



## RESEARCH ARTICLE

### LABORATORY PERSISTENCE STUDY OF BISPYRIBAC-SODIUM IN ACIDIC, NEUTRAL AND BASIC WATER

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#### ABSTRACT

Laboratory persistence studies were performed in water at pH 4.0, 7.0, and 9.0 using bispyribac-sodium 40% SC (w/v) formulation at the rates of T0 – Untreated Control, 1.0 (T1) and 2.0 (T2) µg/mL. The different pH Waters samples have been kept under sunlight. The sampling occasions are Acidic water: 0, 5, 10, 20 and 50 days for acidic water (pH 4.0), basic and Neutral water: 0, 5, 10, 20, 50, 70 and 90 days Samples collected on different occasions were analysed for Bispyribac-sodium content by a validated HPLC method. The average percent recovery for Bispyribac-sodium was  $86.33\% \pm 1.74$ ,  $87.50\% \pm 1.20$  and  $89.0\% \pm 1.23$  at 0.05 µg/mL and  $91.33\% \pm 1.33$ ,  $93.0\% \pm 1.67$  and  $94.83\% \pm 1.82$  at 0.5 µg/mL fortification level for acidic, neutral and basic water respectively. The method has a limit of quantification (LOQ) 0.05 µg/mL. The DT50 (Half Life) of bispyribac-sodium calculated by regression analysis from the dissipation data.

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## INTRODUCTION

Bispyribac sodium is used to treat the invasive Eurasian watermilfoil (*Myriophyllum spicatum*) as well as hydrilla (*Hydrilla verticillata*), an invasive submerged plant not currently found in Wisconsin. Sago pondweed (*Stuckenia pectinata*), a desirable native species, may also be affected by treatment (Shimin Wu et al., 2011) Bispyribac sodium is a systemic herbicide that moves throughout the plant tissue and works by interfering with production of a plant enzyme necessary for growth, acetolactate synthase (ALS). Bispyribac-sodium, sodium 2,6-bis [(4, 6- dimethoxy- 2-pyrimidinyl) oxy] benzoate which was first developed by Japan Kumiai Chemical, belongs to the pyrimidinyl oxybenzoic acid group (Tamilselvan et al., 2014) In rice planting, bispyribac- sodium is absorbed through the leaf surface and translocated throughout the plant to inhibit the activity of acetolactate synthase, which results in death of the weeds. The present research was aimed to investigate the fate of residues of Bispyribac sodium in three different aqueous buffer solutions under sun light.

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## MATERIALS AND METHODS

Reference analytical standards of bispyribac sodium (purity 99.8%) were obtained from Sigma Aldrich. The test item bispyribac sodium 40% aqueous suspension concentrates (SC) was purchased from local market. Acetonitrile, Water HPLC grade, formic acid AR grade, Sodium hydroxide LR grade, Potassium chloride GR grade, Boric acid GR grade, Potassium biphthalate GR grade and Potassium phosphate AR grade were obtained from the Merck India limited. Distilled water was purified by using the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA).

### Standard stock solution

Accurately 25.55 mg of bispyribac sodium reference standard, purity (99.8 %) was weighed into 50 mL volumetric flask. The content was dissolved in 10 mL of acetonitrile, sonicated and made up to the mark with the same solvent. The concentration was 510 µg/mL solution and stored in a freezer at -20°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 acetonitrile, immediately prior to sample preparation.

### Sample stock solution

Accurately 124.2 mg of test item (purity 40.26%) of bispyribac sodium was taken into a 50 mL volumetric flask. The content

was dissolved in 10 mL of acetonitrile, sonicated and made up to the mark with the acetonitrile. The concentration was 1000 mg/L solution. The stock sample solution was used for preparation of dose samples (T1 and T2) in different aqua's buffers.

#### Preparation of acidic water (pH 4.0)

About 8g of potassium dihydrogen phosphate was dissolved in distilled water and diluted to 1000 mL. The pH was adjusted to 4.0 with the same.

#### Preparation of neutral water (pH 7.0)

About 6.3 g of disodium hydrogen was phosphate and 5g of potassium dihydrogen phosphate was dissolved in distilled water and diluted to 1000 mL. The pH of the buffer was adjusted with disodium hydrogen ortho phosphate and potassium dihydrogen phosphate.

#### Preparation of basic water (pH 9.0)

12.5g of boric acid & 15g potassium chloride was dissolved in distilled water and diluted to 1000 mL. The pH was adjusted to 9.0 using 0.1 M sodium hydroxide solution.

