Available online at www.elixirpublishers.com (Elixir International Journal)

Organic Chemistry

Elixir Org. Chem. 40 (2011) 5277-5281

ISSN: 2229-712X

Determination of herbicides applied to soil ecosystem using thin layer chromatographic methodology

S.Afful[1], C. K.Akpabli[2] and P. O. Yeboah[1]

[1]Department of Chemisrty, NNRI/GAEC, BOX LG. 80, Legon

[2]Department of Chemistry, University of Ghana, Legon.

ARTICLE INFO

Article history:

Received: 17 August 2011;

Received in revised form:

18 October 2011;

Accepted: 28 October 2011;

Keywords

Chromatographic,

Adsorbent-solvent systems,

Herbicides,

Recovery efficiency extraction.

ABSTRACT

Thin layer chromatographic technique with silica gel – ethyl acetate adsorbent - solvent system has been validated for the determination of herbicides. Precision of the method determined in terms of reproducibility yielded relative standard deviation of 0.3 % and 0.5 % for the R_f of the herbicides and diameter of spots respectively. The minimum detectable quantity (MDQ) of the herbicides ranges from 0.20 – 0.50 ng. The method has been applied to determine the efficiencies of acetone, acetonitrile, methanol, hexane and acetone/hexane (4:1) for the recovery of the herbicides, atrazine, ametryne, propanil, diuron and nitrofen in a forest zone soil in Ghana. Acetone and acetonitrile were found to have approximately equal efficiency as extraction solvents. Extraction with acetone, acetonitrile, acetone/hexane (4:1) yielded more than 90% recovery for all the herbicides. The highest recovery efficiency of 96.2% was obtained for atrazine using acetone. Extraction with methanol and hexane gave relatively low recoveries particularly, with the clean up extracts. In all, the clean up procedure with SPE cartridge equipped with C-18 as adsorbent reduced recovery by 9 – 13 %.

Introduction

Herbicides are type of pesticides commonly known as weedkillers, are used to kill unwanted weeds especially in agriculture.[1]. In the developed countries the use of herbicides in agriculture has replaced human and mechanical weeding [2]. Apart from agricultural use, herbicides can also be used for non-agricultural purposes, i.e. as weeds killers for lawns and flower gardens. Selective herbicides kill specific targets while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often synthetic "imitation" of plant hormones. Herbicides used to clear waste grounds, industrial sites, railways are non selective and kill all plants material with which they come into contact. Smaller quantities are used in forestry, pasture systems, and management of areas set aside as wildlife habitat [3].

In recent years, there has been a considerable increase in the use of herbicides in Ghana and this has come about as a result of the emphasis on the promotion of non-traditional agricultural exports. Information available indicates that 21 different kinds of herbicides were imported into Ghana for agricultural purposes between 1995 and 2000[4]. Herbicides use for farming makes food production convenient and to some extent easy. This is because they selectively kill the target weeds and leave the cultivated crops/plants intact. This thus saves the farmer the problem of having to use farm implements to clear the unwanted weeds. In Ghana herbicides such as atrazine, diuron, simazine, glyphosphate etc are now being used for commercial cultivation of food crops such as rice, pineapple, banana and vegetables [5]. Despite the immense advantages associated with the use of herbicides, there is the other side of it, the toxicity and persistency they may pose to the environment as a result of their usage. Herbicides have widely variable toxicity. In addition to acute toxicity from high exposures, there is concern of possible carcinogenicity [6] as well as other long-term problems such as contributing to Parkinson's disease. The pathway of attack can arise from intentional or unintentional direct consumption, improper application resulting in the herbicide coming into direct contact with people or wildlife, inhalation of aerial sprays and food contamination by residues of herbicide applied on the field. Herbicide residue can also be transported via surface run off to contaminate near and distant water sources.

It is against this background that it is always necessary to investigate the fate and behaviour of these chemicals after application. Chromatographic techniques such as thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC) have been recommended for analysis of herbicides residues in the environment [7]. Hyphenated techniques such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography\mass spectrometry (LC- MS) are now becoming popular for analysis of herbicides and related compounds. There is a more recently GC x GC two – dimensional gas chromatography available for analysis of herbicides [8].

