivalent;

Centrifuge—Bench-top, capable of 1,500 r/min, head sized to accommodate 16 x 125 mm tubes;

Centrifuge tube—Borosilicate glass, culture tube, disposable, 16 x 125 mm;

Extraction cartridge—3-mL syringe-barrel cartridge (polypropylene or glass) with Stainless Steel frits, containing 500-mg spherical packing material, n-octadecyl (C18) or cyclohexyl (CH), with male Luer-tip fitting, Analytichem BondElut p/n 607313 and 610313, or equivalent. For enhanced recovery of desethylatrazine and desiso-propylatrazine, 6-mL, 1000-mg cyclohexyl cartridge is recommended, Analytichem BondElut p/n 610416, or equivalent;

Extraction manifold—Multi-port vacuum manifold, with female Luer-tip fittings for liquid-tight seal, Baker 10 extraction system, or equivalent;

Filtration apparatus—All glass vacuum-filtration system, for use with 0.20 µm porosity Nylon 66 membranes (Rainin Instrument, Catalog No. 38-111, or equivalent);

High-performance liquid chromatograph—Liquid chromatograph capable of both isocratic and gradient separations, with multiple wavelength photometric detectors or photodiode-array detector, auto-injection system, computer-controlled data-processing terminal, dot-matrix printer and X-Y plotter; Waters Model M6000A pump, Waters Model 45 pump, Waters Model 660 Solvent Programmer, Waters Model 440 Dual Channel Absorbance

[1]The use of trade or product names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey or the University of Wisconsin.

Detector equipped with an Extended Wavelength Module, Waters Model 710B Autosampler, Hewlett-Packard Model 1040M Diode-Array Detection System (including ChemStation), or equivalent;

pH Meter—Capable of pH measurement within from 0 to 14 range, with combination saturated calomel/glass electrode, 0.1-pH-unit accuracy, Fisher Accumet Model 825 MP, or equivalent;

Sample reservoir—Polypropylene or glass, containing permanently-seated 70-micron coarse porosity frit, with male Luer-tip fitting, from 75 to 125 mL capacity, Analytichem, or equivalent;

Vacuum pump—Vacuum pump, oilless, capable of sustaining pressure reduction of about 0.0152 kPa;

Volumetric flask—Class A, actinic, 10-, 25-, and 50-mL capacity for preparation of stock- and working-standard solutions.

Reagents

Reagents used in this method include the following:

Triazine stock solutions—Individual standards of atrazine, simazine, propazine, cyanazine, and their desalkylated degradation products prepared in methanol at concentration of 100 mg/L in actinic volumetric flasks and stored in freezer at -10 °C. Purity-certified crystalline standards were obtained from the Pesticide Repository, U.S. Environmental Protection Agency, Research Triangle Park, N.C., or from the Biochemistry Department, Ciba-Geigy Corporation, Greensboro, N.C.:

Hydroxyatrazine—Stock standard prepared in water by dropwise addition of concentrated hydrochloric acid; 10 mg of hydroxyatrazine dissolved in 50-mL water containing from 4 to 5 drops of acid, diluted to volume in 100-mL volumetric flask; concentration is 100 mg/L; stored at 4 °C;

Triazine working-standard solutions—Mixtures of atrazine and degradation products (including hydroxyatrazine) were diluted together with methanol in actinic volumetric flask at concentrations from 0.2 to 20 mg/L and stored at 4 °C;

Methanol—HPLC grade, or pesticide-residue grade, unpreserved, Burdick and Jackson, or equivalent;

Terbuthylazine (internal standard)—Stock solution prepared in methanol at concentration of 100 mg/L in actinic volumetric flask and stored in a freezer at -10 °C; 100 µL for each mL of final eluate added to autosampler vial;

Water—HPLC grade, or equivalent organic-free water, free of ultraviolet absorbing compounds as determined by blank-gradient analysis; deionized water treated through the Millipore Organic-Pure system, or equivalent, is acceptable.

Procedure

The following method is used on surface-water or ground-water samples.

Calibration—A calibration table is developed prior to sample determinations by injection of 25 µL aliquots of working standard mixtures ranging from 0.2 to 20 mg/L into the instrumental system. Raw data from each injection, using 220-nm and 230-nm wavelengths, is recorded. An integration file (table), consisting of integrated data from both detector wavelengths, provides retention times and peak areas, which also are recorded. Internal-standard quantitation is based upon the 220-nm data in the integration file and is used to compute response ratios for each analyte. Peak identification and verification is based upon a window limit of 7.5 percent of the retention time and ± 5 percent of the 220-nm/230-nm peak-area ratios.

Sample Filtration—Immediately upon receipt, water samples should be filtered through 0.70-µm mean porosity glass-fiber depth filters by using an all-glass vacuum filtration system, or a gravity-flow system.

Extraction—Volumes used for extraction may vary from 0.100 to 2.00 L. Generally, 0.500 L is recommended. Prior to extraction, each water sample is adjusted to a pH between 7.0 and 7.5 using magnetic stirring and dropwise addition of dilute ammonium hydroxide or phosphoric acid, as needed. For each sample, an appropriate BondElut cartridge (cyclohexyl) is activated by delivering 10 mL of methanol followed by 10 mL of water through the cartridge with a glass syringe. Although most of the recovery data presented in this report was generated using 3-mL, 500-mg cartridges, the use of 6-mL, 1,000-mg cartridges will furnish greater recoveries for the more polar analytes, specifically desethylatrazine and desisopropylatrazine (see tables 4 and 6). The activated cartridge(s) is positioned on a multi-port vacuum manifold and equipped with a Luer adaptor and a 50 to 125 mL reservoir. Using applied vacuum of about 0.0152 kPa, the water sample is drawn through the extraction cartridge at a nominal flow rate of 15 ± 5 mL/min. When the entire water sample has been passed through the cartridge, the cartridge is placed in a disposable culture tube and centrifuged for 1 min at approximately 1,500 revolutions per minute. Using a volumetric pipet, 2.00 mL of HPLC-grade

methanol is transferred to the cartridge and the analytes are eluted into a clean, dry disposable culture tube by air displacement delivered from a hypodermic syringe equipped with the Luer-tip adaptor. The cartridge in the culture tube is centrifuged for 1 min at 1,500 r/min to remove traces of eluant. Using a micropipettor, 200 µL of terbuthylazine 100 mg/L stock solution is delivered into the eluant in the culture tube and is swirled, to mix it. Internal standard is added at this point to normalize corrections for variability in volume of liquid recovered as eluate. A portion of this solution is then transferred to an autosampler vial and the septum is sealed.