#### Application data

Name of the buffers	Acidic, Neutral and Basic
Replications	Three
Method of fortification	T <sub>0</sub> ( Untreated Control ) T1 (1 mg/L Level) 1.0 mL of test item stock solution was fortified in to 1000 mL (1Liter) different buffer samples to get the uniform concentrations. T2 (2 mg/L Level) 2.0 mL of test item stock solution was fortified in to 1000 mL (1Liter) different buffer samples to get the uniform concentrations.

#### Sampling data

Samples Exposure	Under direct sunlight
Sample preparation	During each sampling occasion, water samples were mixed thoroughly and sub sampled 20mL using a pipette.
Occasion (Days)	p <sup>H</sup> 4 (0, 5, 10, 20 and 50) and p <sup>H</sup> 7 and 9 (0, 5, 10, 20, 50, 70 and 90)
Laboratory condition	Temperature
	Minimum 20.1°C
	Maximum 25.7°C

#### 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 150 mm x 4.6 mm and particle size 5 µm (Zorbax SB- C18) Column oven temperature was maintained at 30°C. The injected sample volume was 10µL. Mobile Phases A and B was Acetonitrile and 0.1% formic acid (55:45 (v/v)). The flow- rate used was kept at 0.8 mL/min with a detector wavelength at 240 nm. The external standard method of Calibration was used for this analysis.

#### Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and Limits of Detection (LOD) and Quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 µg/mL (Karri, Apparao *et al.*, 2015; Nageswara Rao *et al* 2013; Tentu Nageswara Rao *et al.*, 2015) Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0 and 2.0, 5.0 µg/mL) which were prepared by diluting the stock solution. The Limit of Detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample (Steven J. Lehotay, 2000). The Limit of Quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise<sup>13-15</sup>.

## RESULTS AND DISCUSSION

#### Specificity

Specificity was confirmed by injecting the Mobile phase solvents i.e., Acetonitrile and 0.1% formic acid, HPLC water, sample solution standard solution and buffer controls (acidic, neutral, basic) There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in Figure 1, Figure 2 and Figure 3. Furthermore, the retention time of bispyribac-sodium was constant at 4.6 ± 0.2 min. Different known concentrations of fungicides (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared into a different 10 mL volumetric flasks by diluting the stock solution. The serial dilution details were presented in Table 1. These standard solutions were directly injected into a HPLC. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six standard concentration solutions. The peak areas obtained from different concentrations of standards were used to calculate linear regression equation. This was  $Y=4342.8X + 1.1$  with correlation coefficient of 0.9997 respectively. A calibration curve is showed in (Figure 4).

#### Accuracy and Precision

The analytical method was validated for the recovery of the test item at two concentration levels with acidic, neutral and basic water.

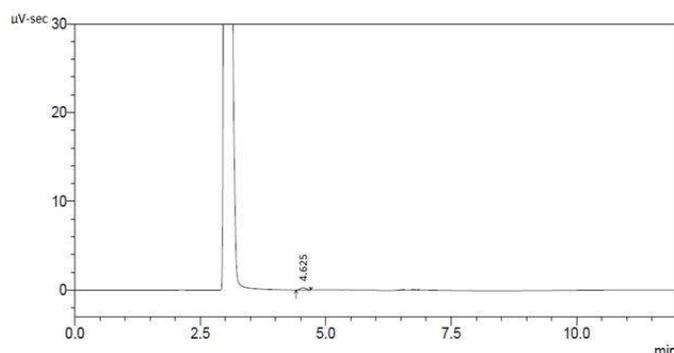


Figure 1. Representative chromatogram of bispyribac-sodium test item in acidic water - 20<sup>th</sup> day

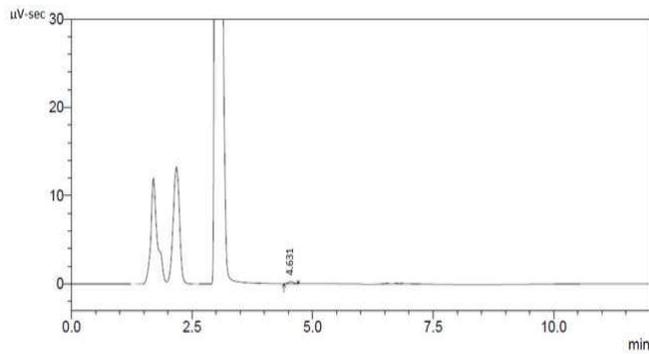


Figure 2. Representative Chromatogram of bispyribac-sodium test item in neutral water - 70<sup>th</sup> day