Analysis of herbicide residues in the soil and other environmental and biological samples using chromatography typically involves a vigorous extraction of the residues using suitable extraction solvent. Selection of suitable extraction solvent may thus be one the most important factor in optimization of herbicide extraction from soils and other related matrices. The primary criteria for choosing a solvent or solvent system have been such as the extraction efficiency, minimal amount of co-extractives and reproducibility of residue recovery

Tele:

E-mail addresses: affulsammy@yahoo.com

[9]. Common solvents recommended for multi-residue analysis of pesticides include acetone, acetonitrile, chloroform, ethy acetate and hexane [9]. Methanol and methanol/water have also been recommended for herbicide extraction from soils [10]. In this study, thin layer chromatographic technique using silica gel – ethyl acetate adsorbent – solvent system has been validated for the determination of five herbicides and the method has been used to determine the efficiencies of extraction solvents for the recovery of the herbicides applied to a forest zone soil in Ghana. The herbicides were selected for the investigation as they are among the commonly used herbicides in Ghana [11]

Materials and methods

Chemicals and Reagents

The chemicals used were obtained from Merck, Darmstadt, Germany and Fluka, Switzerland. They were of analytical grade. The herbicides primary standards were obtained from Dr. Ehrenstorfer GmbH. They were of 98 – 99.5% purity. Borax buffer which was used to prepare 2, 6-dichlorophenol-indolphenol (DCPIP) reagent, was prepared by dissolving 3.325 g of borax in 175mL of distilled water and the solution was added to 75 mL of 0.1 MHCl. DCPIP reagent was used to prepare the detection reagent and was prepared by dissolving 0.02 g of 2,6 dichlorophenol-indolphenol sodium salt in 500 mL borax solution.

Detection reagent used for the detection of herbicides was prepared by mashing 30 g of *panicum maxima* leaves and 5 g of sea sand in a mortal with pestle. 15 ml of distilled water and 3 mL of glycerine were added. These were mixed thoroughly and the liquid squeezed into 50 mL flask. 20 mL of this was added to 13 mL of the DCPIP to give the spraying reagent.

Apparatus

TLC plates (20 cm x 20 cm) coated with silica gel and silica gel F254 were purchased from Merck, Germany. TLC basic set including application guide, atomizer and development tank were obtained from Camag Chemie- Erzeugnisse und Adsorptionstechnik AG, CH-4132, Muttenz, Switzerland. Micro syringes (10 µL) with needle (Hamilton) were obtained from Supelco Inc., Supelco Park, Bellefork, U.S.A.

Soil Sampling and Treatment

Soil samples were taken from a field at Kwame Nkrumah University of Science and Technology (KNUST) at Kumasi in the Ashanti Region of Ghana. A sketch map, showing the sampling site S2 is presented in Fig. 1. Sampling was done with an augur to a depth of 10cm randomly on about 50 m x 50 m plot size marked out for the sampling. The soil samples collected were put together, wrapped in aluminium foil and placed in black polyethylene bag. In the laboratory the sample was screened to pass through 2 mm mesh size sieve. The sample was then air –dried and used for the investigation.