Instrumental analysis—While samples are being prepared, the HPLC system is equilibrated. The detector and lamp should be ON and the lamp allowed a warm-up time of at least 1 h. The final mobile phase ratio of 65 to 35 methanol to water (pH=7) should be recirculated through the column at a flow rate of 1.5 mL/min for at least 1 h. An analysis method, including calibration table, data acquisition parameters, chromatography parameters, integration parameters, and report parameters should be loaded from the hard disk. The autosampler carousel should be loaded with standards first followed by extracts from samples. Using the ChemStation, a sequence file and sequence table are prepared to fit the order of samples on the carousel. Generally, it is recommended that the first standard (usually 5 or 10 mg/L) be injected manually to check retention times and peak areas. If all appears to be functioning properly, begin the automated sequence. The calibration table will be automatically updated using integration data obtained from the standards. It is suggested that the programming function of the autosampler be used to rerun the first standard after every four samples in order to check for calibration drift.

Calculations and Reporting Results

Using the calibration table and internal-standard quantitation, the instrument calculates the concentration of each analyte in the final extract, expressed as ng/µL. A matrix-recovery correction is applied by the following equation and the concentration of each analyte in a water sample is given by:

$$C = \frac{(A)(B)}{(D)(E)}$$

where

C = concentration in sample, in micrograms per liter;
A = concentration in eluate, in nanograms per microliter;
B = volume of eluate, in milliliters;
D = volume of sample, in liters, and
E = recovery factor (E = Recovery Percent/100).

Report concentrations of atrazine and individual degradation products as follows: Less than 10 µg/L, one significant figure; 10 µg/L and greater, two significant figures.

Precision and Accuracy

Validation of this method was done by precision and accuracy experiments conducted by spiking surface-water and ground-water samples at several levels in the µg/L concentration range using standard mixtures. All determinations were done in quadruplicate as a minimum on the following matrices:

MATRIX 1 A clean water matrix, such as tap or distilled.

MATRIX 2 An authentic surface-water matrix that drains agricultural land receiving field-applied herbicides. The Mississippi River at Winona, Minn., receives flow from the Chippewa, Red Cedar, and St. Croix Rivers, along with other small tributaries of west-central Wisconsin. Each of these Wisconsin rivers drains farmland that is extensively cultivated.

MATRIX 3 Several authentic ground-water matrices, known to be contaminated with atrazine and desalkylatrazines, were used for direct recovery studies by the method of standard additions. Sites chosen are located in Rusk and Osseo, Wis., and other locations identified by the Department of National Resources, State of Wisconsin, as atrazine-contamination areas.

Information on precision and accuracy is provided in tables 2 to 4.

RESULTS

Although atrazine has been studied extensively, the details of its many pathways to complete degradation and detoxification are not well understood. However, there is a consensus among researchers that major routes for breakdown are N-desalkylation and hydrolytic dechlorination followed by N-desalkylation. Although the abiotic hydrolysis reactions occur more rapidly, the N-desalkylation reactions are probably more important for water-quality studies, because they occur in the soil where they are promoted by microorganisms, and because the products of these reactions are more water soluble than atrazine and hydroxyatrazine. Therefore, desalkylated atrazines are more likely to occur in water than hydrolytic dechlorination products. Studies that emphasize the fate and movement of atrazine in surface water and ground water are better served with a method that can detect and quantify the major desalkylation and hydrolysis products simultaneously and provide a detection limit

Table 2.—*Dependence of percent recovery on volume of methanol used to elute cyclohexyl cartridge*[1]
[µg/L, micrograms per liter; mL, milliliters]

Analyte[2] concentration (µg/L)	Sample volume (mL)	Methanol elution volume (mL)	Desethyl, desisopropyl-atrazine percent recovery	Desisopropyl-atrazine percent recovery	Hydroxy-atrazine percent rcovery	Atrazine percent recovery
200	100	2.0	1 ± 1	58 ± 2	96 ± 5	99 ± 2
100	100	1.0	3 ± 2	67 ± 3	95 ± 2	98 ± 3
50	100	.5	0	49 ± 1	49 ± 1	38 ± 9

[1]The uncertainties are standard deviations from the mean of quadruplicate determinations.
[2]Final concentration of each analyte in spiked sample.

Table 3.—*Percent recovery from distilled water as a function of volume of sample extracted through a cyclohexyl cartridge*[1]
[µg/L, micrograms per liter; mL, milliliters]

Analyte[2] concentration (µg/L)	Sample volume (mL)	Methanol elution volume (mL)	Desethyl, desisopropyl-atrazine percent recovery	Desiso-propyl-atrazine percent recovery	Desethyl-atrazine percent recovery	Hydroxy-atrazine percent recovery	Atrazine percent recovery
200	100	2.0	3 ± 1	53 ± 1	97 ± 2	96 ± 3	99 ± 4
80	250	2.0	2 ± 1	25 ± 1	93 ± 5	99 ± 5	102 ± 4
40	500	2.0	1 ± 1	14 ± 1	64 ± 2	102 ± 2	95 ± 9
20	1000	2.0	0 ± 1	7 ± 1	33 ± 2	98 ± 1	99 ± 2

[1] The uncertainties are standard deviations from the mean of quadruplicate determinations.
[2] Final concentration of each analyte in spiked sample.

required by the district hydrologist for the study objectives. This method, which allows for the simultaneous detection of atrazine, two desalkylatrazines, and hydroxyatrazine, was developed in order to meet that need.

