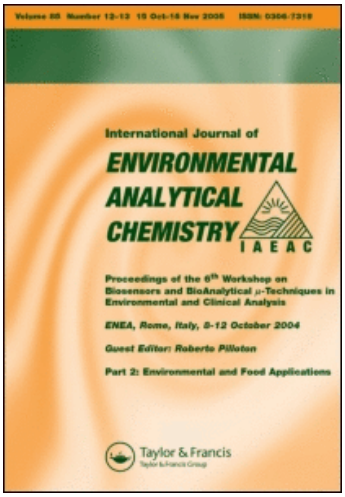

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Development and Evaluation of a Gas Chromatographic Method for the Determination of Triazine Herbicides in Natural Water Samples

T. R. Steinheimer[a]; M. G. Brooks[a]
[a] U.S. Geological Survey, Denver, Colorado

To cite this Article Steinheimer, T. R. and Brooks, M. G.(1984) 'Development and Evaluation of a Gas Chromatographic Method for the Determination of Triazine Herbicides in Natural Water Samples', International Journal of Environmental Analytical Chemistry, 17: 2, 97 — 111
To link to this Article: DOI: 10.1080/03067318408079923
URL: http://dx.doi.org/10.1080/03067318408079923

Intern. J. Environ. Anal. Chem., 1984, Vol. 17, pp. 97–111
0306-7319/84/1702-0097 $18.50/0

Printed in Great Britain

Development and Evaluation of a Gas Chromatographic Method for the Determination of Triazine Herbicides in Natural Water Samples

T. R. STEINHEIMER† and M. G. BROOKS

U.S. Geological Survey, P.O. Box 25046, Mail Stop 407, Denver Federal Center, Denver, Colorado 80225

(*Received August 24, 1983; in final form November 18, 1983*)

A multi-residue method is described for the determination of triazine herbicides in natural water samples. The technique uses solvent extraction followed by gas chromatographic separation and detection employing nitrogen-selective devices. Seven compounds can be determined simultaneously at a nominal detection limit of 0.1 μg/L in a 1-litre sample. Three different natural water samples were used for error analysis via evaluation of recovery efficiencies and estimation of overall method precision. As an alternative to liquid–liquid partition (solvent extraction) for removal of compounds of interest from water, solid-phase extraction (SPE) techniques employing chromatographic grade silicas with chemically modified surfaces have been examined. SPE is found to provide rapid and efficient concentration with quantitative recovery of some triazine herbicides from natural water samples. Concentration factors of 500 to 1000 times are obtained readily by the SPE technique.

KEY WORDS: Triazines, determination, methodology, chromatography, water.

INTRODUCTION

Triazines are the major herbicides used to increase agricultural production. Positive results from their usage are commonly

†Author to whom correspondence and proofs should be addressed.

accompanied by negative aspects of water pollution. Contamination of wells and streams from spills or direct spraying operations, or from agricultural run-off has been observed in Canada and in Europe as well as in the United States. It has been shown that *s*-triazines cause mutagenic and sometimes pathogenic effects on living organisms.[1] The persistence of triazines in soil has been linked to decreased production of certain crops.[2] As a result, environmental protection has emerged as a factor in determining the desirability of these materials. Many governments have enacted legislation that rigidly controls the introduction of these materials into the hydrologic environment, but more information is needed regarding their occurrence, distribution, and fate. Thus, analytical methods that can provide reliable data on a variety of natural water samples are essential to proper impact assessments and regulatory decisions.

Gas chromatography continues to be the technique of choice for the analysis of triazine herbicides in many environmental and tissue samples. Historically, analyses were performed on packed columns with moderately polar liquid phases. More recently, microbore columns have been utilized to achieve lower detection limits and greater resolution.[3,4] Nearly every type of detector available has been used for herbicide analysis, with several providing some specificity for triazine identifications. The advent of element-selective detectors has greatly improved the reliability of pesticide residue determinations.

