ISSN : 0974 - 7532 Volume 10 Issue 10

Research & Reviews in

BioSciences

Regular Paper

RRBS, 10(10), 2015 [394-399]

Determination of oxadiazon herbicide residues in different ecotox samples

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ABSTRACT

A simple and inexpensive high performance liquid chromatography method was developed for the determination of oxadiazon residues in different ecotox mediums. The test mediums are blended water for fish, M4 Medium for Daphnia magna, OECD TG 201 medium for Alga and 20XAAP Medium for lemna. The oxadiazon residues estimated by a validated High Performance Liquid Chromatography with Ultra Violet detector (HPLC-UV) at a wave length of 230 nm. The method showed recovery 91 $\pm$ 5% recovery in ecotox mediums. The method has limit of quantification (LOQ) 0.05 mg/L. The proposed validated method can be applied successfully for the determination of oxadiazon residues in different aquatic solutions having pH range 5-9 and high ionic concentration.

KEYWORDS

Oxadiazon;
Ecotox mediums;
HPLC-UV and LOQ.

INTRODUCTION

Oxadiazon,5-tert-butyl-3-(2,4-dichloro-5-isopropoxyphenyl)- l,3,4-oxadiazol-2(3H)-one is an effective herbicide for control of obnoxious grasses and broad leaf weeds in a wide variety of crops, e. g.,citrus fruit, vines, cotton, rice, soya beans and onions[1,2]. The relatively low toxicity by ingestion and by dermal route of this herbicide is one of the reasons for its widespread use[3,4]. Oxadiazon is stable inneutral or acid media but unstable in alkaline media, DT 50 (half-life) 38 d (pH 9, 25 °C). Maximum concentration of oxadiazon allowed is 0.01 mg/kg[5, 6, 7].

In this particular study the work describes in detail the evaluation of dithiocarbamates in aquatic media solutions prepared according to regulatory guideline, OECD 201 (Alga medium), OECD 202 (Daphnia medium), OECD 203 (Fish medium) and OECD 221 (Lemna medium) with a duration of 5-8 hours to as low as 0.05 mg/L.

MATERIALS & METHODS

Reference standard, reagents and solutions

The reference standard oxadiazon (Purity 99.9 %) was obtained from Sigma Aldrich. The orthophosphoric acid, ethylene diaminetetraacetic acid disodium salt (EDTA) used were Analytical Reagent grade. HPLC grade water and acetonitrile were pur-

chased form Merck india Pvt Ltd and Milli-Q water was obtained from Millipore India Ltd, Bangalore, India.

Ecotoxic mediums

The test medium is a mixture of different macro nutrients, salts and vitamins. This helps in the survival of different organisms during exposure of different compounds. A mixture of well water and reverse osmosis water in the ratio of 1:1.7 liters was used as a blended water. The M4 Medium was prepared in combination of different trace elements, marco nutrients and vitamins. The OECD TG 201 medium and the 20X AAP medium contain nutrients and other useful salts that help the growth and multiplication of aquatic organism[8,9,10,11].

Experimental

Method validation

The method for the determination of oxadiazon residues in ecotoxic samples were validated in terms of method specificity, linearity, assay accuracy, precision, limit of determination and quantification.

RESULTS AND DISCUSSION

Specificity

Specificity was confirmed by injecting the Fish Medium (Blended water), Daphnia (M4 Medium), Algae medium (OECD – TG201 Medium), acetonitrile, HPLC water and standard There were no matrix peaks in the chromatograms to interfere with the analysis of main peak shown in Figure. 1 to 5. Furthermore, the retention time of oxadiazon was relatively constant at 5.7 ± 0.2 min.

