DETERMINATION OF ATRAZINE IN AQUEOUS SOIL EXTRACTS BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A high performance thin layer chromatography (HPTLC) method for the determination of atrazine in aqueous soil extracts was studied. To verify the applicability of the method in environmental concern studies, soil samples having different organic matter (OM) contents (1.4-24.0 %) were used. Development of aqueous extracts was done on HPTLC silica gel F 254 plates and quantification was carried out by UV scanning densitometry. The method was linear between 5-200 ng with an analytical sensitivity of 0.53 ng. Limit of detection (LOD) and limit of quantification (LOQ) were 1.48 and 4.94 ng, respectively. Intra-assay variation was between 1.48 and 5.97 % (RSD). Recoveries ranged from 98.7 to 103.5%.

KEY WORDS: Atrazine, HPTLC-AMD, densitometry, soils, adsorption.

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a member of triazines, a kind of herbicides widely used in agriculture. Its presence as a water pollutant has been recognized in agricultural areas of the world for many years (1). It is considered a moderately persistent herbicide and research on its sorption and desorption properties on soils with different physicochemical properties is needed for environmental fate assessment and to prevent the presence of residues in surface and ground waters. The equilibrium concentration of atrazine in the soil-water system in adsorption-desorption studies or in residue analysis can be determined by gas chromatography-nitrogen phosphorus detection (GC-NPD); however, this technique demands a sample cleanup procedure before the introduction into the chromatographic system (2). Reversed-phase HPLC with spectrophotometric or photodiode array detection (HPLC-DAD) can also be used for rapid and sensitive determination (3, 4, 5). Aqueous extracts from soils contain soluble humic substances that usually generate the greatest degree of interference in the determination of low concentration of pesticides with this technique. This interference must be separated through gradient programs and analysis would be more time-consuming. Recent advances in Thin-Layer Chromatography using modern sample preparation methods combined with automated instrumental techniques for sample application, plate development, and densitometric scanning of spots have allowed sensitive and quantitative residue analysis of environmental samples (6,7). In this study a simple analytical method using high-performance thin layer chromatography (HPTLC) with UV detection on silica gel plates for the determination of atrazine in aqueous extracts of soils is described as an appropriate alternative. Method validation was carried out through the determination of atrazine in the presence of aqueous matrices originated in soils with different organic matter contents.

EXPERIMENTAL

Reagents and standard solutions.

Pure analytical Atrazine (Pestanal®, chemical purity > 99%) was obtained from Riedel de Häen (Seelze, Germany). Its solubility in water is 33 µg cm³ at 20ºC. A stock solution (1mg/mL) was prepared in acetonitrile and further dilutions were prepared in methanol and water for calibration and spiking purposes. Methanol, dichloromethane and acetonitrile, HPLC grade, were purchased from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Water was purified with a NANO pure™ analytical deionization system (Barnstead, Thermolyne Corporation, Dubuque, Iowa, USA).

Chromatographic conditions
1. Sample application

Chromatography was performed on 10 x 20 cm Kieselgel 60 F254 TLC plates (layer thickness, 0.25 mm), and Kieselgel 60 and 60 F254 HPTLC plates (layer thickness, 0.20 and 0.10 mm, respectively) (Merck, Darmstadt, Germany). Prior to development, they were pre-washed twice with methanol and dried at 120ºC for 30 minutes. Plates were stored in a desiccator under vacuum until they were required. Samples were applied with a Linomat III applicator equipped with a 25 µl syringe and developments were carried out in an AMD chamber both from CAMAG, Muttenz, Switzerland.

2. Development

A two-step preliminary program based on dichloromethane-methanol = 98+2, V/V was used to assess the influence of application parameters such as volume and band width and to do calibration-related studies using the three different adsorbents. The elution program applied to aqueous soil matrices started with 10 short isocratic runs (0.8 min) with acetonitrile-dichloromethane = 30+70, V/V. Mixer was emptied after the 10th step and refilled to continue with 4 successive isocratic runs (2.5, 5.0, 7.5 and 25 min) with dichloromethane. The plate was dried for 1 min between each step and for 3 min after the last one. This was also preconditioned with nitrogen for 15 s before each run.