Those detectors that have been used include flame ionization,[5,6,7] electron capture,[4,8] microcoulometric,[4,8,9] alkali flame ionization,[10,11] flame photometric,[12] and electrolytic conductivity devices.[13,14] The thermionic plasma detector or alkali flame ionization detector (AFID) devices currently are the most popular choice for triazine determinations. Extraction and concentration of chloro- and alkylthio-triazines from spiked distilled water by sorption on solid sorbents has recently been reported.[15]

In this report a multi-residue method for the simultaneous determination of seven triazine herbicides in surface and ground water is described. The procedure has been evaluated using authentic samples. As an alternative, solid-phase extraction was compared with liquid–liquid partitioning. Both approaches were evaluated on three different water types.

LIQUID–LIQUID EXTRACTION METHOD

Apparatus

Balance, top-loading, 2000 g capacity; boiling chips, Hengar, hexane prewashed and oven-baked; compressed gases, helium, hydrogen, and air, high purity; concentrator, Kuderna–Danish, consisting of a 10 mL concentrator tube, 500 mL K–D flask, and a 3-ball Synder column; Erlenmeyer flask, 250 mL glass stoppered; volumetric flask, 25 mL, glass stoppered; powder funnel, stainless steel, 65 mm diameter; separatory funnel, glass stoppered with Teflon stopcock, 2000 mL or equivalent glassware capable of extraction of 900 to 1200 mL volumes; microcolumn, disposable Pasteur pipet, 15 cm in length with 0.4 to 1.3 mm taper of more than 5 cm length on one end; multi-channel solvent blow-down manifold; pH paper, indicator range 5 to 9.

Reagents

Aluminum oxide, Woelm W-200, neutral, activity I. Prepare activity V (16% deactivation) by mixing 100 g activity I with 19 mL water. Mix on wrist action shaker for 2 hr and store overnight in sealed container (prepare fresh weekly). Borosilicate glass wool, filtering grade, pre-washed with hexane and baked overnight; potassium hydroxide, 37% (W/V) aqueous solution prepared from reagent grade KOH and reagent water; sodium chloride, reagent grade; sodium sulfate, granular, anhydrous, heated overnight at 300°C and stored at 130°C; solvents, benzene, ethyl ether, hexane, and methylene chloride, distilled in glass; sulfuric acid, 25% (V/V) prepared from high purity concentrated H_2SO_4 and Type II reagent water (ASTM D1193-79); triazine standards, analytical reference grade. Dissolve 5 mg of standard material in benzene in a 25 mL volumetric flask, dilute to volume with benzene, and mix. Dilute stock standard to working concentration of 0.5 to 5 μg/mL with hexane.

Instrumentation

The gas chromatograph used is a Hewlett-Packard Model 5880A dual-column system equipped with a Model 7672A Automatic Sampler and Model 5880A GC Terminal with LEVEL 4 BASIC

programming capabilities.† This instrument enables on-column injection through a glass-lined port. Dual nitrogen–phosphorus detectors are used for all triazine determinations. Columns are of Pyrex glass, 1.8 m × 2 mm inside diameter, rendered inert by treatment with silanizing agent. The packings used were 1% OV-101 on UltraBond^R 20M, 100/120 mesh; and UltraBond^R PEGS, 100/120 mesh. An acceptable alternate packing is 1% SE-30 on UltraBond^R 20M, 100/120 mesh. These columns need to be conditioned according to the manufacturer's suggestions for bonded materials because the nitrogen specific detectors are extremely sensitive to contamination from columns/septum bleed.