HPLC Chromatographic Conditions for the determination oxadiazon

System	Shimadzu – prominence HPLC series equipped with LC – 20AT pump module, CTO – 20 A oven module
Detector	UV – vis detector and interfaced with LC solution software
Column	Phenomenex – C18
Column Specifications	(250 mm length x 4.6 mm I.D., x 5.0 µm particle size)
Mobile phase	A – Acetonitrile (90%) B – pH-2.5 adjusted with dilute H_3Po_4 (10%)
Wave Length (nm)	230
Flow Rate	1 mL / min
Injected Volume	10 µl
Retention time (approximate)	5.7 minutes

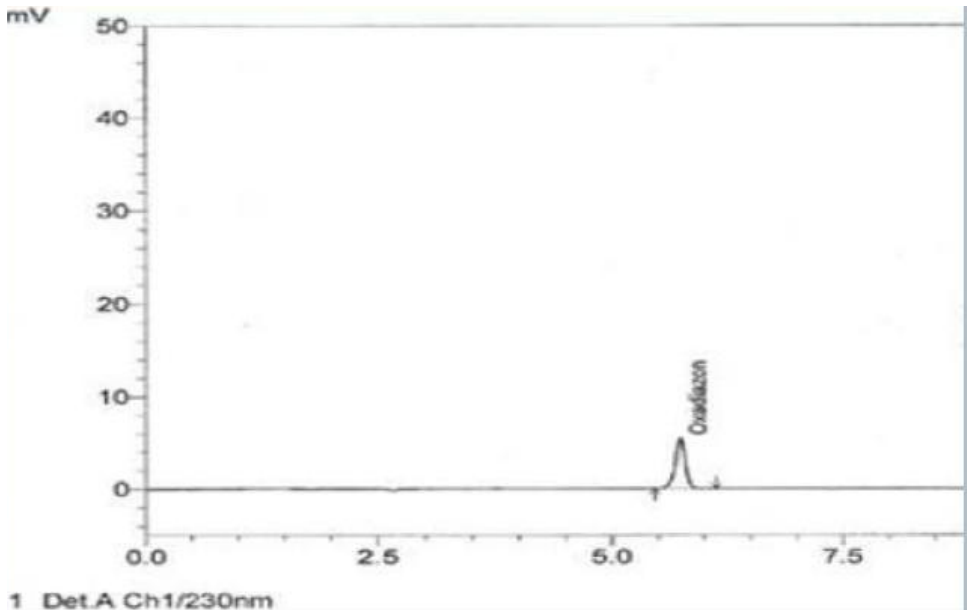

Figure 1 : Calibration solution of oxadiazon standard solution (1 mg/L)

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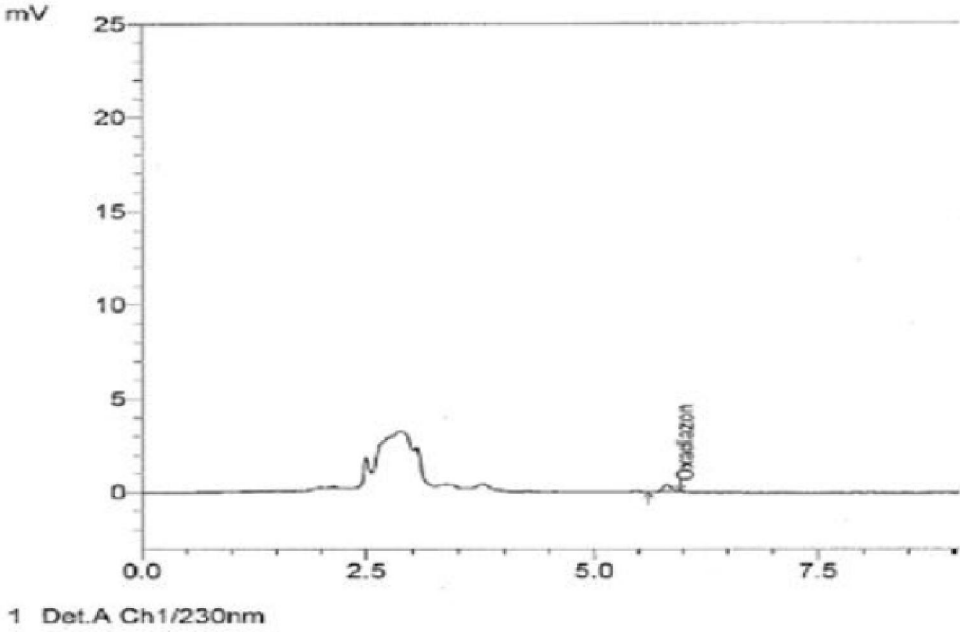

