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Development and validation of HPLC method for the analysis of bifenox in bulk and commercial dosage forms

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ABSTRACT

A simple, economic, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Bifenox in bulk and commercial formulations was developed and validated in the present study. The mobile phase consists of a mixture of Acetonotrile and water in proportion 50:50 and adjust the pH to pH to 6.0 ± 0.05 with sodium hydroxide solution. This was found to give a sharp peak of Bifenox at a retention time of 3.637min. HPLC analysis of Bifenox was carried out at a wavelength of 300 nm with a flow rate of 1.0 mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 50 µg ml-1 to 150 µg ml-1. The linear regression equation was y = 3416x-124.4. The developed method was employed with a high degree of precision and degradation for the analysis of Bifenox. The developed method was validated for precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Bifenox.

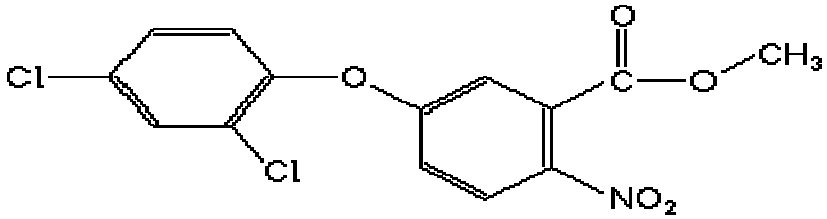
Keywords: Bifenox, HPLC, Development and Validation

INTRODUCTION

A survey of the literature revealed that different analytical techniques for the assay of Bifenox have been reported. [1]Single solid phase extraction method for the simultaneous analysis of polar and non-polar pesticides in urine samples by gas chromatography and ultra high pressure. Determination of multiple pesticides in fruits and vegetables using a modified quick, easy, cheap, effective, rugged and safe method with magnetic nanoparticles [3]spectrophotometric assay for the hepatitis C virus serine protease has been reported (68). ...[4]Electrochemical oxidation of herbicide bifenox acid in aqueous medium using diamond thin film electrode[5]Determination and quantization of sulfonylurea and urea herbicides in water samples using liquid ...Analysis of selected herbicide metabolites in surface and ground water 7. Separation of a mixture of hydrophilic and hydrophobic herbicides by ion-interaction reversed-phase HPLC[6]Spectrophotometricdetection at 228 nm. Simultaneous determination of the herbicides isoproturon, dichlorprop-p, and bifenox in soils using RP- [7]Kinetic determinations and some kinetic aspects of analytical chemistry[8]Voltammetric Determination of Insecticide Thiamethoxam on Silver Solid Amalgam Electrode[9]QSPR modeling of soil sorption coefficients (K oc) of pesticides using SPA-ANN and SPA-MLR[10]Electrochemical degradation of some herbicides in aqueous solution on boron doped diamond anodes[11]Development and validation of a multiresidue method for the analysis of 151 pesticide residues in strawberry by gas chromatography coupled to a triple quadrupole …[12]A new method was developed and validated for the simultaneous determination of 151 pesticide residues in ... The aim of this study was to develop and validate

a MRM in GC-MS ...Pesticide-free samples were used as blanks for validation studies and matrix-matched standard ... [13] ATR FTIR measurement of supersaturation during solution crystallization processes. Calibration and applications on three solute/solvent systems[14]Validation of a multiclass multiresidue method and monitoring results for 210 pesticides in fruits and vegetables by gas chromatography-triple quadruple mass …[15]. To validate the method, studies were carried out of recoveries, linearity, limits of detection [16]validation procedure. The suitability of the method was properly validated prior to its application in real samples in order to ensure that the obtained results were reliable. [17]Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid …[18]Comparison of different solid phase extraction materials and techniques by application of multiresidue methods for the determination of pesticides in water by high- …[19]Validation of multiresidue screening methods for the determination of 186 pesticides in 11 agricultural products using gas chromatography (GC)

Early, analysis of Bifenox in Human plasma by HPLC with fluorescence detection , HPLC determination of Bifenoxpolyglutamates after Low-Dose Bifenox therapy in patients with Rheumatoid arthritis ,Quality control of Bifenox by HPLC and Polarographic and voltammetric methods for the quantitation of Bifenox in pharmaceuticals and plasma samples have been published. There is however no reported HPLC method for the analysis of Bifenox in its technical grade and formulations. This is describes a validated HPLC method for the quantitative determination of Bifenox.