Procedure

Rinse all glassware with methylene chloride before using. Weigh sample and capped bottle to the nearest 0.1 g and transfer to a 2000 mL separatory funnel using a stainless steel powder funnel. Weigh empty sample bottle with cap to the nearest 0.1 g and calculate sample weight. Dissolve 5 g of sodium chloride in the sample and adjust the pH between 7 to 9 using the potassium hydroxide solution or sulfuric acid solution as necessary. Add 75 mL of methylene chloride to sample bottle, and swirl to rinse the sides of the bottle. Drain the bottle into the separatory funnel. Shake the stoppered funnel vigorously for 1 minute, venting the system several times. Let stand undisturbed while layers clarify, and draw off the methylene chloride into a 250 mL glass stoppered Erlenmeyer flask. Most whole water samples containing some particulate material can cause emulsions and may need treatment as follows: If emulsions occur, add a volume of hexane, 50 to 75 mL. Shake the separatory funnel again to mix the solvents and allow the layers to separate (the organic phase is now the upper layer). Draw off the aqueous phase into the sample bottle. The remaining emulsion can be eliminated by vigorous shaking. Decant the extract into a 250 mL glass stoppered Erlenmeyer flask and return the sample to the separatory funnel. Repeat the extraction with two additional 50 mL portions of methylene chloride, collecting the organic layers in the flask. Add 5 g

†Use of brand/firm/trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

of anhydrous sodium sulfate to the combined extracts, stopper, and shake. Quantitatively transfer the combined extract to the K–D apparatus, add approximately 3 mL of hexane as keeper and a boiling chip, and concentrate to 3–5 mL in a water bath maintained at 80°C. Concentration either by K–D evaporation at 80°C or by rotary vacuum evaporation at 40°C is acceptable provided the analyst document the choice with recovery data using natural water samples. Continue evaporation to approximately 0.5 mL in a warm water bath under a stream of nitrogen or helium using the solvent blow down apparatus. During this step wash the walls of the tube with at least two 5 mL portions of hexane. Increase the volume to 1 mL with hexane and proceed to microcolumn clean-up on alumina, if necessary.

Microcolumn clean-up on alumina

Prepare the column by plugging a Pasteur pipet with silanized glass wool. Fill to a depth of 10 mm with sodium sulfate, followed by 30 mm of alumina, and top with another 5 mm of sodium sulfate. Tap gently to promote settling to a uniform bed. Using hexane, quantitatively transfer the extract to a column and elute with hexane until at least 6 mL has been collected and discarded. When the last of the hexane has just entered the upper layer of the sodium sulfate, elute the column with hexane–ethyl ether (2:1) until a 4 mL fraction has been collected. Reduce this volume to 0.5 to 1.0 mL under a stream of nitrogen or helium and proceed to gas chromatographic analysis. Chromatograph and identify the triazines by retention time matching against standards ($\pm 3\%$ window for identification), using at least two of the packings described.

Calculations

Triazine concentrations are calculated by an external standard method using response factors that are updated continuously in a calibration table. A multi-point calibration procedure was used to compensate for non-linearity of response factors and also for response versus quantity injected curves not passing through the origin. The method reports concentrations of pesticide for each peak matched from the calibration table. The use of the auto-sampler assures reproducibility of injection volumes.

SOLID-PHASE EXTRACTION METHOD

A 3 mL prepacked C8 extraction cartridge (J. T. Baker Co.) is prepared by washing first with 2 to 3 mL of HPLC grade methanol and then with 1 to 2 mL of HPLC grade methanol and then with 1 to 2 mL of HPLC grade water. The vacuum manifold extraction apparatus (Baker 10) is assembled under aspirator vacuum, and a 200 mL portion of spiked water is passed through the column at a flow rate of 10 to 20 mL/min. The cartridge then is centrifuged at 1000 rpm for 1 min to remove part of the entrapped water. The cartridge column is placed inside a 15.0 mL graduated centrifuge tube, 1.0 mL of methanol added to the cartridge from a Drummond microdispenser, and the column eluted by centrifugation at 1000 rpm for 1 min. The eluate is transferred to a 2.0 mL septum-sealed autosampler vial and analyzed by gas chromatography.