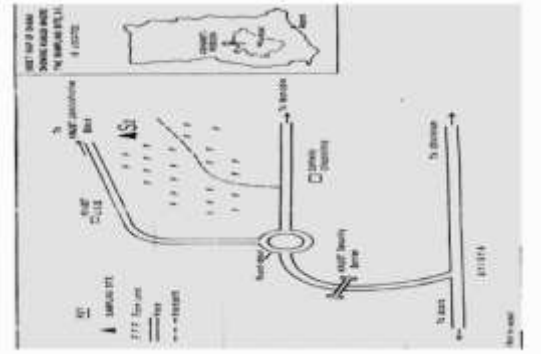

Fig 1: Sketch map of a section of KNUST showing the sampling site S2

Validation of the method

Selection of suitable TLC adsorbent-solvent system

Six adsorbent-solvent systems, silica gel – ethyl acetate, silica gel – chloroform, silica gel – dichloromethane, silica gel F254 – ethyl acetate, silica gel F254 – chloroform and silica gel F254 - dichloromethane were tested for their suitability for analysis of the herbicides. 5 µg/mL of each pesticide solution prepared in acetone were used for testing each adsorbent – solvent system. The corresponding Rf obtained was calculated. Spots were detected by spraying developed TLC plate with the detection reagent. The result proved that silica gel – ethyl acetate was most suitable for separation of herbicides and was selected and was selected for the investigation.

Precision of the method

Precision of the method was determined in terms of reproducibility of the Rf and the size of spots for each herbicide (diameter of spot), Six (6) replicates determination was carried out for each herbicides. 5 µL of 5 µg/mL of the herbicide standard was injected using 10 µL micro syringe. Silica gel – ethyl acetate adsorbent – solvent system was used.

Minimum detectable quantity (MDQ)

Minimum detectable quantity (MDQ) considered as the smallest quantity of the standard materials resulting in definite visible spot on the TLC plate [12] was determined for each of the herbicide by preparing and analyzing 0.5 ng/µL and varying the volume injected from 0.1 – 1 µL. The least volume among the various analyzed that resulted in visible spot noted and corresponding amount of herbicide computed.

Linear Range

Linear range for the herbicides was determined by analyzing various calibration solutions in a concentration range of 10 – 200 ng for each herbicide and calibration curves obtained by plotting response (average diameter of spot) against concentration.

Determination of Herbicides Recovery

Acetone, acetonitrile, hexane, methanol and acetone/hexane (4:1) were investigated for their efficiency for the extraction of the herbicides from the soil samples spiked with known amount of the herbicides standard solution.

Procedure for soil spiking and extraction

Triplicate soil samples (20 g) were accurately weighed and packed into a cellulose extraction thimble and the soil sample was spiked with 2 mL of 50 µg/mL of the herbicide standard solution to generate herbicide - soil concentration of 5 µg/g. The spiked soil was subjected to soxhlet extraction for 5 h with 150 mL of the solvent to be investigated.

After the extraction the extract was concentrated to almost dryness on rotary evaporator. The residues were re-dissolved in 10 ml of the acetone. The extract was then concentrated to 2 ml by subjecting the extract to streams of nitrogen gas blown from nitrogen cylinder. The unclean extract was then subjected to analysis for recovery.

The extraction procedure was repeated but this time the 2 ml unclean extract obtained cleaned on SPE cartridge, equipped with C-18 as adsorbent which was earlier preconditioned with 2 ml acetone/water (1:9) [13].

The cartridge and its content were vacuum dried for 15 minutes after which the herbicide was eluted with 8 mL acetone. The cleaned up extract was adjusted to 2 mL by blowing in streams of nitrogen gas and the extract subjected to analysis for recovery. The procedures were repeated for the other herbicides and solvents.

Analysis of Extracts for Recovery of herbicides

The herbicides recovered in the soil were quantitatively determined using the validated TLC methodology. Ready made silica gel plates were activated in an oven at 105^0C for 30 minutes. The development tank was saturated by the vapour from ethyl acetate used as development solvent. With a calibrated microsyringe, 5 µL of each extract was applied to the sorbent layer of the TLC plate. The same volume of the standard herbicide solution was analyzed concurrently. The developed plate was sprayed with the detection reagent. Spots were seen as blue in colour on greenish background. The Rf and the diameters of the spots were measured to ascertain the identity and to determine the concentrations respectively.

Results

Validation of TLC method

Rf values obtained when the six adsorbent – solvent systems were tested for their suitability for separation of the herbicides are presented in Table 1. Margin of errors are standard deviation based on replicate determination of each herbicide.

The minimum detectable quantity (MDQ), considered as the smallest quantity of the standard material resulting in a definite visible spot on the TLC plate, obtained for the herbicides are presented in Table 2. The results in general suggest that the method is quite sensitive for the determination of the herbicides.