Scanning

Quantitative evaluation was carried out with a Densitometer CD 60 (Desaga GmbH, Heidelberg, Germany). Instrument control, data acquisition and processing were performed with the corresponding standard software, 4.0 version. Chromatograms were scanned using

the remission-extinction mode detection in the following conditions: monochromator wavelength, 210 nm (deuterium lamp); slit width, 0.4 mm; slit length, 2 mm; resolution, 0.1mm. Selected chromatograms obtained for aqueous spiked soil samples and, aqueous and methanolic standard solutions were further scanned for their *in situ* UV spectra from 200 to 350 nm, with a resolution of 1 nm.

Calibration studies in methanolic solutions

The Kieselgel 60 plate was chosen to perform a linearity study by applying eleven atrazine standards over the range 25-500 ng. In addition, calibration curves were determined using 8 working standards over the range 25-200 ng for each type of plate. A 5 μL volume of the corresponding standard solution in methanol was applied in duplicate as bands of 2 mm, 10 mm from the lower edge, with the following application parameters: band velocity 10 mm s^{-1}, delivery speed 200 nL s^{-1}, distance between bands 7 mm.

Determination of sensitivity, limit of detection and limit of quantification in aqueous solutions

These parameters were calculated from the data set obtained from a linear calibration curve in the range 5-20 ng (two replicates for each standard). For this purpose, a 10 μL volume of the corresponding aqueous standard solution was applied in duplicate as bands of 2 mm. Application parameters were the same as above, except for delivery speed, which was changed to 50 nL s^{-1} in order to obtain an appropriate evaporation.

The corresponding slope and regression standard deviation ($S_{Y/X}$) values were used to establish sensitivity ($S_{Y/X}$ / b). LOD was calculated with the following equation (8):

$$LOD = 3\left(\frac{S_{Y/X}}{b}\right)\sqrt{\frac{n-2}{n-1}}$$

LOQ was determined by multiplying by 10 in this equation. A linear calibration curve in the range 5-100 ng (two replicates for each aqueous standard) was also calculated.

Reproducibility and repeatability

To evaluate the precision of area measurements three volumes containing 75 ng atrazine (5, 10 and 15 μL) from aqueous standards were run five times within the same plate, the procedure being repeated two times to evaluate run-to-run variation in the method.

Accuracy

This parameter was determined on the basis of recovery tests. Solutions of atrazine at four concentration levels (5-10-15 and 20 μg/mL) were prepared in the presence of aqueous matrices obtained from three different soils having different physicochemical properties, the most relevant being OM content: Pocuro (PCR) 1.4%; Quillota (QLT) 3.1% and Temuco (TEM), 11.4%. To this end, 16 g of air-dried soil samples were equilibrated with 80 mL of CaCl$_2$ 0.01M in a sealed tube by shaking mechanically at ambient temperature for 20 h. After equilibration each suspension was centrifuged at 4000 rpm for 30 min and filtered through a 0.22 μm membrane (GV, Durapore). The resulting solutions were used as dilution solvent to prepare the standards. All tests were performed in duplicate, and a 10 μL volume was applied

both for soil matrices and their corresponding aqueous standards, which were used to calculate recoveries. Additionally, to verify the applicability of the method, a batch equilibrium adsorption isotherm was determined for a high organic matter soil (24 %). Duplicate 2 g of air-dried soil samples were equilibrated with 10 mL of standard solutions (2, 4, 6, 8 and 10 μg ml-1 in distilled water) in 30 ml sealed tubes by shaking mechanically at 30ºC ± 0.5ºC for 14 h. After equilibration the suspensions were centrifuged, the supernatants were diluted in acetonitrile (1:1) and then filtered as above. The amount of sorbed herbicide was considered to be the difference between the initially present and that in the equilibrium solution determined by the HPTLC method. Kf and the corresponding exponent (*1/n*) from the empirical Freundlich relationship describing the adsorption behavior were calculated and compared with the corresponding values obtained by applying an HPLC-DAD method, previously validated.