APPLICATION TO WATER SAMPLES

Natural surface and ground water samples were used for evaluation of method performance. Three natural water samples were tested. Two surface water samples were selected to be representative of the types of matrix normally encountered in triazine analysis. They included creek water (Clear Creek, Colorado Rocky Mountains, Front Range near Denver), and an agricultural drainage river water (Withlacoochee River, south central Georgia, near Valdosta). The latter site is in an agricultural area which is susceptible to pesticide contamination. A groundwater sample taken at a depth of approximately 91.4 m (lower aquifer, Piceance Basin, Colorado) also was used as a test matrix. Clear Creek samples were spiked at three levels covering the tenfold range from 0.5 μg/L to 5.0 μg/L. All other samples were spiked at the 1.0 μg/L level only. Samples were spiked from dilutions in acetone of the same stock solution containing a known mixture of seven triazines. All spikes were 1.0 mL in volume. Unspiked samples were analyzed simultaneously to insure that other organic constituents in the sample did not bias the results. The sensitivity of the method is considered to be 0.1 μg/L. Minimum detectability approaches 0.01 μg/L and can be achieved by appropriate adjustment of the final extract volume and/or injection volume without alteration of instrument conditions.

The results of chemical analyses are given in Table I for the major constituents in each matrix used for method evaluation. All laboratory determinations were completed within 3 weeks of sampling. The dominant water quality characteristics of the Withlacoochee River sample are color and dissolved organic carbon (DOC). The stream is greatly impacted by organic compounds contributed from both man's activities and terrestrial inputs. The distinguishing chemical characteristics of the Piceance Basin ground water are high pH and alkalinity.

TABLE I

Water quality characteristics of samples used for method evaluation.[a]

Chemical property	Clear Creek	Withlacoochee River	Piceance Basin groundwater
pH	7.4	6.9	9.1
Solids[b]	205	102	1060
Alkalinity	65	12	750
Organic carbon[c]	1.6	18.7	3.6
Sodium	19	8.9	420
Potassium	3.5	2.1	0.9
Magnesium	8.5	2.2	2.4
Calcium	31	5.9	3.8
Fluoride	0.8	0.2	24
Chloride	7.8	14	110
Nitrite/nitrate	0.6	0.6	0.1
Sulfate	84	9	6
Silica	10	7.6	16
Iron	ND[d]	0.8	ND[d]
Manganese	ND[d]	0.02	ND[d]

[a]Concentrations expressed as mg/L of dissolved constituent. Alkalinity calculated as equivalent to mg $CaCO_3$/L.
[b]Dissolved.
[c]Total.
[d]ND Not Detected.

RESULTS AND DISCUSSION

Gas chromatographic separations for standard mixtures of all seven compounds are shown in Figures 1a and 1b. The chemically-bonded UltraBond[R] packings were found to be superior to conventional coated supports for triazine separations. When coated with low

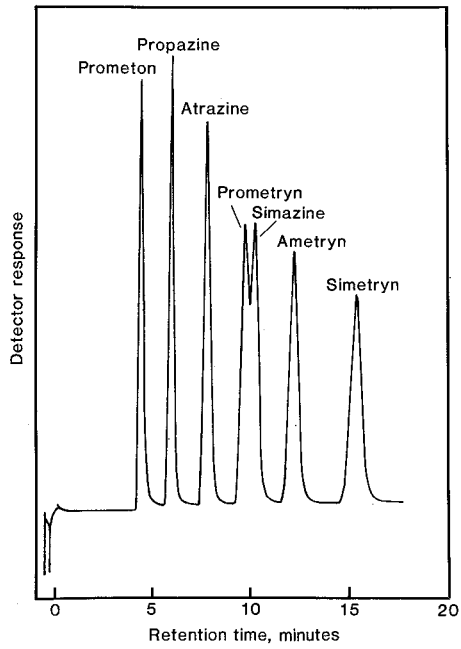

FIGURE 1(a) Gas chromatographic separation of seven triazines using 1% OV-101 on UltraBond[R] 20M. Column Temp. 175°C. Standard mixture at 1 ng/μL.