Sample Preparation by Bonded-Phase Extraction

Water samples are extracted by solid-phase extraction on BondElut cartridges (Van Horne and others, 1985; J.T. Baker Chemical Company, 1982, 1984). These devices consist of small quantities of silica-based chromatographic packing sandwiched between two Stainless Steel frits and contained in a small polypropylene syringe barrel fabricated with a standard Luer-Lok tip. The reversed-phase silica gel packings with carbon loading between 10 to 12 percent are the better choice for extraction of pesticides from water. Authors of this work have determined that the cyclohexyl packing material is the bonded-phase best suited for removal of atrazine and its degradation products from water. In the course of development of this method, several other surface chemistry specificities were evaluated. The authors have determined that the cyclohexyl material was superior to the n-octadecyl, n-octyl, phenyl, cyano, amino, and aliphatic diol reversed-phase cartridges for all analytes. For those determinations that may focus on the most water-soluble congeners, such as desisopropylatrazine, a 1,000-mg cyclohexyl reversed-phase cartridge is suggested. Using this approach, the authors have obtained the greatest recoveries from spikes for all analytes. For most natural water samples, those with dissolved organic carbon levels

Table 4.—*Percent recovery from spiked Mississippi River samples*[1]
[µg/L, micrograms per liter; mL, milliliters]

Analyte[2] concentration (µg/L)	Sample volume (mL)	Methanol elution volume (mL)	Cyclohexyl cartridge description	Desethyl, desisopropyl-atrazine (percent recovery)	Desiso-propyl-atrazine (percent recovery)	Desethyl-atrazine (percent recovery)	Hydroxy-atrazine (percent recovery)	Atrazine (percent recovery)
1.6	500	2.0	2.8 mL, 500 mg stainless steel frits	<5	3 ± 6	54 ± 6	97 ± 7	101 ± 8
4.0	500	2.0	2.8 mL, 500 mg stainless steel frits	<5	12 ± 2	49 ± 1	88 ± 2	93 ± 2
2.0	500	2.0	2.8 mL, 500 mg stainless steel frits	<5	<5	43 ± 6	89 ± 7	102 ± 5
2.0	500	2.0	6.0 mL, 1,000 mg stainless steel frits	<5	30 ± 1	102 ± 2	91 ± 9	94 ± 1

[1]The uncertainties are standard deviations from the mean of quadruplicate determinations.
[2]Final concentration of each analyte in spiked sample.

less than 5 mg/L, the use of a test-sample volume of 0.5 L is recommended. To recover all material sorbed onto the packing, the elution volume becomes a very critical step. Data in table 2 show the results of experiments designed to examine the dependence of recovery upon elution volume. In all instances, the sample volume remains unchanged, while the elution volume was varied from 0.5 to 2.0 mL. Recoveries were substantially improved when elution was done with larger volumes of methanol, while recognizing that the recommended 2.0 mL elution volume from 500-mg cartridges may not be optimized for recovery from 1,000-mg cartridges.

Under operating parameters consisting of a 6-mL, 1,000-mg extraction cartridge and 2.0-mL elution volume, the method provides a nominal detection limit of 0.4 µg/L for atrazine, hydroxyatrazine, and desethylatrazine, and 1.0 µg/L for desisopropylatrazine, based upon a 0.5-L test sample volume. Data in table 3 indicate results of experiments carried out on distilled water and designed to determine the optimum volume of sample for recovery of all analytes through the cartridge. Although these compounds represent a broad range of polarities and water solubilities, complete and quantitative recovery for each from a single pass through the procedure is not possible. As a result, optimum sample volume for each analyte is compromised. For atrazine, hydroxyatrazine, and desethylatrazine, the 500-mL sample volume provides good recovery at the spiking level of 200 µg/L. Improved recoveries of the two most hydrophilic compounds, desisopropylatrazine and desethyl,desisopropylatrazine, are observed when smaller volumes are taken for extraction. Table 4 gives results of recovery experiments for all analytes spiked at concentrations between 1.6, 2.0, and 4.0 µg/L (ppb). The water matrix used for this evaluation was the Mississippi River. Samples were collected from the lock and dam near Winona, Minn., during June, 1988. All samples were filtered prior to spiking with methanol solution of standards. Mean recovery for atrazine, hydroxyatrazine, and desethylatrazine are greater than 50 percent at all spiking levels. Mean recovery from this matrix for the remaining two most polar compounds are very low. The data in table 4 also indicate that BondElut cartridges containing 1,000 mg of cyclohexyl bonded-phase substantially increase recovery of desethyl- and desisopropylatrazine.

Frequently, triazine weed-control agents are applied to cultivated land in conjunction with other pesticides, including other herbicides. Other classes of chemicals that are recommended by manufacturers for co-application with

Table 5.—*Retention times of s-triazine herbicides and desalkylated and hydroxylated metabolites*[1]

Triazine derivatives	Abbreviation used for triazine derivatives	Retention time, in minutes
Desethyl,desisopropylatrazine	DEDIA	2.13
Desisopropylatrazine	DIA	3.47
Hydroxysimazine	HS	3.77
Desethylatrazine	DEA	5.42
Hydroxyatrazine	HA	6.50
Cyanazine	C	8.13
Simazine	S	9.67
Hydroxypropazine	HP	10.18
Atrazine	A	11.62
Propazine	P	14.03
Terbuthylazine	TBA	14.63

[1]Separation conditions:
Flow rate = 1.5 milliliters per minute
6-minute gradient, 45 to 65 percent methanol
Solvent programmer, curve 10
Mobile phase pH = 7.2

triazines are the urea, dinitroaniline, and chloroacetamide compounds. Data in table 5 list the liquid-chromatographic retention times of several triazine herbicides that are likely to occur together in a water sample with atrazine and its degradation products. Specifically, simazine and propazine, and their corresponding hydroxyl derivatives, along with cyanazine, are shown not to interfere with chromatographic detection of any of the method analytes. Differentiation based upon retention time would permit recognition of each. Although not included in this table, the herbicides, alachlor and trifluralin, have been shown to produce no co-eluting or peak-overlap interference.

The liquid-chromatographic separation parameters were chosen so that all analytes and internal standard would be resolved from each other and to the baseline within a 15-min analytical run cycle. Figure 1, which corresponds to 250 ng on column of each component, illustrates the separation and peak symmetry to be expected by using the flexible-walled radial-compression-column technology. The upper curve represents 220-nm detection, and the lower curve represents the 230-nm detection. In the case of each compound, the shorter detection wavelength provided larger responses. Authors of a recent report discuss the reversed-phase liquid-chromatographic-separation behavior of a series of triazines along with their hydroxyl derivatives in terms of mobile-phase composition and pH (Pacakova and others, 1988). The use of photometric detectors is preferred over electrochemical devices for low-level residue measurements.

