ISSN : 0974 - 7451 Volume 8 Issue 7

Environmental Science
An Indian Journal
Ecotoxicology

ESAIJ, 8(7), 2013 [263-267]

Efficient method development for atrazine determination in soil samples

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ABSTRACT

Atrazine is a worldwide herbicide used to control pre- and post-emergence broadleaf and grassy weeds in major crops. Being both effective and inexpensive, it is well-suited to production systems with very narrow profit margins, as is often the case with maize. However, due to atrazine's ground water contamination potential and its association with birth defects and menstrual problems when consumed by humans at concentrations even below government standards; environmental media monitoring for atrazine are inevitable. The objective of this study was to investigate a proposed "safe, cheap and relatively fast" analytical technique for the routine monitoring of atrazine in soil samples by high performance liquid chromatography equipped with ultra-violet detector and/or gas chromatography mass spectrometry. The method uses acetonitrile as the extracting solvent, and a self-packed activated silica gel for clean-up of extract.

The proposed method exhibited good sensitivity and recovery, and allowed for rapid analysis. For soil analysis, a single chemist could prepare test solutions from 15 corresponding homogenized samples within 4 hours.

KEYWORDS

Atrazine;
Herbicide;
Contamination;
Liquid chromatography;
Clean-up;
Silica gel.

INTRODUCTION

In agricultural cultivation, a group of pesticides that plays an essential role in the control of weeds which compete for nutrients with the desired crop on a farm is herbicide. 1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine or simply called atrazine, is a worldwide known herbicide. It is used to control pre- and post-emergence broadleaf and grassy weeds in major crops. Being both effective and inexpensive, it is well-suited to production systems with very narrow profit margins, as is often the case with maize. Atrazine is a banned chemical in the European Union[1] and other countries, but it still continues to be widely used as a preferred broad-

leaf and grassy weeds herbicide. For instance, in Ghana, atrazine is registered for the control of annual perennial grass, broadleaf weeds and as a contact herbicide under several trade names including Trazine 80 WP, Trazine 500 SC, Sun-Atrazine 500 SC, Kb Super Traz 500 SC and Cotraxine 500 SC[2]. However, due to atrazine's ground water contamination potential and its association with birth defects and menstrual problems when consumed by humans at concentrations even below government standards, environmental media monitoring for Atrazine are inevitable[1,3]. There is also the need for more residue data for atrazine in order to use modeling software to verify the contamination levels of atrazine in the environment.

Determinations of pesticide residues in media such as soil come with some complications, especially in extraction and clean up steps due to the complex nature of soil samples[4]. Techniques such as soxhlet, ultrasonication and solid phase microextraction[4], and others have been used and continue to be use for soil samples with different solvent systems. Each technique has its own advantages and deficiencies over the other. Large amounts of solvent usage to long extraction hours bedevil soxhlet extraction technique, while solid phase microextraction technique is not very common for residue analysis in soil. For detection and quantification, gas chromatograph mass spectrometer and gas chromatograph nitrogen phosphorous detector have been used for atrazine in soil and water samples[4,5]. Liquid chromatography techniques have also been used for atrazine in water determinations[6,7]. This study investigates a proposed safe, cheap and relatively fast analytical method for the routine monitoring of atrazine in soil by high performance liquid chromatography equipped with ultra-violet detector. However, with slight modification of the clean up procedure, gas chromatography mass spectrometer (GC/MS) could also be use to confirm and quantify the atrazine extracted from the soil samples.

MATERIAL AND METHODS

Sampling and processing

Beach soil samples were collected into zip lock plastic bags. They were then transported into the laboratory for sample processing. Soil samples were then transferred into Pyrex beakers and placed in an oven set at 150°C and was left overnight. In addition some clay and loamy soil samples were also collected to check for robustness of the method.

Reagent and chemicals

Acetonitrile and Methanol were pesticide grade and obtained from BDH, England. Silica gel adsorbent, polypropylene cartridges, distilled water and Whatman filter paper no. 1.