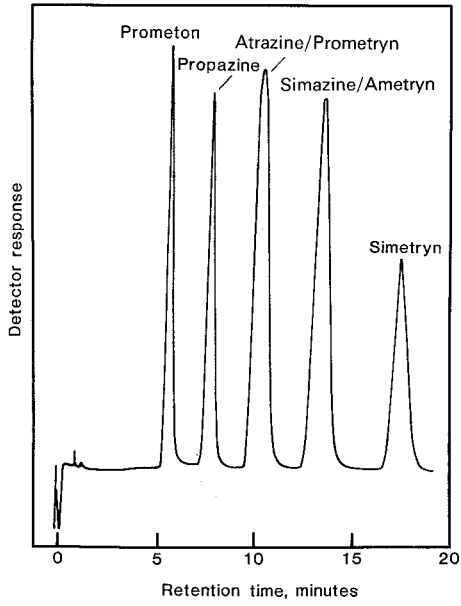

FIGURE 1(b) Gas chromatographic separation of seven triazines using UltraBond[R] PEGS. Column Temp. 195°C. Standard mixture at 1 ng/μL.

loading (1%) of silicone oil (OV-101), this packing provides an efficient separation of all seven compounds in less than 20 min. Other advantages important for triazine residue analysis include good efficiency, temperature stability, and minimal column bleed. Relative retention times (atrazine) for a mixture of seven triazines on two columns are given in Table II. Reproducibility of retention time for injection of standard compounds over a period of months has consistently remained within ± 0.01 min, thus enabling distinct identifications to be made for closely eluting components. Both columns used in this study repeatedly furnished chromatograms showing the peak symmetry and resolution needed for reliable, quantitative measurements.

TABLE II

Retention times for triazine herbicides.[a]

Compound	Relative retention time, (atrazine)[b]	Relative retention time, (atrazine)[c]
Prometon	0.56	0.61
Propazine	0.77	0.77
Atrazine	1.00	1.00
Prometryn	1.03	1.23
Simazine	1.30	1.31
Ametryn	1.32	1.55
Simetryn	1.71	1.96

[a]Injector Temp. 200°C; Detector Temp. 300°C. Helium Carrier at 35 mL/min. Detector Flows: Hydrogen at 3.2 mL/min; Air at 100 mL/min.
[b]1% OV 101 on UltraBond[R] 20M, 100/120 mesh. Oven Temp. 175°C.
[c]UltraBond[R] PEGS, 100/120 mesh. Oven Temp. 195°C.

The OV-101 column separates five of the triazines completely, leaving prometryn/simazine as a partly resolved pair. However, the separation distinguishes between the two and is adequate for tentative identification or confirmation of the presence of either material (if both are present and the ratio of one to the other is great, one component may be obscured). The polyethylene glycol succinate column separates three of the components, leaving two pairs of co-eluting doublets. Atrazine/prometryn and simazine/ametryn each co-elute as two-component peaks with no apparent separation. In practice, all triazine determinations need to be made on two packing materials and the results compared.

Retention data from two dissimilar columns in addition to the appropriate response from nitrogen selective detectors constitute the three elements necessary for triazine analysis in water extracts.

Recovery data obtained on creek water samples spiked at three residue concentrations are summarized in Table III. At the lowest spiking level, mean recovery exceeded 95%, with the coefficient of variation (CV) less than 9% for each pesticide. Recoveries from samples spiked at greater concentrations were diminished somewhat; however, CV values remained less than 10%. The lesser recoveries observed for all triazines spiked at the greatest level is believed to be a result of solubility limits in hexane which are approached during final concentration steps. Data in Table III were obtained from the method described herein, but without the alumina microcolumn chromatography. The clean-up procedure is effective for samples that

TABLE III

Recovery of seven triazine herbicides from Clear Creek: ten replicates spiked at three levels (liquid–liquid extraction).