Mol. Formula $C_{14}H_9Cl_2NO_5$ Mol.Wt : 342.13 grams

Bifenox having the IUPAC name is methyl 5-(2, 4-dichlorophenoxy)-2-nitrobenzoate.It is a Nitrophenyl ether herbicide. Bifenox is a protoporphyrinogen, Oxidase inhibitor and acts on the young parts, leaves and partially on the roots of weeds and grass. This leads to a release of peroxides that destroy the cell membranes of the weed plants and lead to tissue death. Bifenox also inhibits photosynthesis. The effect is enhanced by High light intensity and metabolic activity.

The HPLC method described here is simple, sensitive, and reproducible for Ametridione determination in Formulations with low background interference. An attempt has been made to develop and validate to ensure their , precision and other analytical method validation parameters as mentioned in various gradients. One method reported for the HPLC determination for developed based on the use of a C-18column, with a suitable mobile phase, without the use of any internal standard. For formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in formulations.

MATERIALS AND METHODS

1. **Experimental instrumentation**

HPLC Analytical column ChromolithRP - C18, 25mm x 4.6mm x 5µ

Stationary phase	Mobile phase	Flow rate (ml min^{-1})	Run time (min)	Column Temp (0^c)	Volume of injection loop (µl)	Detection wavelength (nm)	Retention time (min)
RP - C18, 25mm x 4.6mm x 5µ	Acetonotrile and water 50: 50	1..0	10	25	20	300	3.637

2. Analytical methodology

1. Preparation of Mobile phase

For isocratic system, prepare a mixture of Acetonotrile and water in the proportion 50:50 respectively. Mix well, adjust the pH to 6.0 ± 0.05 with sodium hydroxide pellets. Filter through 0.2 µ Nylon membrane filter paper and degas prior to use.

2. Chromatographic conditions

Separation was performed on C -18, 25 mm x 4.6 mm x 5µ Column. Dimethyle Sulfoxide used as a Diluent and Mobile phase consists of mixture of Acetonitrile and water in the proportion 50:50. Injection volume of 20 µl was used. Mobile phase was filtered before use through 0.5 µm Nylon membrane filter paper and degassed with helium purge for 10 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.0 ml min^{-1} and wavelength was set to 300 nm. The column temperature was set at 25^{o}C.

3. Preparation of Bifenox Standard Solution:

Weigh accurately about 25 mg of Bifenox working Standard and transfer to a 25 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.

(Dilution scheme: 25mg → 25.0 ml → 1 ml /10.0 ml)

4. Preparation of Test Solution:

Weigh accurately about 50 mg of sample and transfer to a 25 ml volumetric flask. Add 10 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix.(Dilution scheme: 50mg → 25.0 ml → 1 ml /10.0 ml)

5. System Suitability Solution:

Use Bifenox Standard working solution as system suitability solution.

6. Procedure:

Separately inject equal volumes of blank, five replicate injections of system suitability solution (Bifenox Standard working solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Bifenox Standard working solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability solution (Bifenox Standard working solution).

7. Degradation studies:

The forced degradation studies are performed to establish the stability indicating nature of the assay method and to observe any degraded compounds. BFN and sample Bifenox SG 700g/l) are subjected to stress with 5NHCl , 5N NaOH , thermal degradation and UV degradation . All the above solutions are chromatographed and recorded the chromatograms. The following stress conditions are followed for degradation.

Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60°C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60°C for 10 min on a water bath.
Thermal degradation	105ºC for 12 hours
UV degradation	expose to UV-radiation for 7 days

RESULTS AND DISCUSSION

The appropriate wavelength in UV region has been selected for the measurement of active ingredient in the proposed method. This method was validated by linear fit curve and all the other parameters were calculated.

