



Environmental Science

An Indian Journal

Current Research Paper

ESAIJ, 12(4), 2016 [174-178]

Determination of some herbicide residues in pond water using dispersive liquid-liquid micro-extraction followed by high performance liquid chromatography

SNVS.Murthy¹, Tentu.Nageswara Rao^{2*}, G.Kumar², N.Krishna Rao², Karri.Apparao², K.Raghu Babu³

¹DLR PG College, G Mamidada, Andhra Pradesh, (INDIA)

²Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, (INDIA)

³Department of Engg Chemistry, Andhra University, Visakhapatnam, Andhra Pradesh, (INDIA)

E-mail: tentu6581@rediffmail.com

ABSTRACT

A rapid and simple extraction and preconcentration method for the determination of herbicide residues (2,4-D Sodium, Ethoxysulfuron and Halosulfuron methyl) in pond water samples using dispersive liquid-liquid micro extraction (DLLME) technique coupled with HPLC-UV was developed. The significant factors in dispersive liquid-liquid microextraction such as volume of extraction, disperser solvent, pH, ionic strength as well as extraction time were investigated and optimized in detail. The optimum experimental conditions of the proposed method were 5.00 mL aliquot of the sample solution, 5 mL acetonitrile, natural pH containing 5% (w/v) NaCl, while keeping centrifugation time and speed fixed at 10 min, 4000 rpm respectively. The calculated calibration curves gave high-level linearity for all target analytes with correlation coefficients ranging between 0.9997 and 0.9999. The reproducibility and recovery of the proposed method, expressed as relative standard deviation, varied between 85 % to 95 % and 2% to 5%, respectively. The obtained LOQ values were in the range of 0.05 µg/mL.

© 2016 Trade Science Inc. - INDIA

KEYWORDS

Herbicides;
Dispersive liquid-liquid
micro extraction;
HPLC-UV and LOQ.

INTRODUCTION

Herbicides are widely used in agriculture, industry, and non crop areas for weed management. Herbicides can provide cost effective weed control while minimizing labor. However, improper herbicide use may result in crop injury, poor weed control, evolution of herbicide resistant weeds, environmental contamination, or health risks^[1,2].

Herbicides present about 50% of the demand for agricultural chemicals; their prolonged use represents not only an environmental risk but also a health hazard for their retention in crops^[3]. The intensive application of herbicides has resulted in the contamination of the atmosphere, ground and waste waters, agricultural products and, consequently, in the direct or indirect pollution of food and food products and biological systems.

Herbicides present a 50% of the demand of agricultural chemicals; their prolonged use represents not only an environmental risk but also a health hazard for their retention in crops. The intensive application of herbicides has resulted in the contamination of the atmosphere, ground and waste waters, agricultural products, consequently, in the direct or indirect pollution of food and food products and biological systems^[4,5].

At present, the most commonly used analytical method for the analysis of herbicide residues is HPLC with different detectors. Analyte extraction and pretreatment is the most challenging and time-consuming step in an analytical procedure are available, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) techniques. Recently, solid-phase micro extractions (SPME) and liquid-phase micro extractions (LPME) techniques have emerged as new attractive alternatives for sample preparations^[6,7]. Dispersive liquid-liquid micro extraction (DLLME) as a novel liquid-phase micro extraction technique was developed by Assadi in 2006^[8,9]. The advantages of DLLME method include simplicity of operation, rapidity, low cost, high-recovery and high-enrichment factor^[10]. Until now, DLLME methods coupled with liquid/gas chromatography have been mainly and successfully used for the extraction and determination of herbicide residues in environmental water samples. To our knowledge, its application to the analysis of herbicide residues has not been reported.

In the present study, we have developed a simple and rapid method for the determination of three herbicide residues in water samples by DLLME coupled with high performance liquid chromatography-ultraviolet detection (HPLC-UV).

EXPERIMENTAL

Standards, reagents and samples

Reference standards of 2,4-D Sodium (98.6%), Ethoxysulfuron (99.5%) and Halosulfuron methyl (99.8%) were obtained from Sigma Aldrich. Acetonitrile and waater – HPLC grade were purchased from Rankem, New Delhi, Analytical grade solvent, orthophosphoric acid and chloroform

supplied from Merck Limited, Mumbai, and AR grade sodium chloride from Merck Limited, Mumbai.