Compound	Spiking level, μg/L	Mean recovery, %	Standard deviation of the mean	Relative standard deviation, %
Prometon	0.5	95.1	5.83	6.13
	1.0	96.9	2.08	2.15
	5.0	84.6	4.58	5.41
Propazine	0.5	96.2	5.46	5.68
	1.0	100.9	2.06	2.05
	5.0	87.6	4.62	5.28
Atrazine	0.5	96.0	7.34	7.65
	1.0	98.1	2.11	2.15
	5.0	85.5	5.14	6.01
Prometryn	0.5	98.0	5.09	5.20
	1.0	77.9	1.67	2.15
	5.0	83.5	3.83	4.57
Simazine	0.5	96.9	4.90	5.05
	1.0	98.8	4.91	4.97
	5.0	82.8	7.69	9.29
Ametryn	0.5	101.1	8.82	8.73
	1.0	101.5	3.83	3.77
	5.0	87.8	4.03	4.58
Simetryn	0.5	98.7	7.17	7.27
	1.0	94.7	2.08	2.19
	5.0	86.4	4.23	4.90

contain other polar nitrogen and phosphorus compounds that may obscure triazine peaks. The procedure is intended primarily for those samples containing great DOC concentrations. In the ground water sample used for these studies (pH = 9.1, DOC = 3.6 ppm), the calculated concentration for each triazine was reduced by 10 to 25% when the clean-up step was included in the procedure. However, when a known triazine mixture at the 1 μg/L level was subjected to the clean-up procedure only, and not extracted, the individual components were recovered in nearly quantitative yield of 94%.

The apparent effect of DOC concentration and pH (see Table I) upon the accuracy of triazine residue determinations is indicated in Tables IVa and IVb. Most of the large DOC concentration is

TABLE IV(a)

Recovery of seven triazine herbicides from Withlacoochee River water: ten replicates spiked at approximately 1 μg/L (liquid–liquid extraction).

Compound	Mean recovery, %	Standard deviation of the mean	Relative standard deviation, %
Prometon	72.5	2.82	3.88
Propazine	74.3	3.37	4.54
Atrazine	87.9	3.69	4.20
Prometryn	62.3	1.73	2.78
Simazine	79.1	4.12	5.21
Ametryn	78.7	2.77	3.52
Simetryn	75.5	3.15	4.17

TABLE IV(b)

Recovery of seven triazine herbicides from Piceance Basin ground water: ten replicates spiked at approximately 1 μg/L (liquid–liquid extraction).

Compound	Mean recovery, %	Standard deviation of the mean	Relative standard deviation, %
Prometon	88.1	13.0	14.6
Propazine	84.0	12.3	14.7
Atrazine	83.0	12.7	15.3
Prometryn	63.8	8.81	13.8
Simazine	83.0	12.2	14.7
Ametryn	83.4	10.7	12.8
Simetryn	79.9	9.93	12.4

presumed to be of terrestrial origin and consist mainly of hydrophilic organic acids of fewer than ten carbon atoms in chain length. These compounds may participate in various solute–solute or solute–particulate interactions with the weakly basic triazine materials. The effect of alkaline pH upon triazine determinations is shown by the recovery data for ground water. Although these two water quality characteristics seem to control recovery efficiency, the effect of other dissolved constituents cannot be ignored. Solids content and nature and type of DOC may also be important. Overall, the differences in recoveries are likely due to the different compositions of the water samples. In the case of the river and ground water samples studied, the gas chromatograms were readily interpreted without difficulty. Figures 2(a) and 2(b) show that none of the triazine peaks were obscured or masked. The ground water sample co-extracted more early eluting material than the river sample, resulting in an elevated baseline. However, the correct identification of prometon (the first triazine to elute) was made from the calibration table without modification of instrument conditions.

As an alternative to classical liquid–liquid partition for the sample extraction segment of pesticide residue analysis, solid-phase sorption techniques were examined. This approach offers several advantages including efficiency, selectivity, portability, and speed. In addition, the analytical chemist can appreciate the convenience attendant to smaller solvent volumes, freedom from emulsions, reduced glassware needs, and fewer method-blank associated problems. Liquid chromatographic separations of triazine mixtures have been reported for both normal,[16] and reversed-phase[17] columns. The most effective sorbent for triazines determined by this method appears to be the moderately polar *n*-octyl material.[18] Extraction cartridges packed with C-8 material were chosen for the evaluation. The polyethylene cartridge had a volume of 3 mL with approximately 200 mg of packing between porous polypropylene discs. The packing consisted of end-capped irregular 40 μ silica particles carrying a 12.5% C8 loading. Unfiltered water samples (200 mL) were spiked with a seven component triazine mixture (200 ng of each constituent) and analyzed by the method described herein. Results in Table V indicate that C8 cartridges can be used for extraction and concentration of triazine residues from the raw water samples used in this study. In addition, the eluates are suitable for gas