Figure 2 : Representative chromatogram for M4 medium (0.05 mg/L)

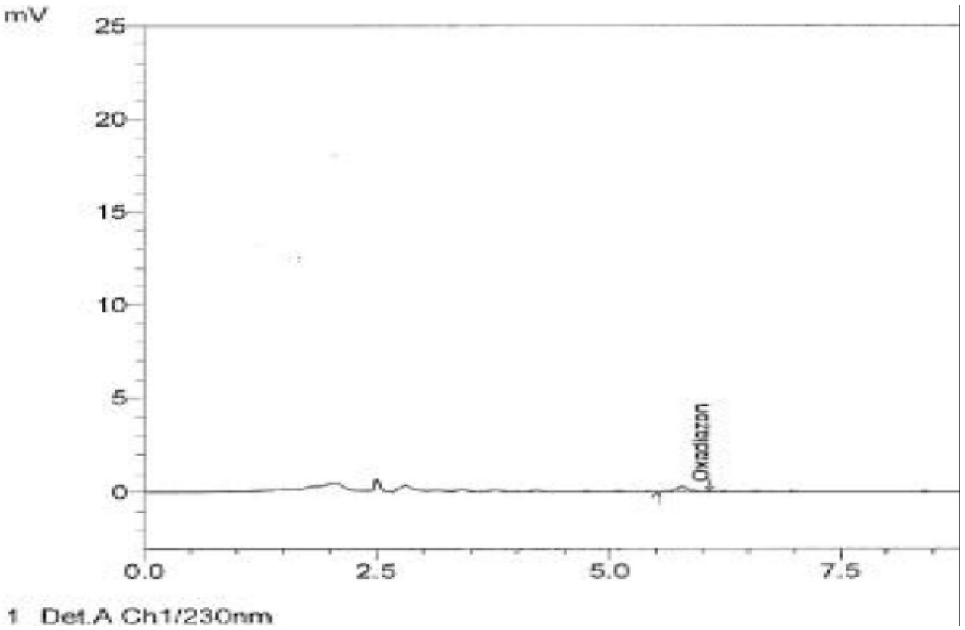

Figure 3 : Representative chromatogram for Blended water medium (0.05 mg/L)

LINEARITY

Preparation of linearity solutions

100.6 mg of oxadiazon reference item was taken into 10 mL volumetric flask and dissolved in acetonitrile, sonicated and made upto the mark with the same solvent. The concentration of the solution was 10050 mg/L. The dilution details are presented in TABLE 1.

Each of these solutions (2000, 1000, 100, 10, 1.0 and 0.05 mg/L) were injected into HPLC under the given conditions and the peak area was recorded and a graph of detector response (peak area) versus concentration in mg/L was plotted. The values for slope (m), intercept (b) and the linear regression coefficient (R2) were calculated.

The detector response to varying concentrations of acetaminophen was found to be linear (R2 = 1.0000) in the range of 0.05 to 2000 mg/L. The plot of concentrations versus detector response along with the regression parameters was attached (Refer