Detection and Quantitation by Photodiode-Array Spectrophotometry

The photodiode-array detector system is well suited for identification of triazines, because it is an information-rich measurement. Complete ultraviolet absorption spectra can be recorded instantaneously by using the hardware and associated computer-assisted software described in this report. Pesticide identifications are made reliably by comparison of retention time and ultraviolet absorption spectra to authentic standard compounds contained in a user-generated library. The advantages and limitations of photodiode-array detectors have been discussed by several authors (Jones, 1985; Fell and others, 1982; Fell, 1983; Fell and others, 1983; Fell, 1985). The signal-to-noise ratio of the detector used in this method can be improved by nearly an order of magnitude by careful choice of data-acquisition frequency (signal averaging, time domain) and spectral bandwidth (George and Elgass, 1984). Both of these instrumental parameters are programmable and can be optimized for each pesticide analyte. The influence of processing speed on spectral data acquisition also is important. The 10-ms acquisition time has been determined to be sufficiently brief to allow spectral averaging for enhanced signal-to-noise ratio, without distorting the spectra from large concentration changes during elution (Martin and others, 1984).

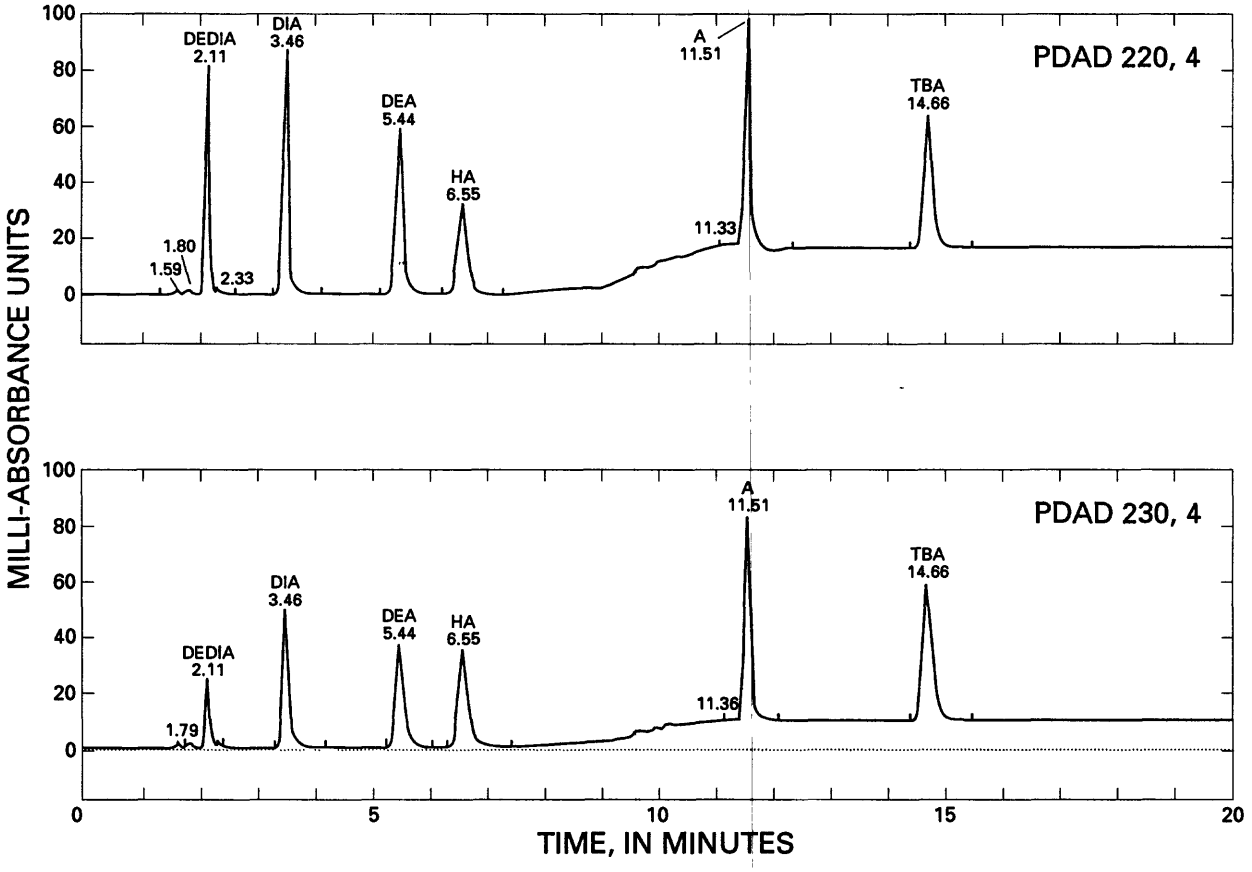


Figure 1.--Chromatogram from a 25 microliter injection of a 10.0 milligram per liter standard mixture using separation conditions described in footnote to table 5.

Data collected from a photodiode-array detector can be plotted in several formats to visually determine existence of overlapping peaks. Figure 2 illustrates a spectro-chromatographic plot of all five analytes together with the internal standard. Also referred to as a pseudoisometric (three-dimensional) plot, absorbance wavelength in nanometers is displayed on the y-axis, retention time in minutes is displayed on the x-axis, and intensity of absorbance (unitless) is displayed on the z-axis. These plots are used to determine optimum wavelengths for detection and quantitation, as well as to indicate peak purity for all analytes. The high sensitivity of the photodiode-array system is illustrated in figure 3, which shows the response obtained from 10 ng on column of each of the analytes from table 2 measured at 220 nm. Peak symmetry is very good, with an integratable area clearly visible for each analyte.

Method Performance

The true performance of this method is illustrated in figure 4. Mississippi River water was spiked at the 4-µg/L level with all five analytes together with the internal standard. Each compound was correctly identified within its retention window. Internal-standard quantitation furnished concentration values greater than 50 percent recovery for atrazine, desethylatrazine, and hydroxyatrazine; and recoveries from 5 to 30 percent for the most-polar congeners, desisopropylatrazine and desalkylatrazine, as shown in table 4. Figure 5 is a chromatogram using the extended-wavelength module (229-nm filter) of a river-water blank to which internal standard had been added after extraction and elution. A small atrazine peak can be seen at a retention time of 11.5 min. By using the same filter photometer as detector, figure 6 shows the chromatogram of a series of triazine herbicides and degradation products that are likely to occur in water samples adulterated with residues of atrazine metabolites or degradation compounds or both. Because of the differences in retention time between all of these candidate co-contaminants in a water sample, an experienced analyst would probably not mis-identify any of the target analytes.