Chromatographic separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC-20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed Phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 μm (PhenomenexLuna-C18) Column temperature was maintained at 30°C. The injected sample volume was 10 μL . Mobile Phases A and B was Acetonitrile and pH 3.0 adjusted with H_3PO_4 (80:20(v/v)). The flow- rate used was kept at 0.8 mL/min. A detector wavelength was 235 nm. The approximate retention times of 2,4-D Sodium, Ethoxysulfuron and Halosulfuron methyl in minutes i.e., 4.7, 5.4 and 7.2 respectively.

Standard stock solutions

The herbicide standard stock solutions were individually prepared in acetonitrile at a concentration level 100 $\mu\text{g/mL}$ and stored in a freezer at 4°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using methanol, immediately prior to sample preparation.

Sample preparation

A mixture stock solution containing each of 2,4-D Sodium, Ethoxysulfuron, and Halosulfuron methyl at 10 $\mu\text{g/mL}$ was prepared in acetonitrile. The mixed standard solution was prepared by combining equal volumes of the stock solution and then diluting them to an appropriate concentration with the double distilling water. All the standard solutions were stored at 4°C in the dark.

The rain, surface and ground water samples were filtered through 0.45 μm polyamide filter to remove suspended particles prior to the analysis by the proposed method.

Extraction procedure

For the DLLME (Figure 1), a 5.00 mL aliquot of the sample solution was placed in a 10 mL screw