RESULTS AN DISCUSSION

Preliminary calibration studies using the three different adsorbents are shown in Figure 1. The linear regression equations were calculated from the densitometric area measurements, the corresponding standard error for each parameter is expressed in parentheses. In spite of the correlation coefficient obtained for the observed data with Kieselgel 60 plate over the range 25-500 ng, the lack of fit test, from the analysis of variance, used to determine whether the linear model was adequate to describe these data, gave a p-value <0.01, indicating a statistically significant lack of fit at the 99% confidence level. On the other hand, the calibration curves exhibited good linearity in the range 25-200 ng for the three adsorbents under study, the lack of fit p-value was (0.10. As expected, a significant higher slope value was obtained for Kieselgel 60 F254 HPTLC plates (layer thickness 0.10 mm).

LOD and LOQ determined for aqueous solutions were 1.48 and 4.94 ng, respectively. These values were obtained after the optimization of several application parameters (band width and delivery speed) and scanning parameters (slit width and slit length) for measurements carried out with a 10 μL volume. The lowest noise levels, and the highest peak heights and peak areas were achieved with a slit width, 0.4 mm and a slit length, 2 mm for a spot wide of 2 mm. Similar LOD's for atrazine and simazine determination by using a 40 μL volume of methanolic extracts originated in a solid-phase extraction method for surface waters have been mentioned in literature (9). Analytical sensitivity indicating the least variation of concentration (or amount) that the analytical method is able to discriminate (8) was 0.53 ng. The calibration equation for aqueous standards (5-100 ng) calculated from area measurement was Y= 3.190 (0.053) X + 10.66 (2.595) (r= 0.998, S$_{Y/X}$ = 6.753). In spite of the different conditions employed (volume and solvent), slope was closely similar to that from the preliminary methanolic calibration study.

From the study of precision, the contribution of the three variance components was estimated considering each factor after the first nested in the one above: between run precision (sb^2), between volume precision (sv2), and within run precision sw^2, the total variance of measurements being expressed as the sum of sb^2 + sv^2 + sw^2. The total variance in area was 333. A 26.86 % was estimated to be due to variation within run, 73.14 % to variation between volumes and 0% to variation run-to-run. Intra-assay variation for each applied volume was between 1.48 and - 5.97 % (RSD). A significant interaction between volume and
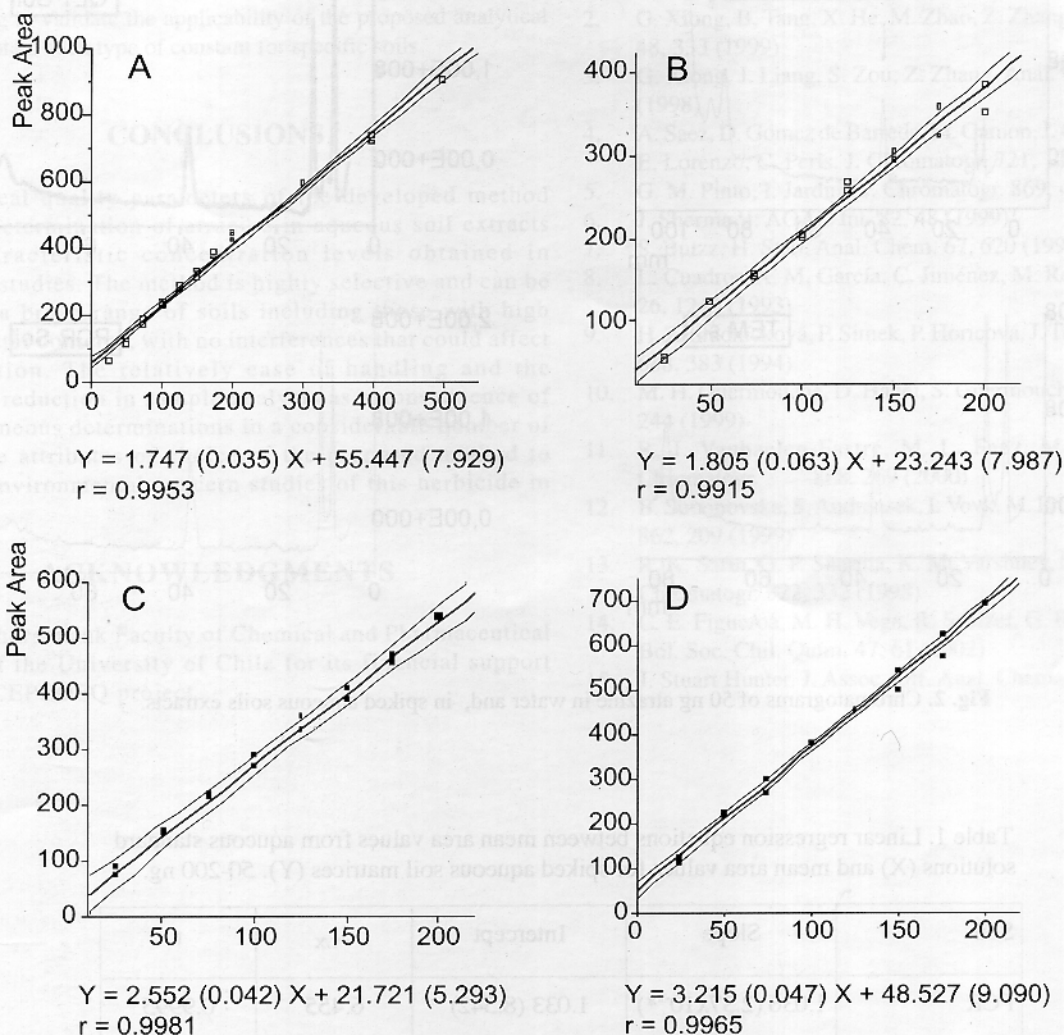
Fig. 1. Calibration curve for atrazine obtained with different adsorbents. A: Kieselgel 60 (HPTLC, layer thickness 0.20 mm), B: Kieselgel 60 F254 (TLC, layer thickness 0.25 mm), C: Kieselgel 60 (HPTLC, layer thickness 0.20 mm) D: Kieselgel 60 F254 (HPTLC, layer thickness 0.10 mm).