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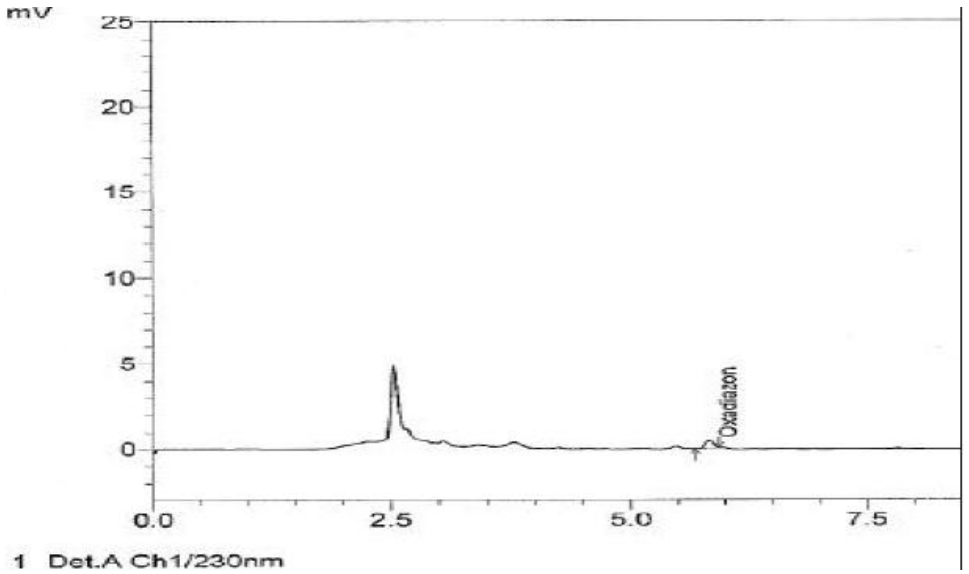

Figure 4 : Representative chromatogram for OECD TG 201 medium (0.05 mg/L)

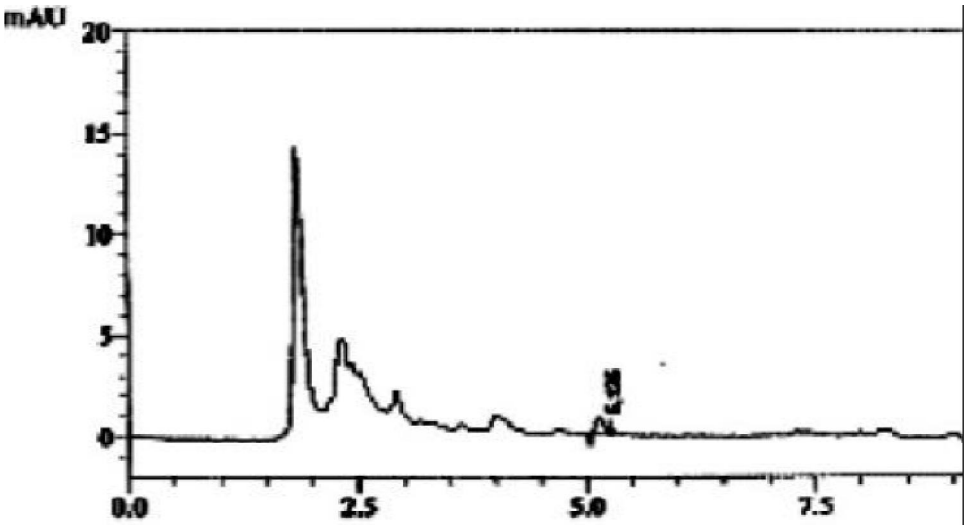

Figure 5 : Representative chromatogram for 20XAAP medium (0.05 mg/L)

TABLE 1 : Preparation of linearity solutions

Stock solution concentration (mg/L)	Volume taken (mL)	Final volume (mL)	Obtained concentration (mg/L)
10050.0	1.99	10	2000
10050.0	0.95	10	1000
10050.0	0.1	10	100
100.0	1.0	10	10
10.0	1.0	10	1
1.0	0.5	10	0.05

Figure 4 and TABLE 2).

Assay accuracy and precision

Recovery studies were carried out at 0.05 and 0.5 mg/L fortification levels for oxadiazon (n=5 for each at two fortification levels) by spiking 10 mL (aquatic mediums meant for fish, daphnia, alga and lemna) samples with the appropriate volumes of standard solutions. After spiking, samples were handled and processed as described.