Certified reference standard; atrazine used for the identification and quantification was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Preparation of stock reference and calibration standards solution

25.0 mg of 99.0% atrazine standards was weighed into 25 mL volumetric flask. Methanol was used to dissolve the standard and further diluted to the mark to form 1000 mg/L atrazine stock standard solution. An aliquot of 0.1 mL of the 1000 mg/L stock solution was transferred into 10 mL volumetric and diluted to the mark using methanol to obtained 10 mg/L. 0.01 mL, 0.05 mL, 0.10 mL, 0.50 mL and 1.0 mL aliquots of the 10 mg/L standard solution were diluted with methanol to obtained 0.01, 0.05, 0.10, 0.50 and 1.0 mg/L standard solutions of atrazine for instrument calibration.

PROCEDURE

Extraction

10 g of soil sample was weighed and quantitatively transferred into a 250 mL separating funnel. 10 mL of acetonitrile was added to the soil sample in the funnel and ultra-sonicated for 2 minutes. An additional 10 mL acetonitrile was added, and the separating funnel closed tightly and placed on a horizontal shaker. It was then set to shake continuous for 30 minutes at 300 mot/min and finally allowed to stand for 5 minutes to sufficiently separate the phases. 10 mL of the supernatant was carefully taken and dried over 5 g anhydrous sodium sulphate through filter paper into 50 mL round bottom flask. This was then concentrated to about 1 mL using the rotary evaporator, and made ready for silica clean up step.

Clean up

1 g of silica gel that previously had been activated at 130°C for 10 hours was carefully packed into 10 mL polypropylene cartridge column and 6 mL acetonitrile was used to condition the cartridge. The concentrated extract was then loaded onto the column and 50 mL pear shape flask was placed under the column to collect the eluate. 10 mL acetonitrile was used to elute the column afterwards, and the total filtrate collected concentrated to just dryness using the rotary evaporator set at 38°C. The residue was re-dissolved in 1 mL methanol and transferred into a 2 mL standard vial prior to quantitation by high performance liquid chromatography.

NB

For GC/MS determination, the packed 1 g activated silica gel was sandwiched between two 1 g of anhydrous sodium sulphate before the column was conditioned with the 6 mL acetonitrile and the extract loaded onto the column afterwards. This was done in order to take care of any residual moisture in the extract.

Instrumentation

HPLC analysis

A Varian Incorporated (USA) High Performance Liquid Chromatography (HPLC) with prostar ultra-violet, photodiode array and fluorescence detectors, equipped with model 410 Varian autosampler, and a three system 210 pump; all coordinated by a galaxy workstation software. The separation was done on Luna C18, (5 μm, 250 x 4.6 mm) stainless steel column at room temperature operation. The mobile phase was water-methanol (40:60, v/v) at a flow rate of 1.5 mL/min. UV detection was realized at 257 nm, and the injection volume was fixed at 50 μL for partial loop filling. The total run time was 10 min.

GC/MS analysis

A Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with 1177 type injector, Saturn 2200 Mass Spectrometer (MS) as detector and 8400 Varian auto-sampler was used for GC analysis. Sample extract of 2 μL aliquots was injected and the separation was performed on a fused silica gel capillary column (VF- 5ms, 30 m + 10 m column guard x 0.25 mm id., 0.25 um film thickness). The carrier gas was ultra pure helium at flow rate of 1.2 mL/min. The temperature of the injector operating in splitless mode was 250°C and the MS detector with an ion trap mass analyzer was set to scan mass range of 50 m/z – 350 m/z at auto EI. The mass 200 m/z was selected as the detection and quantification ion, while 215 m/z was used for confirmation of the detected atrazine. The manifold, ion trap and transferline temperatures were set to 80°C, 210°C and 260°C, respectively. The column oven temperature was programmed as follows; 70°C for 1 min, then at 30°Cmin^{-1} up to 240°C and finally at 5°Cmin^{-1} to 300°C held for 0.3 min. The total run time for a sample was 28 min.