Current Research Paper

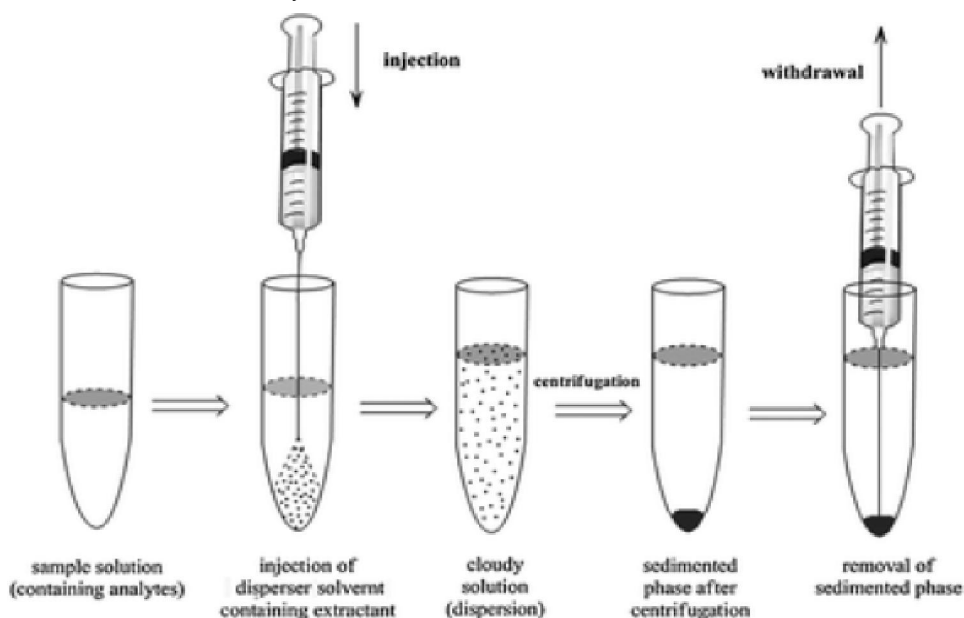


Figure 1 : DLLME technique

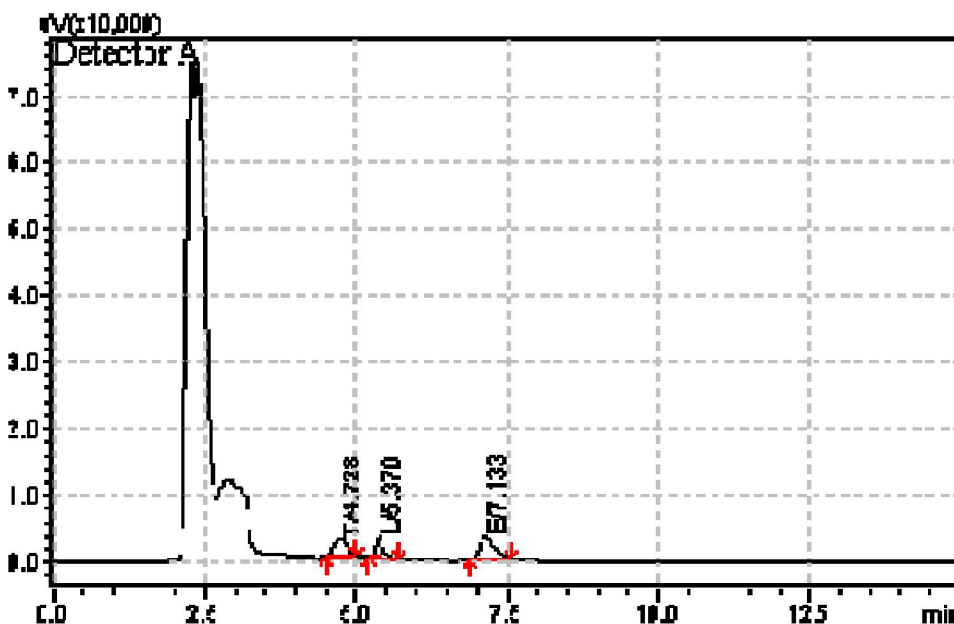


Figure 2 : Representative chromatogram at fortification level of 10 $\mu\text{g}/\text{mL}$ in pond water

cap glass tube with conic bottom and 0.5g NaCl was added into the solution. A mixture of 5.0 mL of acetonitrile (as disperser solvent) and 100 μL of CHCl_3 (as an extraction solvent) was injected into the sample solution by a syringe, and then the mixture was vortexed for 50 sec. A cloudy solution that consists of very fine droplets of CHCl_3 dispersed into an aqueous sample was formed, and the analytes were extracted into the fine droplets. After centrifugation at 4000 rpm for 5 min, the CHCl_3 phase was sedimented at the bottom of the centrifuge

tube. The sedimented phase was completely transferred to another test tube with a conical bottom using 100- μL HPLC syringe and blown to dryness with a mild nitrogen stream. The residue was dissolved in 5 mL acetonitrile, and 10.0 μL was injected into the HPLC system for analysis.

RESULTS AND DISCUSSION

In order to obtain the optimum DLLME conditions for the determination of herbicides, the influence of

TABLE 1 : The linear ranges, regression equations, detection limits and enrichment factors of the method

Herbicide	Linear range (µg/mL)	Linear equation	Correlation coefficient(r ²)	Enrichment factor	LOQ (µg/mL)
2,4-D Sodium	0.05-100	Y=75417X+10.59	0.9999	90	0.05
Ethoxysulfuron	0.05-100	Y=58954+5.23	0.9997	102	0.05
Halosulfuron methyl	0.05-100	Y=78152X+37.19	0.9998	153	0.05

TABLE 2 : Recoveries of three herbicide residues in pond water sample (n=6)

Herbicide	Portified Concentration (µg/mL)	Recovered Concentration (µg/mL)	Recovery in %	RSD in %
2,4-D Sodium	Control (Pond water)	Below detectable limit	-	-
	10	21.3	84.92	4.15
	50	45.3	94.17	3.26
Ethoxysulfuron	Control (Pond water)	Below detectable limit	-	-
	10	20.9	85.25	3.98
	50	46.1	94.89	2.15
Halosulfuron methyl	Control (Pond water)	Below detectable limit	-	-
	10	20.9	86.69	4.95
	50	46.1	95.06	3.47

different experimental parameters including the type and volume of the extraction solvent and disperser solvent, the presence of salt, and the extraction time on the performance of DLLME was investigated. Under the optimum conditions, the enrichment factors of this method were 90, 102 and 153 for 2,4-D Sodium, Ethoxysulfuron, and Halosulfuron methyl respectively.