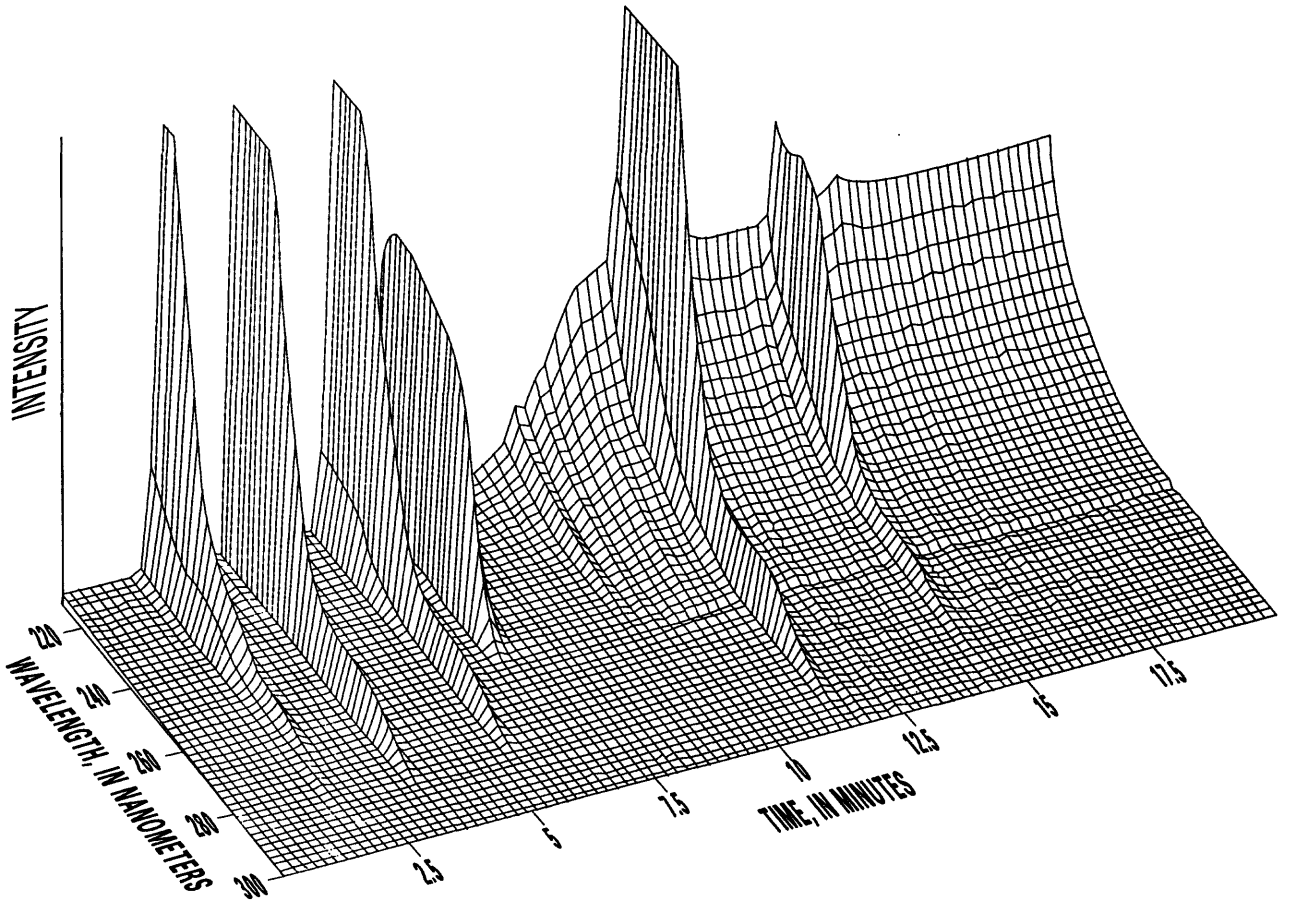


Figure 2.--Spectrochromatographic plot generated from the standard mixture injection in figure 1.

Test well samples were analyzed from the site of an atrazine spill near Osseo, Wis., in Trempeleau County. These determinations were made on 0.5-L samples using extraction cartridges containing 500 mg of cyclohexyl bonded-phase material. Although several samples had relatively high concentrations of atrazine and desethylatrazine, none contained hydroxyatrazine or desisopropylatrazine at detectable concentrations. In order to validate this method, atrazine and desethylatrazine concentrations in selected samples were independently confirmed by chemical analysis at the Wisconsin State Laboratory of Hygiene using solvent extraction and packed-column gas chromatography (GC) with an NPD. The data in table 6 show that good agreement was observed between the two methods.

Additional ground-water samples from private wells located in southern Wisconsin delivering a potable supply known to contain from 0 to 10 µg/L of atrazine were provided by the Wisconsin State Laboratory of Hygiene. This is an area impacted by heavy application of herbicides associated with field cultivation of corn and soybeans. Extraction of 0.5-L samples with cartridges containing 1,000 mg of cyclohexyl bonded-phase yielded measurable concentrations of atrazine, desethylatrazine, and desisopropylatrazine. Propazine was also detected in one of the samples. The data in table 7 shows that this method is capable of providing residue data comparable to that obtained from a classical solvent-extraction GC method.

CONCLUSIONS

A liquid-chromatographic method is described for the determination of atrazine, two desalkylatrazines, and hydroxyatrazine simultaneously in surface-water and ground-water matrices. The technique uses liquid chromatography and ultraviolet detection with a programmable photodiode-array system. Terbuthylazine is chosen for internal-standard quantitation. A nominal sensitivity of 1 µg/L is estimated for most water samples, based on a 0.5-L test sample. Sample preparation for instrumental analysis uses bonded-phase extraction on BondElut cartridges containing 500 mg of

Table 6.—Results of the analysis of ground-water samples obtained from a commercial laboratory in November 1988, taken from the site of an atrazine spill near Osseo, Wisconsin

Extraction of 500-milliliter samples using 3-milliliter, 500-milligram cyclohexyl cartridges

[milligrams per liter, µg/L, micrograms per liter; mg/L]

Well number	Run number	Desethylatrazine			Atrazine		
		Concentration in extract (mg/L)	Concentration in water[1] (µg/L)	Independent confirmation[2] (µg/L)	Concentration in extract (mg/L)	Concentration in water[1] (µg/L)	Independent confirmation[2] (µg/L)
MW-1	1	8.0	66		20.0	82	
MW-1	2	8	67		20	80	
MW-2	1	.6	5		2	8	
MW-2	2	.6	5		2	7	
MW-4	1	.1	1		.9	4	
MW-4	2	.2	1	1.6	.9	4	3.8
MW-5	1	.4	3		4	16	
MW-5	2	.4	3		4	17	
MW-6	1	.1	1		.5	2	
MW-6	2	.1	1	1.0	.5	2	1.7
MW-7	1	.5	4		1	5	
MW-7	2	.4	3	5.6	1	5	4.6
MW-8	1	.2	2		.7	3	
MW-8	2	.2	1		.7	3	
MW-9	1	1.8	14		16	62	
MW-9	2	1.8	14		16	63	

[1]Calculated concentrations are based on an atrazine recovery factor of 100 percent (1.0) and a desethylatrazine recovery factor of 50 percent (0.50).