run-to-run factors at the 95% confidence level was identified. In fact, slightly higher area responses were obtained for the 5 µL volume with the second plate. Variation attributed to different volumes was more important for the 10-15 µL pair. This effect can be explained by the lateral broadening of the spot when higher volumes are applied and it could be modified by controlling the delivery speed to slower rates to facilitate water evaporation.

Typical chromatograms obtained for spiked aqueous soil extracts are shown in Figure 2. No peak from blank samples of soils at the atrazine retention factor was detected. In situ UV spectra in spiked samples were found to be identical with

standard atrazine. No band broadening during migration was observed due to the band re-concentration effect achieved through the initial short isocratic runs with acetonitrile-dichloromethane 30+70, V/V also allowing the extraction of atrazine from the sample application area, away from water soluble humic materials of soils. The subsequent lower eluent strength of dichloromethane allowed migration of atrazine without any other interference from the soil matrix, leaving impurities mostly on the start and front positions of the eluent. Width at the base of the peaks (< 5 mm) was similar or lower than those obtained for several HPTLC applications, including automated multiple development (10-14).
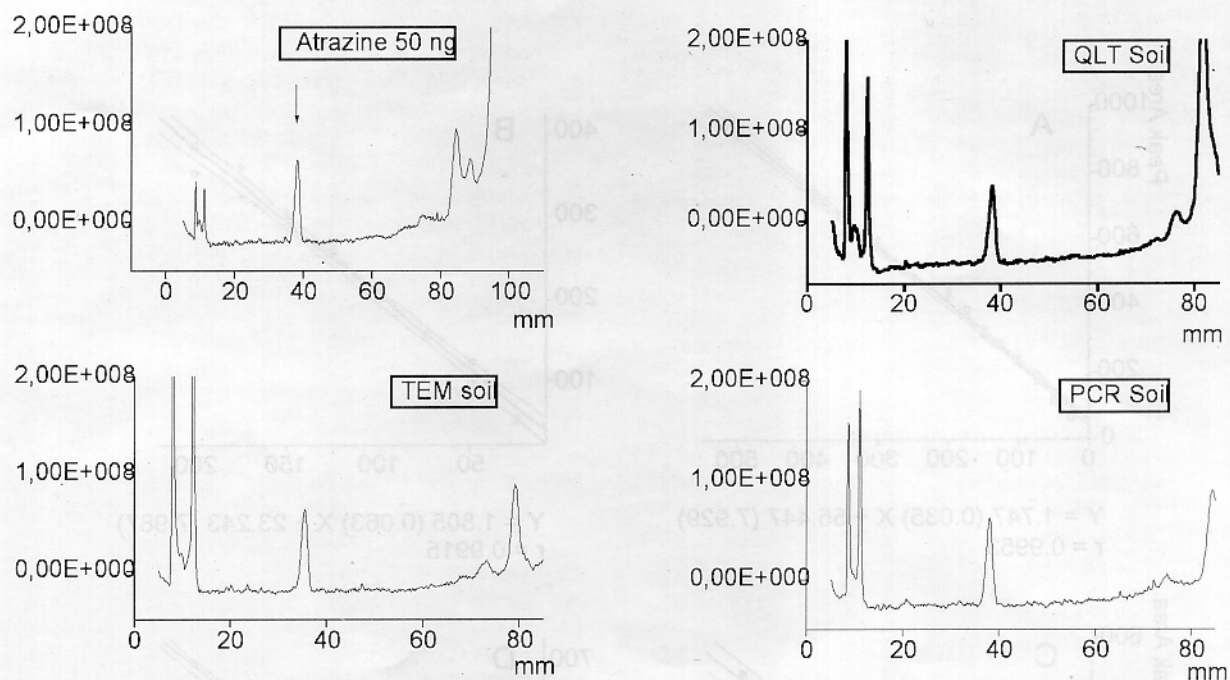
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Fig. 2. Chromatograms of 50 ng atrazine in water and, in spiked aqueous soils extracts.

Table 1. Linear regression equations between mean area values from aqueous standard solutions (X) and mean area values for spiked aqueous soil matrices (Y). 50-200 ng.

Soil	Slope	Intercept	$S_{Y/X}$	r
PCR	1.036 (2.37×10^{-2}*)	1.033 (8.542)	6.455	0.9995
QLT	0.999 (2.41×10^{-2})	2.430 (7.475)	5.424	0.9994
TEM	0.973 (1.43×10^{-2})	8.210 (4.310)	3.430	0.9998

(*) Standard error