A series of working solutions containing each of 2,4-D Sodium, Ethoxysulfuron, and Halosulfuron methyl six concentration levels of 0.05, 1, 5, 10, 50 and 100 µg/ mL were prepared for the establishment of the calibration curve. For each concentration point, three parallel extraction and analyses were performed under the optimum conditions. The linear regression equations showing the relationship between the signal of the herbicides (y, peak area) and the concentration of the herbicides in the water sample (X, µg mL⁻¹ and other relevant results are given in TABLE 1. The limits of detection for the three herbicides, which were evaluated on the basis of a signal-to-noise ratio of 3(S/N=10) of limit of quantification (LOQ) was 0.05µg /mL for 2,4-D Sodium, Ethoxysulfuron, and Halosulfuron methyl respectively. The performance of the method was tested by performing the analysis of pond water sample with 2,4-D Sodium, Ethoxysulfuron, and

Halosulfuron methyl each spiked at concentration levels of 10 and 50 µg/ mL according to the established procedures. Six parallel replicate analyses were performed for each spiked concentration level. The reproducibility (expressed as the relative standard deviations (RSD) and the recoveries (expressed as the mean percentage between the amounts found and the ones added) are shown in TABLE 2. It can be seen from TABLE 2 that both satisfactory recovery values and good reproducibility's were achieved with the method. The overall recoveries for the eight herbicides were in the range from 85-95% and RSDs fell in the range from 2 % to 5 % respectively.

The described method has been successfully employed to determine the herbicide residues in rain, surface and ground water samples. There were no matrix peaks in the chromatograms to interfere with the analysis of herbicide residues shown in Figure.2. Furthermore, the retention times of 2,4-D Sodium, Ethoxysulfuron, and Halosulfuron methyl were constant at 4.7±0.2, 5.4±0.2 and 7.2±0.2, minutes.

CONCLUSION

This newly developed DLLME method in coupled with HPLC-UV offers a simple and sensitive

Current Research Paper

approach for the determination of herbicide residues in water samples. The results demonstrate that the proposed method has good recoveries and reproducibility's. Compared with other conventional extraction method offers advantages such as rapidity, simplicity, ease of operation, high enrichment factor, and lower consumption of organic solvent. The DLLME combined with HPLC-UV method has been successfully applied to the analysis of herbicide residues in water samples. The application of this novel DLLME method to other more complex matrix samples is currently under further study in our lab.

ACKNOWLEDGEMENT

The authors are thankful to the Dr. B. Gowtham Prasad, SVV University, for providing necessary facility to conduct the Laboratory experiment.

REFERENCES

- [1] W.A.Battaglin, E.T.Furlong, M.R.Burkhardt, C.J.Peter; *The Science of the Total Environment.*, **248**, 123-133 (2000).
- [2] J.L.Belcher, R.H.Walker, E.V.Santen, G.R.Wehtje; *Weed Technology.*, **16**, 575-579 (2002).
- [3] W.Zheng, S.Yates, S.K.papiernik; *Journal of Agricultural and Food Chemistry.*, **56**, 7367-7372 (2008).
- [4] Ryan P.Hultgren, Robert J.M.Hudson, Gerald K.Sims; *J.Agric.Food Chem.*, **50**, 3236-3243 (2002).
- [5] Hanene Chaabane, Jean-Francois Cooper, Lamia Azouzi, Camilla-Michel Coste; *J.Agric.Food Chem.*, **53**, 4091-4095 (2005).
- [6] Yihua Liu, Zhenzhen Xu, Xiaoguang Wu, Wenjun Gui, Guonian Zhu; *J.Jhazmat.*, **01**, 105 (2010).
- [7] Zhenwu Tang, Wei Zhang, Yumin Chen; *J.Jhazmat.*, **12**, 52 (2008).
- [8] Radka Kodesova, Martin Kocarek, Vit Kodes, Ondrej Drabek, Josef Kozak, Katerina Hejtmankova; *J.Jhazmat.*, **11**, 40 (2010).
- [9] Zhi Mei Liu, Xiao Huan Zang, Wei Hua Liu, Chun Wang, Zhi Wang; *Chinese Chemical Letters.*, **20**, 213-216 (2009).
- [10] Cátia M.Bolzan, Sergiane S.Caldas, Bruno S.Guimarães, Ednei G.Primel; *J.Braz.Chem.Soc.*, **26(9)**, 1902-1913 (2015).