[2]Solvent-extraction followed by packed-column GC with NP detection.

cyclohexyl sorbent. A summary of the suggested practice for processing water samples is given in table 8.

This method can be used as a rapid technique for evaluation of a field site suspected of being contaminated with atrazine residues. It is intended to provide the district hydrologist with a tool for planning a field sampling and laboratory-analysis program. If this protocol reveals the presence of atrazine or any of its degradation products in a water sample, the investigator may elect to carry out additional determinations using the more-sensitive, definitive, and costly gas chromatographic-mass spectrometric (GC-MS) technique for desalkylatrazines, but not for the hydroxyl derivatives.

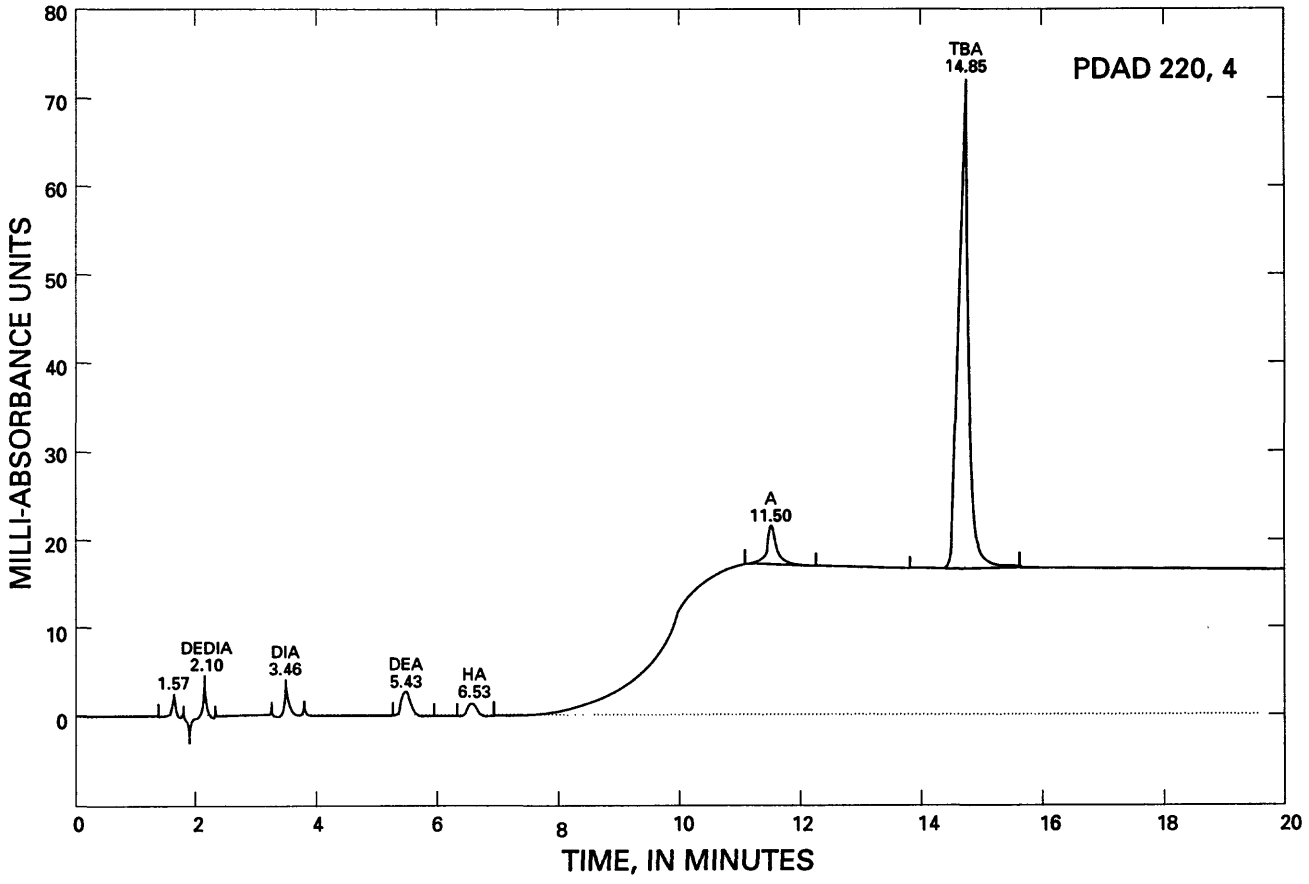


Figure 3.--Chromatogram from a 25 microliter injection of a 0.4 milligram per liter standard mixture.

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Table 7.—*Results of the analysis of samples obtained in April, 1989, from private wells delivering a potable supply in an area impacted by the application of herbicides. Extraction of 500-milliliter samples using 6-milliliter, 1000-milligram cyclohexyl cartridges.*

[mg/L, milligrams per liter; µg/L, micrograms per liter; ND, not detected; NR, not reported]