The recoveries of oxadiazon were > 89%. The method was validated over the fortification level 0.05 – 0.5 mg/L. The repeatability of the method is satisfactory (RSDs <3 %) for five replicate analy-

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TABLE 2 : Linearity details of oxadiazon

Concentration in mg/L	Area in µv*sec
0.05	281
1	2492
10	23792
100	225297
1000	2279125
2000	4552178
Slope	2276.59
Intercept	95.89
CC	1.0000

Detection and Quantification Limits

The limit of quantification was determined to be 0.05 mg/L. This quantification limit was defined as the lowest fortification level evaluated at which acceptable average recoveries were 89-95%, RSD <3%. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at a level approximately 10 times the baseline noise in the chromatogram.

The limit of lowest detection was determined to be 0.01 mg/L at a level of approximately three times

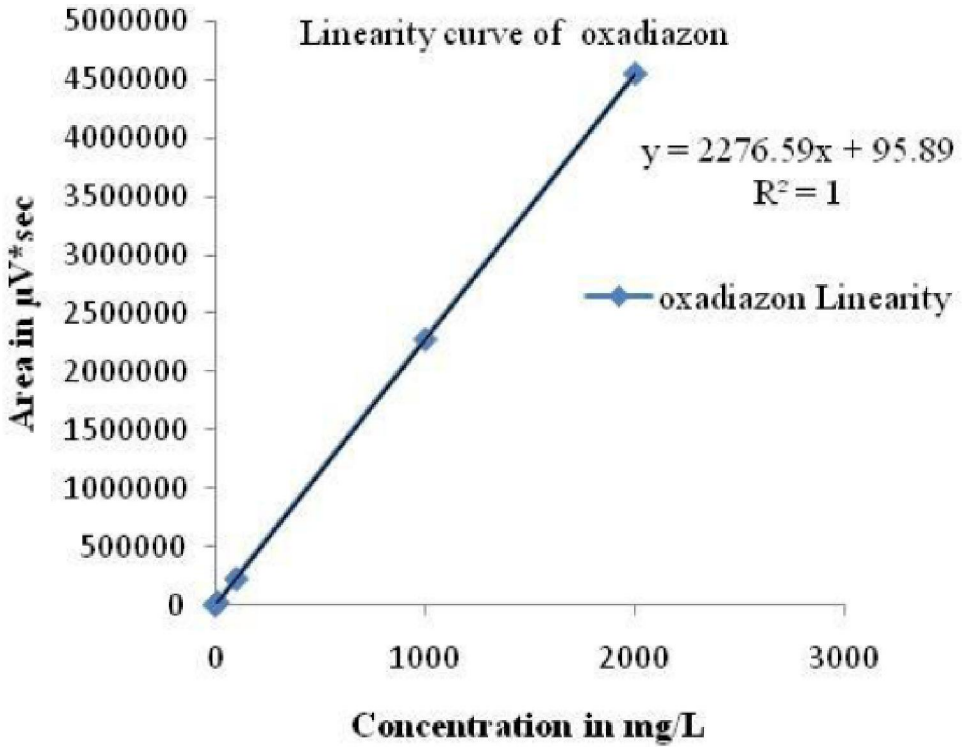

Figure 4 : Linearity curve of oxadiazon reference standard

TABLE 3 : Determination of oxadiazon in different spiked samples

Medium	Fortification level	oxadiazon	
		Mean Recovery (%)*	% RSD
Blended water	0.05	89.25	2.96
	0.5	94.28	1.54
OECD TG 201	0.05	90.52	1.29
	0.5	96.36	2.05
M4	0.05	90.87	1.74
	0.5	95.36	2.48
20XAAP	0.05	92.57	1.52
	0.5	96.14	2.49

* Average of five replicate determinations

sis. The recovery data and relative standard deviation values for various mediums are shown in TABLE 3.

the back ground of control injection around the retention time of the peak of interest. The LOD and LOQ

values were 0.01 mg/L and 0.05 mg/L.

CONCLUSION

This validated analytical method provides fast and accurate results in quantification of oxadiazon residues and has greater advantage over classical methods such as UV-VIS method and GC-FPD and the proposed analytical procedure could satisfactorily be useful for regular monitoring of pesticide residues in different tox medium samples (meant for fish, Daphnia and Algae).

ACKNOWLEDGEMENT

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

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