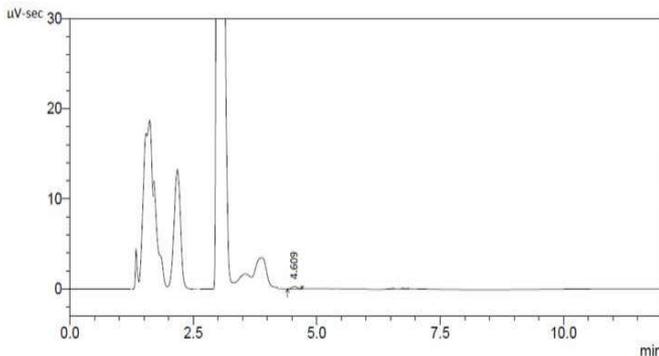


Figure 3. Representative Chromatogram of bispyribac-sodium test item in basic water - 70<sup>th</sup> day Linearity

Table 1. Serial dilutions for linearity standard solutions

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
510	1.960	10	100
100	0.500	10	5
100	0.200	10	2
100	0.100	10	1
5	1.000	10	0.5
5	0.200	10	0.1
1	0.500	10	0.05

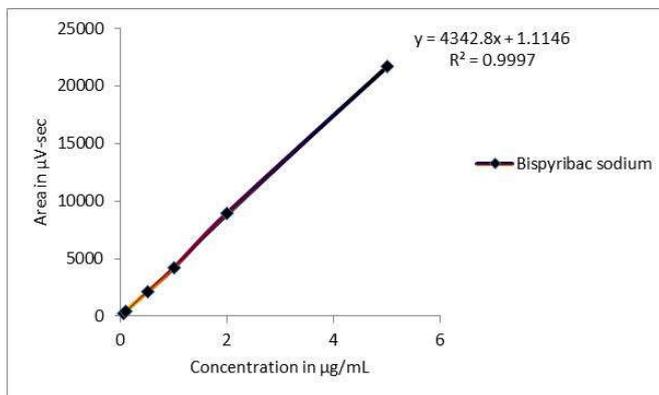


Figure 4. Representative Calibration curve of bispyribac sodium standard

**Preparation of Test item stock solution**

Accurately 6.46 mg of test item (purity 50.12%) of bispyribac-sodium was taken into a 50 mL volumetric flask. The content was dissolved in 5 mL of acetonitrile, sonicated and made up

to the mark with the same solvent. This concentration was 52.0 mg/L solution.

**Fortified test item concentration – 1 mg/L**

0.481 mL of test item stock solution was taken into a 25 mL volumetric flask and made up to the mark with acetonitrile.

**Preparation of 0.05 mg/L Fortification Level**

0.5 mL aliquot of 1.0 mg/L test item solution was fortified into each of the 10mL of buffer solutions (acidic, neutral, basic). This was done in 6 replications.

**Preparation of 0.5 mg/L Fortification Level**

5.0 mL aliquot of 1.0 mg/L test item solution was fortified into each of the 10mL of buffer solutions (acidic, neutral, basic). This was done in 6 replications. The samples were assayed for accuracy and repeatability in HPLC. Accuracy was calculated as %recovery and repeatability as %RSD and the results are mentioned in Table 2.

Table 2. Recoveries of the bispyribac sodium from aqueous buffer solutions samples (n=6)

Fortification Concentration in µg/mL	Replication	Recovery (%)		
		Acidic water	Neutral water	Basic water
0.05	R1	85	88	89
	R2	88	87	90
	R3	87	88	87
	R4	88	86	90
	R5	85	89	89
	R6	85	87	89
	Mean	86.33	87.50	89.00
	STDV	1.51	1.05	1.10
	RSD	1.74	1.20	1.23
	0.5	R1	90	94
R2		93	95	97
R3		92	92	96
R4		90	91	95
R5		91	92	94
R6		92	94	92
Mean		91.33	93.00	94.83
RSD		1.21	1.55	1.72
RSD		1.33	1.67	1.82

**Detection and Quantification Limits**

The limit of quantification was determined to be 0.05 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (86-95%, RSD<2%) were achieved. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.02 µg/mL at a level of approximately three times the background of control injection around the retention time of the peak of interest.