RESULTS AND DISCUSSION

To test the performances of this method of extraction - purification, soil samples from the beach, clay and loamy soils were chosen. Two analyses were carried out respectively; one after addition of a known quantity of atrazine (fortified sample) and the other without addition of atrazine (blank sample). The analysis was repeated twenty times in order to evaluate the reproducibility of the method. The quantities of atrazine added made up fortification levels of 0.01 mg/kg, 0.05 mg/kg and 0.10 mg/kg (quantities which are close to the ones recommended by most monitoring Agencies). The fortified sample was left at rest for 30 minutes before starting the operation of extraction - purification. The residue obtained after this operation was analysed by HPLC-UV, and also by GC/MS.

Analysis of the blank samples (not fortified) of beach, loamy and clay soils after the procedure of extraction–purification indicates the absence of a peak at the time of retention of atrazine.

Under the already quoted chromatographic conditions this peak is well resolved compared to the close peaks in the fortified samples (typical chromatograms are presented in Figure 1 and 2).

The present method of extraction - purification with a relatively safer solvent, acetonitrile, gave very good recovery results (TABLE 1); the calculated coefficients of variation are all lower than 6% indicating a very good reproducibility. Linearity of the developed method was tested in a concentration range from 0.01 to 1.0 mg/kg.

The limit of detection (LOD) was computed as three times the base line noise (S/N = 3) at the lowest detectable concentration. LOD for atrazine studied was equal or less than 0.005 mg/kg (TABLE 1).