A linear regression equation was calculated between mean areas corresponding to 50-100-150 and 200 ng atrazine in aqueous standard solutions as independent variable and mean areas from the respective spiked aqueous soil extracts as dependent variable. Data presented in Table 1 indicated satisfactory correlation between results. When the slope and intercept values for equations are 1 and zero, respectively, systematic errors will not be present. A joint confidence region for slope and intercept for each data set was obtained through the F value of Snedecor's F statistic with 2 and n-2 degrees of freedom for α = 0.05, in order to test whether 1 and zero values were included within this (15). This test led to the conclusion that there was a lack of

systematic errors for all matrices under study, with recoveries ranging from 98.7 to 103.5%.

The soil selected as a good reference as a complex matrix because of its high organic matter content, presented, as expected, a high capability to adsorb atrazine, consequently low concentrations in the equilibrium solutions were obtained. Kf and the corresponding exponent (1/n) from the Freundlich equation calculated were 10.820 (0.653) and 0.770 (0.054), respectively, the standard error in parentheses. The same constants were established by the alternative HPLC method giving the following values: 11.407 (0.223) and 0.779

(0.018). Upper and lower confidence limits at a 95% level for Kf and 1/n were 10.127-11.518 and 0.711-0.825, respectively. Values used as reference are included within the corresponding ranges in both cases, thus allowing to validate the applicability of the proposed analytical method to obtain such type of constant for specific soils.

CONCLUSIONS

Analytical quality parameters of the developed method allow the determination of atrazine in aqueous soil extracts at the characteristic concentration levels obtained in adsorption studies. The method is highly selective and can be applied to a broad range of soils including those with high organic matter contents, with no interferences that could affect quantification. The relatively ease of handling and the significant reduction in sample analysis as a consequence of the simultaneous determinations in a considerable number of samples are attributes of choice of the proposed method to carry out environmental concern studies of this herbicide in soils.

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