Journal of Chromatography, 629 (1993) 55–59
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 672

Automated solid-phase extraction of herbicides from water for gas chromatographic–mass spectrometric analysis

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ABSTRACT

An automated solid-phase extraction (SPE) method was developed for the pre-concentration of chloroacetanilide and triazine herbicides, and two triazine metabolites from 100-ml water samples. Breakthrough experiments for the C_{18} SPE cartridge show that the two triazine metabolites are not fully retained and that increasing flow-rate decreases their retention. Standard curve r^2 values of 0.998–1.000 for each compound were consistently obtained and a quantitation level of 0.05 μg/l was achieved for each compound tested. More than 10 000 surface and ground water samples have been analyzed by this method.

INTRODUCTION

The triazine and chloroacetanilide herbicides atrazine and alachlor are widely used pre-emergent herbicides in the midwestern USA. These herbicides have been reported as common contaminants in both surface and ground water [1–3]. Two dealkylated triazine metabolites, deisopropylatrazine and deethylatrazine, have also been detected [2,3]. As a result of this environmental problem several methods using gas chromatography–mass spectrometry (GC–MS) have been developed to analyze these herbicides [4–6]. However, regional reconnaissance and process studies usually require the analysis of large numbers of samples. Because of the extensive automation available for liquid chromatographic and GC–MS analysis the most time-consuming, expensive, and error-prone step then becomes the pre-concentration of the analytes of interest.

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Solid-phase extraction (SPE) has been successfully used for the extraction of triazine and chloroacetanilide herbicides [7–9]; however, automated methods for the preparation of these frequently analyzed organic compounds are needed to reduce the costs of analysis, diminish sample handling and preparation errors, increase sample through-put, and increase safety. The most common techniques for the extraction of non-ionic, non-polar organic compounds from water are liquid–liquid extraction and SPE [10,11]. Of these techniques, SPE is best suited for automation because the small disposable cartridges containing small amounts of solid sorbent require little organic solvent and are readily fitted into a robotic system. Several other advantages of SPE over liquid–liquid extraction as a sample preparation technique also have been espoused [10,12,13].

Automation of SPE is a relatively new concept as commercial equipment for this purpose has become available within only the last 5–6 years. Furthermore, only within the last 4 years has an automated SPE workstation been available that was capable of processing more than three large samples of 100–1000 ml, which are necessary for environmental

0021-9673/93/$06.00

analysis. This paper presents an automated method for the isolation of chloroacetanilide and triazine herbicides, and two polar metabolites of atrazine, deethylatrazine and deisopropylatrazine, from water using a Millilab 1A workstation (Waters Chromatography, Milford, MA, USA) with an on-line computer. Coupled with GC–MS–selected-ion monitoring (SIM) this is a robust method for the analysis of herbicides from water present at ng/l levels using only a sample of 100 ml.

EXPERIMENTAL

Apparatus

The Millilab 1A workstation with an on-line computer was used for the automated SPE of herbicides from water. Here we describe the pertinent set-up information for implementing this procedure. The two syringe pumps on the fluidics module were outfitted with a 5- and 1-ml syringe. Two multiple intake accessories (MIAs) were added to the 5-ml syringe on the fluidics module to increase the number of water samples that could be processed from 3 to 14. Also a distilled water reservoir, for rinsing the PTFE tubing and XYZ probe, was connected to one of the valves of an MIA. Solvent reservoirs of distilled water, ethyl acetate, and methanol, attached to the 1-ml syringe, were used as working solvents for elution of the SPE cartridges and the pipetting of reagents and spiked sample eluates. Custom-designed Plexiglas racks that could hold up to fourteen 125-ml bottles were used to organize water and quality assurance samples into sets. The bottle racks were keyed to fit into a custom-designed Plexiglas housing that was mounted in front of the fluidics module. PTFE sample lines from the MIAs were held securely in the sample bottles by pre-drilled holes in the top of the Plexiglas housing, through which the hoses were strung.

The transport module of the Millilab workstation contained an XYZ probe used for pipetting and dispensing reagents, and delivering the water sample to the SPE cartridge. Also, the transport module had a test-tube rack, and SPE cartridge rack, which also contained positions to which small reservoirs containing pipetting reagents can be placed. To one of the reagent positions a custom designed Plexiglas rack was attached to hold a 10-ml screw-top test-tube so that an internal standard could be pipetted into each of the sample eluates.