Well number	Run number	Desisopropylatrazine			Desethylatrazine			Atrazine		
		Concentration in extract (mg/L)	Concentration[1] in water (µg/L)	Independent confirmation (µg/L)	Concentration in extract (mg/L)	Concentration[1] in water (µg/L)	Independent confirmation (µg/L)	Concentration in extract (mg/L)	Concentration[1] in water (µg/L)	Independent confirmation (µg/L)
LR-1	1	0.5	5.0	NR	5.0	19	NR	1.0	6.0	NR
LR-1	2	.5	6	2.0	6	22	41	2	7	8.3
LR-2	1	.4	4	NR	3	11	NR	2	7	NR
LR-2	2	.2	2	.4	3	13	20	2	8	8.4
LR-3	1	ND	ND	NR	.2	.7	NR	.1	.4	NR
LR-3	2	.1	.7	ND	.1	.5	.6	.2	.6	ND
LR-4	1	ND	ND	NR	ND	ND	NR	ND	ND	NR
LR-4	2	ND	ND	ND	ND	ND	ND	.1	.3	ND
LR-5	1	.7	8	NR	.1	.4	NR	.1	.3	NR
LR-5	2	.8	9	2.5	ND	ND	.5	ND	ND	ND
LR-6	1	ND	ND	NR	.3	1	NR	.1	.3	NR
LR-6	2	ND	ND	ND	.4	2	1.8	.3	1	ND

[1]Calculated concentrations are based on replicate spikes of 500-milliliter LR-4 and LR-6 samples yielding mean recoveries of 36 percent (0.36) for desisopropylatrazine, 100 percent (1.00) for desethylatrazine, 82 percent for hydroxyatrazine, and 93 percent (0.93) for atrazine. No hydroxyatrazine was detected in any unspiked sample.

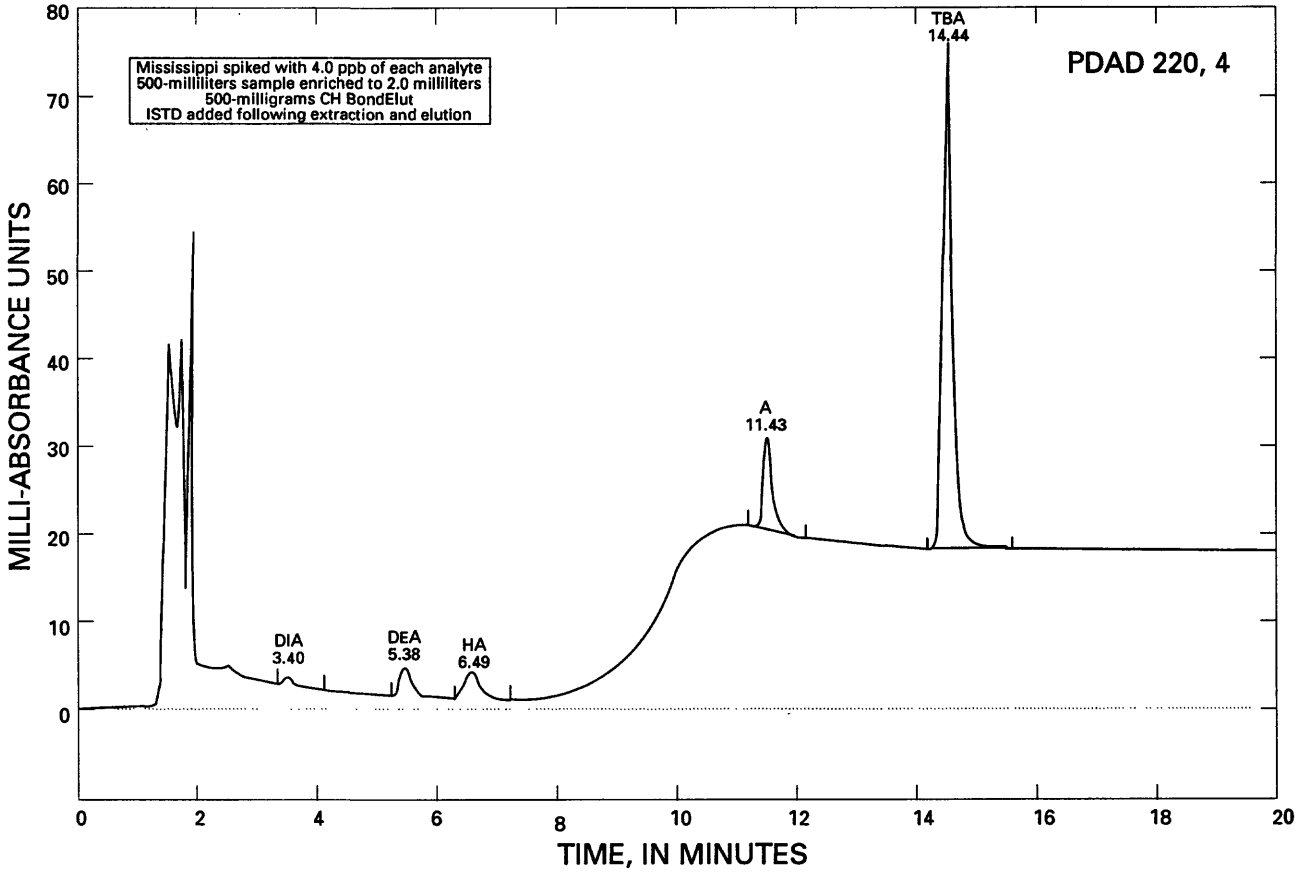

Figure 4.--Chromatogram from a 25 microliter injection of extract from Mississippi River sample spiked at 4 nanograms per milliliter.

Table 8.—*Recommended practice for the processing of surface-water and ground-water samples for instrumental analysis*

Multiport vacuum manifold
6-milliliter, 1000-milligram cyclohexyl BondElut (stainless steel frits)
0.5-liter-filtered water sample
Recommended sample set = 9
Four samples, four field or laboratory spikes[1], one method blank
2.0-milliliter elution with methanol
Instrumental analysis

[1]A spike recovery is performed on a duplicate 0.5-liter aliquot of each water sample for the purpose of calculating a matrix final concentration.