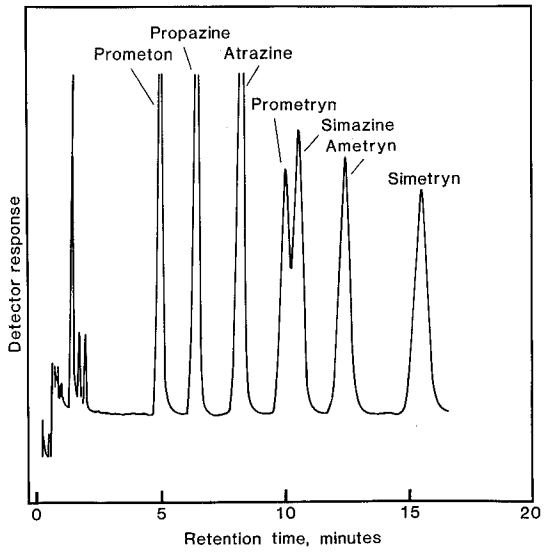

FIGURE 2(a) Gas chromatogram obtained from Withlacoochee River sample spiked at the 1 μg/L level with seven triazines. Column: 1% OV-101 on UltraBond^R 20M at 175°C.

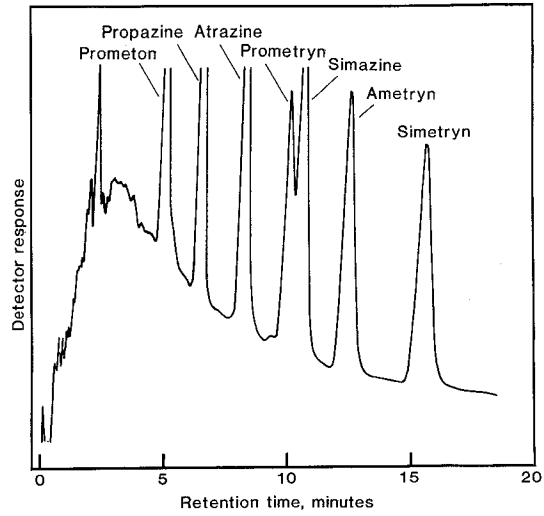

FIGURE 2(b) Gas chromatogram obtained from Piceance basin ground water sample spiked at the 1 μg/L level with seven triazines. Column: 1% OV-101 on UltraBond^R 20M at 175°C.

TABLE V

Recovery of seven triazine herbicides from surface and ground water by solid-phase extraction on C8 columns. Spiking level approximately 1 μg/L. Mean recovery from triplicate determinations.

Compound	Clear Creek water rec. ± SD, %	Withlacoochee River water rec. ± SD, %	Piceance Basin ground water rec. ± SD, %
Prometon	68.0 ± 4.44	64.6 ± 9.08	66.6 ± 9.14
Propazine	81.7 ± 5.15	75.6 ± 7.34	76.7 ± 4.34
Atrazine	63.3 ± 3.39	75.3 ± 10.5	57.1 ± 2.35
Prometryn	82.5 ± 5.06	80.2 ± 10.8	79.3 ± 5.49
Simazine	85.9 ± 8.12	64.7 ± 9.75	70.5 ± 5.60
Ametryn	84.0 ± 7.63	73.3 ± 13.4	80.1 ± 5.66
Simetryn	80.7 ± 12.7	65.0 ± 11.0	65.1 ± 8.44

chromatographic analysis directly, requiring no additional treatment. The chromatograms are free of interfering peaks with triazine retention times falling within a ±3% window, thus minimizing the possibility of false identifications. The data show that good recoveries can be expected from SPE even when analyzing samples of unusually high pH or great DOC concentrations. Generally, recoveries by solid-phase extraction are not significantly different than for solvent extraction. In addition, the higher recoveries from creek water than from river or ground water by SPE further attest to a dependence upon sample type.