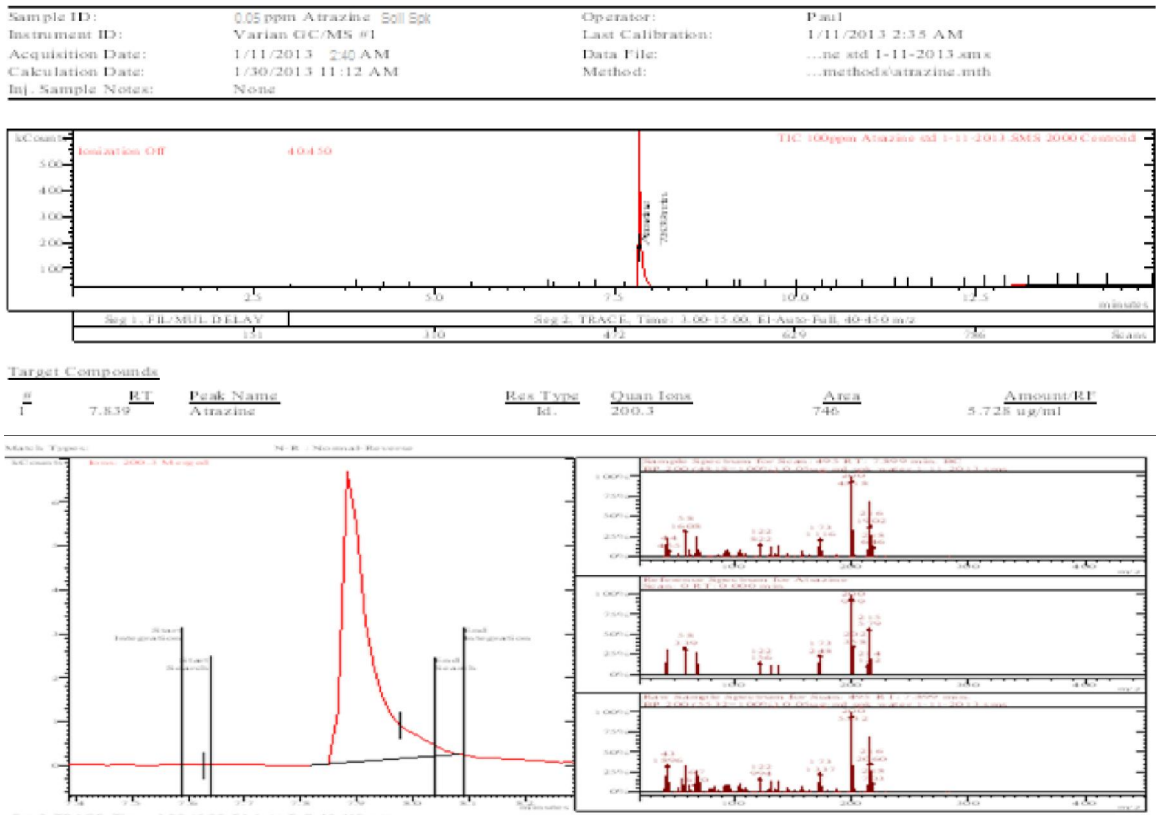

Figure 1: Chromatogram from GCMS determination of atrazine in soil samples; 0.05mg/kg spiked soil sample

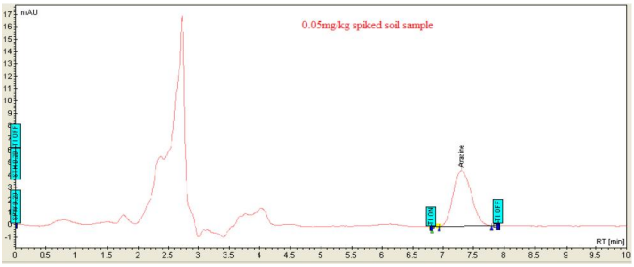

Figure 2 : Chromatogram from HPLC determination of atrazine in soil samples; 0.05mg/kg spiked soil sample

TABLE 1 : Fortification level (mg/kg), correlation coefficients (R), average recoveries (%, n = 20), relative standard deviations (RSDs, %) and limits of detection (LODs, mg/kg) obtained for atrazine study

Soil Type	Fortification Level (mg/kg)	R	Average Recovery (n=20,%)	RSD (%)	LOD (mg/kg)
Beach	0.01 0.05 0.10	0.999	94	4.1	0.003
Loamy	0.01 0.05 0.10	0.998	90	4.6	0.003
Clay	0.01 0.05 0.10	0.992	71	5.9	0.005

The relatively poorer result obtained with clay soil could be explained by a greater affinity of sorption of atrazine to clay soil.

CONCLUSION

Safe, cheap and relatively fast analytical method for the routine determination of atrazine residue in soil had been developed. The method is based on a simple extraction with a relatively safer organic solvent, purification with self-packed activated silica gel cartridge, and determinations with HPLC-UV and GC-MS. The proposed method exhibited good sensitivity and recovery, and allowed for rapid analysis. For soil analysis, a single chemist could prepare test solutions from 15 corresponding homogenized samples within 4 hours. This method does not require special techniques in sample preparation. The major achievements of this "safe, cheap and relatively fast," method would yield immense benefits such as: (a) reduced time and costs for sample extraction, (b) reduced time for mastering the analytical

techniques, and (c) less errors within procedure. The method described here has a high efficiency covering different soil types. Thus, it would be applicable to various soil and sediments suited for use in monitoring works.

The method of extraction – purification which have been adapted and tested, and that consists of an extraction with acetonitrile followed by purification on activated silica gel appears to be completely satisfactory since it leads to atrazine recoveries higher than 70%.

ACKNOWLEDGMENTS

This research was partly supported by the National Nuclear Research Institute of Ghana Atomic Energy Commission. Special thanks to Mr. K. Acheampong, Director of Metrology of Ghana Standards Authority and the entire staff members of the Pesticide Residue Laboratory, Ghana Standards Authority; Mr. Paul Osei-Fosu, Ms. Ernestina Adeenze, Ms. Pearl Mensah, Mr. Duke Henry Ashie and Mr. George Kuntu Blankson for their immense laboratory support. Our gratitude also goes to Mr. John Pwamang, Director of Chemical Control and Management Centre, Environmental Protection Agency, Ghana and the Manager of Nuclear Chemistry and Environmental Research Centre, Ghana Atomic Energy Commission, Dr. Dickson Adomako; for their support and cooperation throughout the entire research.

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