**Dissipation details**

**Acidic water**

The initial concentration of Bispyribac-sodium in acidic water collected on 0 day was 0.992 µg/mL and 1.987 µg/mL in T1 and T2 dosages respectively, which on 5<sup>th</sup> day had dissipated

to 0.874 µg/mL and 1.625 µg/mL. The 10<sup>th</sup> day samples showed the residues 0.712 µg/mL (T1) and 1.408 µg/mL (T2), 20<sup>th</sup> day samples showed 0.425 µg/mL (T1) and 0.856 µg/mL (T2). A complete dissipation of residues of bispyribac-sodium to below detectable level in both the tested dosages was observed on 50<sup>th</sup> day.

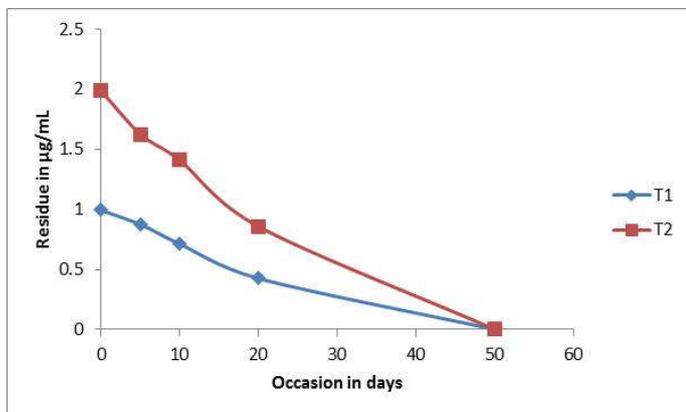


Figure 5. Dissipation curve of bispyribac-sodium in acidic water

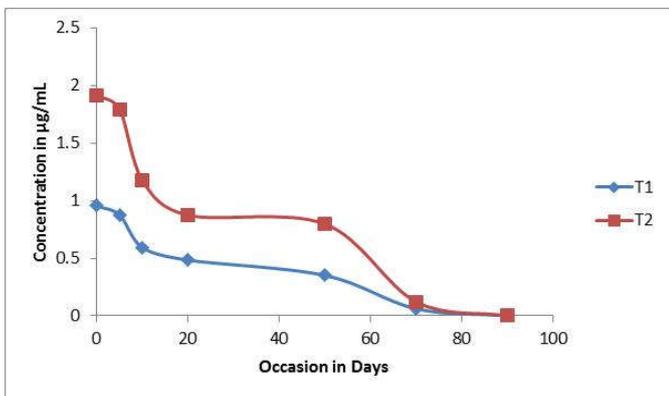


Figure 6. Dissipation curve of bispyribac-sodium in neutral water

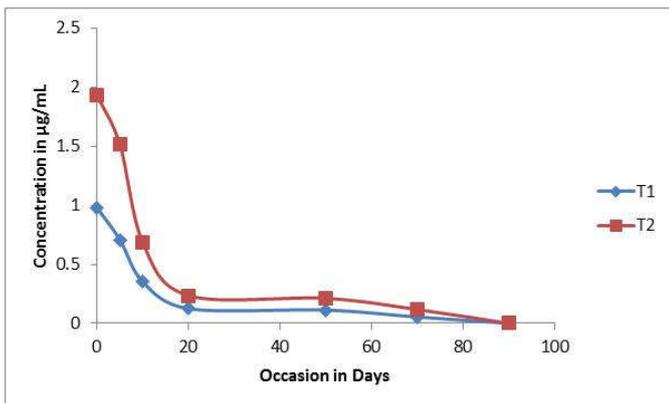


Figure 7. Dissipation curve of bispyribac-sodium in basic water

Table 3. Regression Analysis – Acidic water (pH -4) for of bispyribac-sodium

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.0197*X+0.9663	Y = -0.0383*X+1.8269
Half-life (Days)	16.00	16.57
Correlation co-efficient	0.966	0.968

Table 4. Regression Analysis – neutral water (pH -7) for of bispyribac-sodium

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.0100*X+0.8227	Y = -0.0197*X+1.6448
Half-life (Days)	20.55	20.99
Correlation co-efficient	0.908	0.877

Table 5. Regression Analysis – basic water (pH -9) for of bispyribac-sodium

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.0084*X+0.6254	Y = -0.017*X+1.2633
Half-life (Days)	18.72	18.96
Correlation co-efficient	0.6249	0.6195