Data for the linear range determination for the herbicides are shown in Table 3. Coefficient of correlation between concentration and diameter of spots ranges from 0.9714 – 0.9924. Fig 2 is a sample calibration curve obtained for determination of atrazine. This is a plot of diameter of spot versus amount of herbicide in the linear range.

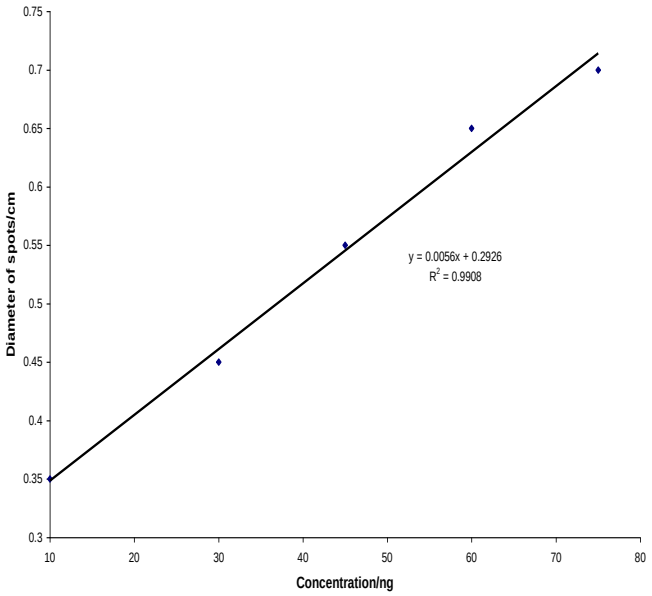

Fig 2: Calibration curve for determination of atrazine

Herbicides Recovery

Recovery data obtained for herbicides when the various solvents were used for extraction are presented in Tables 4 and 5. Margins of errors are standard deviation based on triplicate determination. Highest efficiency of the extraction for almost all the herbicides was achieved with acetone, and acetonitrile, when used as single solvents. In all, it was found that the clean up procedure reduced recovery efficiency by 9 – 13 % as shown in Table 6. Thus with the methodology employed in the study clean up might not be necessary to achieve the desired results.

Discussion

It is obvious from the Rf data (Table 1) of the six adsorbent-solvent systems investigated, that the silica gel – ethyl acetate system appeared the most suitable adsorbent – solvent system for separation of the herbicides. This is because the Rf values obtained using the silica gel – ethyl acetate system are relatively distinct from each other. This means that with the silica gel – ethyl acetate system spots of the herbicides will not overlap in a multi residue analysis involving a mixture of these herbicides. Results obtained for silica gel – chloroform is also suggests that the system could be useful for analysis of propanil, diuron and nitrofen administered in a mixture as their spots will not overlap. However, analysis of atrazine and ametryne in the same sample would not be possible. The herbicides in the silica gel-dichloromethane system gave Rf spread of 0.07 – 0.70. The relatively low Rf values obtained for atrazine, ametryne and diuron indicate that their ascent on the adsorbent were retarded by their relative insolubility in the mobile phase (dichloromethane).

Thus silica gel-dichloromethane could not therefore be recommended for analysis of atrazine, ametryne and diuron. Afful et al [13] also reported low Rf values of 0.08 and 0.09 for atrazine, and diuron respectively using silica gel 60-dichloromethane system. The results of linear range determination show that the method is linear over amount of herbicide range of 10 – 75 ng, 20 – 100 ng, 25 – 75 ng, 20 – 80 ng and 20 – 100 ng for atrazine, ametryne, propanil, diuron and nitrofen respectively. The minimum detectable quantity (MDQ) ranges from 0.2 – 0.5 ng. The least value of 0.2 ng was obtained for diuron. This suggests that the detection method is more sensitive to diuron as the detection reagent could respond to small change in quantity of diuron. The results of herbicides recovery with the extraction solvents showed that both acetone and acetonitride have approximately equal efficiency as extraction solvents for the herbicides. Extraction with methanol and hexane as single solvents gave relatively lower recovery efficiencies, particularly with the clean up extract. However, performance of acetone/hexane mixture (4:1) was not surprising as it was between those of acetone and hexane used as single solvents, but closer to that of acetone. Performance of the two solvents, methanol and hexane with regard to the unclean extracts was satisfactory since in most cases recovery efficiencies were generally above 80 %. However, if the extracts are to be cleaned up on SPE cartridge equipped with C-18 adsorbent the two solvents could not be recommended as low recovery efficiencies were obtained for the herbicides.