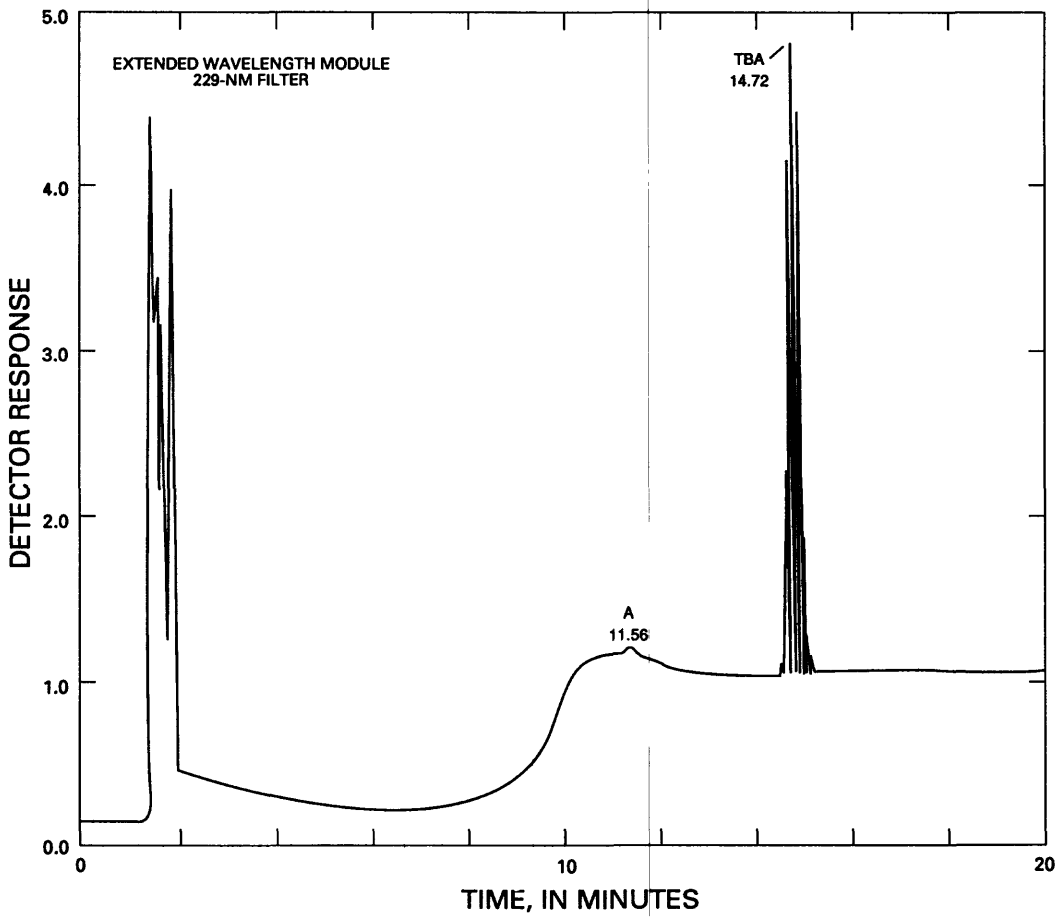

Figure 5.--Chromatogram from a 25 microliter injection of extract from unspiked Mississippi River sample.

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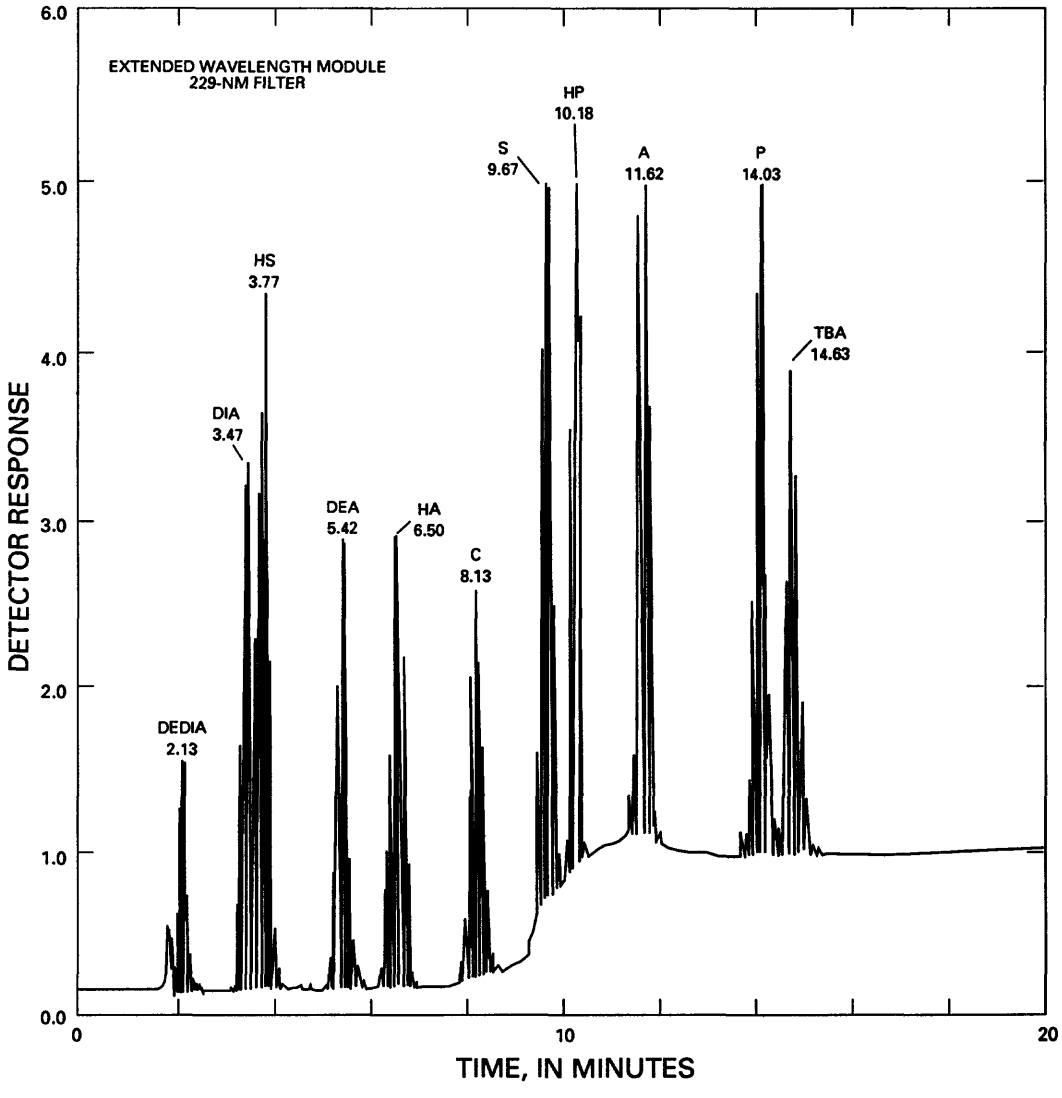

Figure 6.--Chromatogram from a 25 microliter injection of a standard mixture of symmetrical triazine herbicides and derivatives listed in table 5.

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