Neutral water

The initial concentration of Bispyribac-sodium in neutral water collected on 0 day was 0.995 µg/mL and 1.917 µg/mL in T1 and T2 dosages respectively, which on 5<sup>th</sup> day had dissipated to 0.874 µg/mL and 1.791 µg/mL. The 10<sup>th</sup> day samples showed the residues 0.591 µg/mL (T1) and 1.175 µg/mL (T2). Analysis of water samples collected on 20<sup>th</sup> day showed the residues 0.484 µg/mL and 0.874 µg/mL whereas 50<sup>th</sup> day samples showed 0.351 µg/mL and 0.798 µg/mL in (T1) and (T2) tested dosages respectively. By 70<sup>th</sup> day the residue dissipated to 0.061 µg/mL and 0.121 µg/mL in (T1) and (T2) tested dosages. A complete dissipation of residues of Bispyribac-sodium to below detectable level in both the tested dosages was observed on 90<sup>th</sup> day.

Basic water

The initial concentration of Bispyribac-sodium in basic water collected on 0 day was 0.979 µg/mL and 1.932 µg/mL in T1 and T2 dosages respectively, which on 5<sup>th</sup> day had dissipated to 0.706 µg/mL and 1.512 µg/mL. The 10<sup>th</sup> day samples showed the residues 0.349 µg/mL (T1) and 0.682 µg/mL (T2), whereas 20<sup>th</sup> day samples showed 0.124 µg/mL and 0.235 µg/mL in (T1) and (T2) tested dosages respectively. By 50<sup>th</sup> day the residue dissipated to 0.112 µg/mL and 0.212 µg/mL in (T1) and (T2) tested dosages. Analysis of water samples on 70<sup>th</sup> day showed the residues to 0.054 µg/mL in (T1) and 0.117 µg/mL on (T2) tested dosages. A complete dissipation of residues of bispyribac-sodium to below detectable level in both the tested dosages was observed on 90<sup>th</sup> day.

The dissipation curve plotted between concentration of the analyte and sampling occasions is presented in Figure. 5, Figure. 6 and Figure. 7. DT50 value was calculated using the following formula

$$DT50 = \ln 2 / (k)$$

Where,

‘k’ is slope of the curve obtained from the dissipation data.

The analysis changed into carryout in laboratory circumstance at 25°C and shown the half life values around 16.57 days in acidic water. In neutral water the half-life was around 20.99

days. Further the degradation of herbicide in basic water was rapid; the half-life value was around 18.96 days. The calculated DT 50 (Time required to degrade 50% of residues) values of bispyribac sodium in different pH waters (Acidic (pH -4), Neutral (pH -7) and Basic (pH -9)) under the influence of sunlight presented in Table 3, 4 and 5. The rate constant value was calculated by linear regression equation from the first order rate equation (Prem Dureja *et al.*, 2011; Jyot, Gagan *et al.*, 2010)

$$K = \ln a/a-x/dt$$

Where, dt is the time interval between  $t_1$  and  $t_2$  and a, x are the concentration of pesticides at times  $t_1$  and  $t_2$  respectively. A plot of concentration of the residues and rate with the  $R^2$  indicates first order kinetics in dissipation of the fungicide. The DT50 (Half Life) of bispyribac sodium calculated by regression analysis from the dissipation data.

### Conclusion

The Dissipation kinetics of bispyribac-sodium in different pH waters under direct sunlight become very fast whilst as compared with dissipation facts acquired at 25°C. In basic water degradation changed into faster while compared to acidic and impartial water. Dissipation of bispyribac-sodium in different pH waters followed first order kinetics and this paper describes a fast, simple sensitive analytical method based on HPLC-UV to determine the bispyribac-sodium residues in three different types of buffers. The mobile phase Acetonitrile and 0.1% formic acid confirmed excellent separation and decision and the analysis time required for the chromatographic determination of three different type of buffers is very short (around 12 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and LOQ and DT 50 values were established by following South African National Civic Organization and Environmental Protection Agency (EPA) guidelines (Alberto Navalo N *et al.*, 2002; SANCO Guidelines, 2009) Hence, the proposed analytical procedure and dissipation information would be valuable for regulatory monitoring authority, residue labs and research scholars to determine the bispyribac-sodium residues in different commodities (crop, water and soil samples).

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