Conclusion

The method presented is simple, fast and reliable, and can be very useful for routine analysis of herbicides in soil ecosystems using silica gel – ethyl acetate adsorbent solvent system. Silica gel – ethyl acetate system would be efficient in separating the herbicides in a multi residual analysis involving these herbicides. It is therefore recommended for TLC analysis of these herbicides. Acetone and acetonitrile are efficient solvents for extraction of the herbicides in the forest zone soil used for the investigation. Recoveries with methanol and hexane were relatively low particularly, with the clean up extracts, and could not be recommended for the extraction of the herbicides.

References

1.Kellogg R, L; Nehring R; Grube A; Goss D, W and Plokin S (2002). Environmental indicators of pesticides leaching and

runoff from field. United States department of agriculture national resources conservation service. Retrieved on 2010-8-26
2.Baird, C. (1995). Pesticides and the Environment in "Environmental Chemistry", 2nd Edition, W. H Freeman and Co. New York, 241.
3.Kollberg Robert L and Wiles L, J (2002). Effect of steam application cropland weeds. Weed Technology. 4(18): 43-49
4.Afful S (2002). Thin layer chromatographic studies on depletion of some herbicides in two soil ecosystems. M.Phil Thesis in Chemistry, University of Ghana, p. 4
5.Afful, S; Akpabli, C. K and Yeboah, P. O. (2007). Comparison of two methods in thin layer chromatographic analysis of some herbicide in a oastal savannah Soil in Ghana. West Afr. J. of Appl. Ecol. 12: 1 – 7
6.Howard I, M; Kathrym W; Wigle D (1992). Herbicides and cancer. Jour. national cancer institute 84 (24):1866-1874
7Yeboah , P. O. , Lowor, S. T and Akpabli, C. K (2002). Detection and determination of pesticides using thin layer chromatography. J. of Appl. Sci. & Tech, 7:77-83
8.Af;ful S; Enimil E; Blewu B; Adjei M, G; Ewusie E,A (2010). Gas chromatography methodology for the determination of halogenated pesticides. Res. Jour. Of Appl. Sci. Engineer. & Tech 2(6):592-595.
9.Dao T. H. and Dragun, J. (1983). Methods for Pesticide Residues Extraction. Residue Rev. 87:91
10.Ambrus A; L antos J; Visi, E ; Csatlos, L.and Sarvari, L. (1986). J. AOAC 64:733
11.Balinova, A. M and Balinov (1991) Determination of herbicides residues in soil in the presence of persistent Organochlorine insecticides, Fresenius J.Anal Chem. 339: 409 - 412
12.Gerken, A. Suglo, V and Braun, M. (2001).Crop Protection in Ghana for Ministry of Food and Agriculture, Intergrated Crop Protection, Project PPRST/GTS Accra, 162.
13.Ambrus, A. (1998). Development of Cost Effective Screening Methods for
Pesticide Residues in Vegetables. International Atomic Energy Agency (IAEA) Final Report. 8908/R
14.Yeboah , P. O. , Lowor, S. T and Akpabli, C. K. (2001). Comparison of thin layer chromatography determination of propuxur residues in cocoa ecosystems. Afri. J. of Sci. and Tech.4: 24 - 28
15.Afful S; Dogbe S, A; Ahmad K and Ewusie E. A. (2008). Thin layer chromatographic analysis of pesticides in soil ecosystem West Afri. J. of Appl. Ecol. 14:57-64

Acknowledgement

The authors are very grateful to Mr Julius Nortenor of the Soil Science Department of University of Ghana and Mr. B. Q. Modzinuh of Ghana Atomic energy Commission for their technical assistance.