Solid-phase extraction for water samples appears to be broadly applicable to many organic residue analysis problems. Packing materials now available permit extraction and concentration to be carried out via adsorption, partition, or ion-exchange mechanisms. Analytes that can be determined range from very nonpolar to polar compounds. In our laboratory, SPE has been used successfully for isolation of nitrosamines from river water, and for phenols and nitrogen heterocylic compounds from polluted pond water. Furthermore, joining together of several columns of different surface chemistry in sequence offers the potential for clean and efficient separation of acidic, basic, and neutral compounds, or hydrophobic and hydrophilic materials. The value of this approach is readily apparent when performing pesticide residue determinations on

samples from agricultural runoff or irrigation ponds. In such instances, the pesticide composition is complex and represented by several distinct classes of active chemicals. SPE may lend itself well to residue analysis for more recent classes of pesticides such as carbamic acid and urea derivatives and several anilides.

Acknowledgement

The authors wish to acknowledge the assistance of personnel at the U.S. Geological Survey national water quality laboratories in Atlanta, Georgia and Denver, Colorado. Their suggestions proved to be most helpful.

References

1. H. E. Christensen, Editor, *Toxic Substances List*, published by the U.S. Dept. of Health, Education, and Welfare, National Institute for Occupational Safety and Health, pp. 519–20 (1972).
2. H. M. LeBaron, *Residue Reviews* **32,** 311 (1970).
3. E. Matisova, J. Krupcik and O. Liska, *J. Chromatogr.* **173,** 139 (1979); E. Matisova and J. Krupcik, *J. Chromatogr.* **142,** 597 (1977).
4. R. Deleu and A. Copin, *J. High Resolut. Chromatogr. & Chromatogr. Comm.* **3,** 299 (1980).
5. H. G. Henkel and W. Ebing, *J. Chromatogr.* **19,** 283 (1964).
6. E. D. Chilwell and D. Hughes, *J. Sci. Food Agric.* **13,** 425 (1962).
7. C. A. Benfield and E. D. Chilwell, *Analyst* **89,** 475 (1964).
8. J. A. Burke and W. Holswade, *J. Assoc. Off, Anal. Chem.* **49,** 324 (1966).
9. A. M. Mattson, R. A. Kahrs and J. Schneller, *J. Agric. & Food Chem.* **13,** 120 (1965).
10. R. C. Tindle, C. W. Gehrke and W. A. Aue, *J. Assoc. Off. Anal. Chem.* **51,** 682 (1968).
11. S. U. Khan and R. Purkayastha, *J. Agric. & Food Chem.* **23,** 311 (1975).
12. K. Ramsteiner, W. D. Hormann and D. O. Eberle, *J. Assoc. Off. Anal. Chem.* **57,** 192 (1974).
13. W. E. Westlake, A. Westlake and F. A. Gunther, *J. Agric. & Food Chem.* **18,** 685 (1970); D. C. Muir and B. E. Baker, *J. Agric. & Food Chem.* **24,** 122 (1976).
14. H. Roseboom and H. H. Herbold, *J. Chromatogr.* **202,** 431 (1980).
15. M. Popl, Z. Voznakova, V. Tatar and J. Strnadova, *J. Chromatogr. Sci.* **21,** 39 (1983).
16. P. Dufek and V. Pacakova, *J. Chromatogr.* **187,** 341 (1980).
17. D. J. Subach, *Chromatographia* **14,** No. 6, 371 (1981).
18. C. E. Parker, C. A. Haney, D. J. Harvan and J. R. Hass, *J. Chromatogr.* **242,** 77 (1982).