Reagents

Pesticide-grade methanol and ethyl acetate were obtained from Fisher Scientific (Springfield, NJ, USA). Ametryn, atrazine, prometon, prometryn, propazine, simazine, and terbutryn were obtained from Supelco (Bellefonte, PA, USA); alachlor, cyanazine, metolachlor, metribuzin, terbuthylazine (the surrogate, recovery standard), and [$^2H_{10}$]phenanthrene (the internal standard) were obtained from EPA Pesticide Chemical Repository (Research Triangle Park, NC, USA). Two triazine metabolites, deethylatrazine and deisopropylatrazine, were obtained from Ciba Geigy Agricultural Division (Greensboro, NC, USA). All of the standards obtained were greater than 97% pure. Concentrated stock and spiking solutions were prepared in methanol, except for [$^2H_{10}$]phenanthrene which was prepared in ethyl acetate. Distilled water was generated by purification through activated charcoal filtration and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration step and finally distillation in a Wheaton Autostill-5 (Millville, NJ, USA). Sep-Pak plus cartridges, containing 360 mg of 40-μm C_{18} bonded silica packing, were obtained from Waters (Milford, MA, USA).

Extraction procedure

Surface and ground water samples collected for analysis were filtered through 0.7-μm glass-fiber filters (Geotech, Denver, CO, USA), then refrigerated in 125-ml glass bottles. Ten samples were placed into custom-made Plexiglas racks. Two distilled water solutions fortified with a herbicide mix and two blanks were then added to the sample racks for quality control. The concentration of the fortified distilled water solutions ranged from 0.05 to 5.0 μg/l. Then 100 μl of a surrogate standard, terbuthylazine (1.34 ng/μl), were added to each bottle.

The SPE cartridge was conditioned by passing 2 ml of methanol, 6 ml of ethyl acetate, 2 ml of methanol, and 2 ml of distilled water. Reagents were pipetted through the SPE cartridge from the reagent reservoirs on the transport module. The PTFE sample lines were primed with 23 ml of water sample prior to pumping the remaining 100 ml of sample through the SPE cartridge at a flow-rate of 20 ml/min. The probe was then rinsed with distilled water and filtered compressed air then was passed

through the SPE cartridge for 1 min to remove as much water sample trapped in the cartridge as possible. The SPE cartridge was eluted with 2.5 ml of ethyl acetate into a centrifuge tube. Each sample in the set was sequentially prepared in the same way. An internal standard, [$^2H_{10}$]phenanthrene (500 μl, 0.2 ng/μl), was then added to each of the sample eluates in a batch spiking procedure. The Millilab mixed the sample eluate, then pipetted the ethyl acetate into a clean centrifuge tube to separate it from the residual water in the SPE cartridge, which co-eluted with the ethyl acetate. The interior and exterior of the probe were then washed with ethyl acetate after each sample was mixed and pipetted. This cycle was repeated until all the samples were mixed and separated. The eluates were removed from the Millilab, reduced to a volume of approximately 100 μl using a Zymark Turbovap LV evaporator (Hopkinton, MA, USA), and transferred into 200-μl glass-lined polystyrene vials for analysis by GC–MS.

GC–MS analysis

Sample eluates were analyzed using a Hewlett-Packard Model 5890 gas chromatograph and a 5970B mass-selective detector (Palo Alto, CA, USA). Operating conditions were as follows: a direct capillary interface at 280°C, ionization voltage 70 eV, ion source temperature 280°C, electron multiplier 400 V above autotune, tuned daily with perfluorotributylamine. The filament and electron multiplier were turned on 15 min into each sample run. Twenty-nine ions divided into four acquisition groups were monitored during each sample run. The area of the base-peak ion for each compound was divided by the single 188 ion-peak of the [$^2H_{10}$]phenanthrene and the 214 ion-peak of terbuthylazine for quantification. Compound confirmation was based upon the presence of the molecular ion, and one to two confirming ions (with area counts ±20%), and a retention time match of ±0.2% relative to [$^2H_{10}$]phenanthrene.

Samples were injected in the splitless mode into the gas chromatograph. The injector temperature was 280°C. Herbicides were separated on a 12 m × 0.2 mm I.D., HP-1 fused-silica capillary column with a film thickness of 0.33 μm (Hewlett-Packard). The helium carrier gas flow-rate was 1 ml/min with a head pressure of 35 kPa. The column temperature was held at 60°C for 1 min and programmed to ramp at 6°C/min to 250°C.

RESULTS AND DISCUSSION

Table I shows that only the two triazine metabolites were detected in the breakthrough determinations. As the flow-rate was increased from 20 to 60 ml/min the breakthrough of deisopropylatrazine increased from 35 to 40% and deethylatrazine from 5 to 10%. The standard deviations of the mean breakthrough for some adjacent flow-rates overlap for both compounds. Thus, it is not certain that there is discernible difference in breakthrough by increasing the flow-rate from 20 to 30 ml/min. However, there is a trend of increased breakthrough with increasing flow-rate. Furthermore, there is a distinguishable difference in breakthrough from 20 to 60 ml/min for both compounds. These data indicate that for compounds with low sorption capacity precise control of the flow-rates is necessary to ensure consistent quantitative results.