Table 1: Rf values obtained for the herbicides using six adsorbent – solvent systems

Herbicides	silica gel – chloroform	silica gel – ethyl acetate	silica gel – dichloromethane	Silica gel F254 - ethyl acetate	silica gel F254 - chloroform	silica gel F254 - dichloromethane
Atrazine	0.41±0.01	0.62±0.02	0.08±0.01	0.66±0.04	0.40±0.03	0.14±0.03
Ametyne	0.42±0.02	0.73±0.02	0.07±0.01	0.68±0.03	0.43±0.02	0.17±0.04
Propanil	0.32±0.01	0.81±0.01	0.27±0.01	0.64±0.02	0.48±0.02	0.28±0.03
Diuron	0.27±0.04	0.42±0.02	0.09±0.02	0.50±0.03	0.32±0.04	0.29±0.04
Nitrofen	0.72±0.03	0.56±0.03	0.70±0.02	0.71±0.04	0.72±0.03	0.77±0.02

Table 2: Minimum detectable quantity (MDQ) for the herbicides

Herbicides	MDQ/ng
Atrazine	0.25
Ametryne	0.25
Propanil	0.30
Diuron	0.20
Nitrofen	0.50

Table 3: Data on calibration curves for the herbicides

Herbicide	Linear range (amount)/ng	Regression equation	Coefficient of correlation (R^2)
Atrazione	10 – 75	Y = 0.0056X + 0.2926	0.9908
Ametyne	20 – 100	Y = 0.0065X + 0.3395	0.9820
Propanil	25 – 75	Y = 0.0089X + 0.2405	0.9924
Diuron	20 – 80	Y = 0.0075X + 0.2821	0.9714
Nitrofen	20 - 100	Y = 0.0053X + 0.2950	0.9844

Table 4: Percentage recovery (mean of three replicates) of the herbicides with the Extraction solvents using the unclean extracts

Herbicides	acetone	acetonitrile	hexane	methanol	acetone/hexane(4:1)
Atrazine	96.2±3.2	92.6±3.5	84.8±3.5	86.0±3.9	93.6±2.3
Ametryne	95.1±4.2	93.4±3.0	82.9±3.0	84.6±3.7	92.7±2.4
Propanil	93.4±3.9	92.8±3.3	82.1±4.3	85.6±4.1	92.8±3.3
Diuron	92.4±4.2	95.0±4.2	78.6±3.1	83.3±5.3	90.9±3.8
Nitrofen	94.6±3.7	90.6±4.2	79.7±4.1	83.3±5.3	89.7±2.9

Table 5: Percentage Recovery (mean of three replicates) of the Herbicides with the solvents using the clean up extracts

Herbicides	acetone	acetonitrile	hexane	methanol	acetone/hexane(4:1)
Atrazine	84.8±2.7	82.1±4.4	73.4±3.3	74.4±3.3	81.7±5.5
Ametyne	83.6±4.5	83.9±4.2	72.3±3.0	73.3±4.7	80.8±3.7
Propanil	82.3±4.4	82.5±2.9	73.5±2.3	74.0±4.9	82.6±5.5
Diuron	81.3±5.1	85.2±4.3	68.4±2.5	72.5±4.9	81.7±5.3
Nitrofen	83.7±4.4	80.4±3.9	69.3±3.5	69.1±5.6	80.9±3.3

Table 6: Difference in Percentage Recovery between unclean and clean up Extracts

Herbicides	acetone	acetonitrile	hexane	methanol	acetone/hexane(4:1)
Atrazine	11.4	10.5	11.4	11.6	11.9
Ametyne	11.5	9.5	10.6	11.3	11.4
Propanil	12.0	10.3	9.6	11.6	10.2
Diuron	11.3	9.8	10.2	10.8	9.8
Nitrofen	10.9	10.2	10.4	9.7	8.8