Deisopropylatrazine has less sorption capacity than deethylatrazine because it has one less carbon group in the alkyl sidechain and can undergo less hydrophobic interactions with the C_{18} resin. Break-

TABLE I

PERCENT BREAKTHROUGH FOR SEP-PAK C_{18} CARTRIDGE FOR 13 HERBICIDES AS A FUNCTION OF FLOW-RATE

Using 100-ml sample, 1 μg/l concentration for each compound, passed through two cartridges in tandem, cartridges eluted separately using Millilab procedure and analyzed by GC–MS. Breakthrough based on an external standard curve for each compound. Procedure performed for each flow-rate in duplicate.

Flow-rate (ml/min)	% mean breakthrough ± S.D.[a]	
	Deisopropylatrazine	Deethylatrazine
20	35 ± 1.9	5 ± 0.6
30	37 ± 2.5	7 ± 0.7
40	37 ± 1.1	9 ± 0.9
60	40 ± 0.3	10 ± 0.2

[a] Breakthrough not detected for the other 11 herbicides listed in the Reagents section.

through volumes for the triazine and acetanilide herbicides on C_{18} at a flow-rate of 4 ml/min have previously been reported [6]. The results from that study, with 10 and 100% breakthrough for deisopropylatrazine occurring after 75 and 225 ml of sample were passed through a C_{18} cartridge, are in good agreement with those presented in Table I.

The variation of the recovery ratio of terbuthylazine to [$^2H_{10}$]phenanthrene was calculated for 11 historical standard curves. The ratio was calculated by dividing the area of the 214 ion of terbuthylazine by the 188 ion of [$^2H_{10}$]phenanthrene. The relative standard deviation (R.S.D.) for the average ratio calculated from each standard curve varies from ±2 to 6% and the average R.S.D. is 4%. The deviation of the terbuthylazine to [$^2H_{10}$]phenanthrene ratio for the majority of samples measured was, in general, within ±10% of the ratio calculated from the standard curve being used. However, the percent deviation for most of the samples was only 3 to 7% greater than the R.S.D. for the recovery ratios calculated from the standard curves. Most of the deviations greater than ±10% are due to either a spiking error, usually the terbuthylazine, or because the full volume of sample was not pumped through the cartridge. Thus, most of the variation in this method is not from extraction efficiency from complex matrices but is from spiking error, or instrument malfunction.

For each compound r^2 values from 11 standard curves were between 0.998 and 1.000. The high r^2 values, obtained using both the [$^2H_{10}$]phenanthrene and the terbuthylazine as quantification standards, shows that a high degree of reproducible precision has been maintained with this automated method. Furthermore, herbicide concentrations calculated by both standards usually agree within ±10%. Because the 5.0 μg/l standards exerts a lot of leverage on these standard curves it is important to maintain r^2 values of 0.997 or greater to have good control over the 0.05 to 0.2 μg/l range in the standard curve.

Quantitation levels of 0.05 μg/l are achieved for each of the 13 herbicides. However, deisopropylatrazine and cyanazine give significantly less response than the other compounds. A chromatogram of our 13-compound mix published in a previous study [6] illustrates this point. For deisopropylatrazine the response is diminished because 35 to 40% of the compound is not sorbed to the SPE cartridge. Furthermore, cyanazine and deisopropylatrazine are susceptible to losses in the injection port as the injection sleeve becomes dirty [6], which raises the quantitation levels if the injection sleeve is not regularly replaced. The fortified distilled water solutions run with each set were used to detect deterioration in analyte response. When the slope of the standard curve was adjusted by more than 15% to compensate for this loss the injection sleeve was replaced. Finally, analysis of duplicate samples and fortified distilled water solutions by independent laboratories were used as an independent check for method accuracy. In general, the results from the inter laboratory comparisons agree within 10 to 20% of the results obtained from our laboratory.

CONCLUSIONS

The breakthrough experiments show that the C_{18} SPE cartridge has limited sorption capacity for deisopropylatrazine and deethylatrazine and that the sorption capacity for these compounds is reduced with increasing flow-rates. Therefore, for accurate quantitative analysis of compounds with limited sorption capacity, such as deisopropylatrazine, precise control of flow-rate is necessary. The recovery, and standard curve data along with the analysis of thousands of fortified distilled water solutions and several hundred duplicate samples show that the precision (% relative standard deviation) for this method is ±10% for each compound. Furthermore, inter laboratory comparison studies of fortified distilled water solutions, and duplicate samples show that this method is accurate. Also this automated SPE method has reduced the amount of man-hours required for extraction by more than 70%, increased precision by 5%, increased sample through-put by 200% using two Millilab workstations, and reduced significantly technician exposure to solvent fumes. Finally, automated SPE coupled with GC–MS–SIM is a robust and reliable method for the routine detection of herbicides in the sub-ppb levels using only a 100-ml water sample.

ACKNOWLEDGEMENT

We appreciate the financial support of the Surface-Water and Ground-Water Toxics Program of the US Geological Survey, and the Productivity En-

hancemant Funding Program, US Department of the Interior. Brand names are for identification purposes only and do not imply endorsement by the US Geological Survey.

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