1. Parameters fixation:

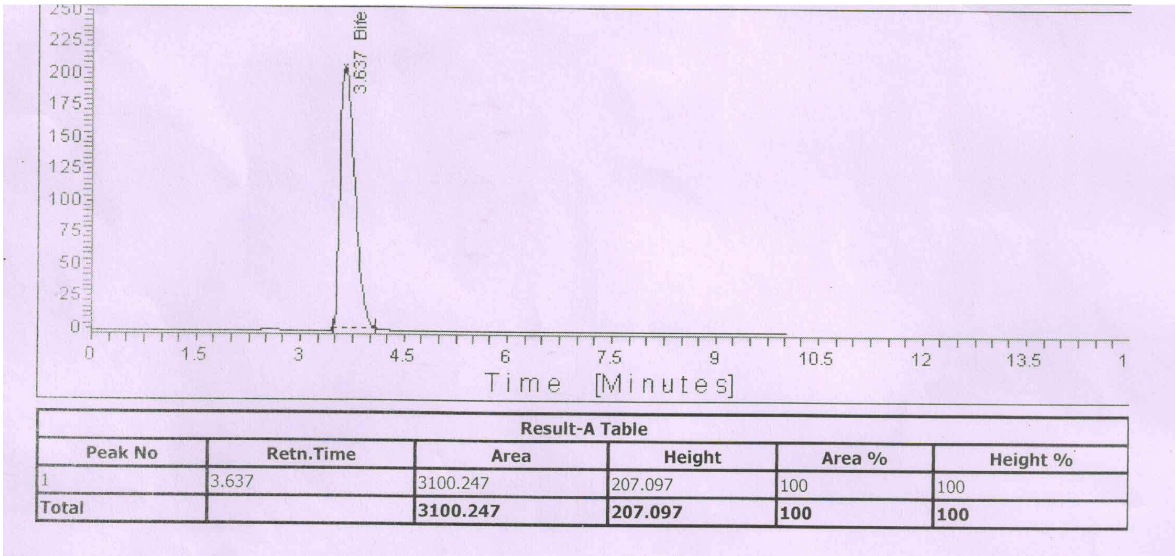
In developing methods, systematic study of the effects of various parameters was undertaken by varying one parameter at a time controlling all other parameters. The following studies were conducted for this purpose.

a. **Mobile phase characteristics**

In order to get sharp peaks and baseline separation of the components, carried out number of experiments by varying different components like percentage of organic phase in the mobile phase, total p^H of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were included in the procedure proposed.

2. **Detection Characteristics**

To test whether Bifenox had been linearly eluted from the column, different amounts of Bifenox were taken and analyzed by the above mentioned procedures. The peak area ratios of component areas were calculated and the values are graphically represented in Fig, the linear fit of the system was illustrated graphically. Least square regression analysis for the method was carried out for the slope, Intercepts and correlation coefficient. The results are presented in Table -



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %
1	3.637	3100.247	207.097	100	100
Total		**3100.247**	**207.097**	**100**	**100**

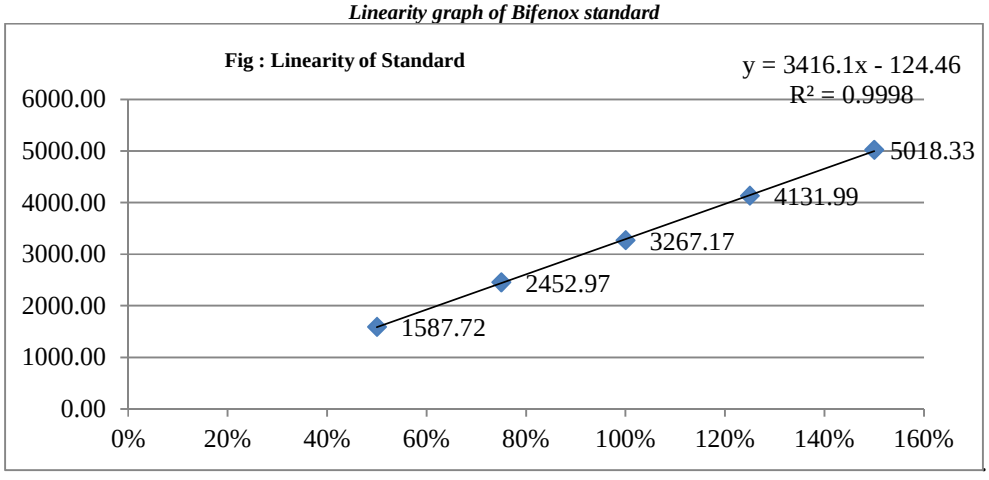
3. **Performance Calculations**

To ascertain the system suitability for the proposed method, a number of statistical values have been calculated with the observed readings and the results are recorded in Table.

Method validations

The UV absorption maximum for Bifenox was fixed at 310 nm respectively. As the final detection was made by the UV - absorption spectrum, each method was validated by linear fit curve.

Linearity graph of Bifenox standard



LinearityTable1: System suitability - Linearity of standard

Sr. No.	Area of Bifenox
1	3130.17
2	3140.23
3	3135.18
4	3113.54
5	3100.25
Mean	**3123.87**
Standard Deviation (±)	**16.58**
(%) Relative Standard Deviation	**0.53**

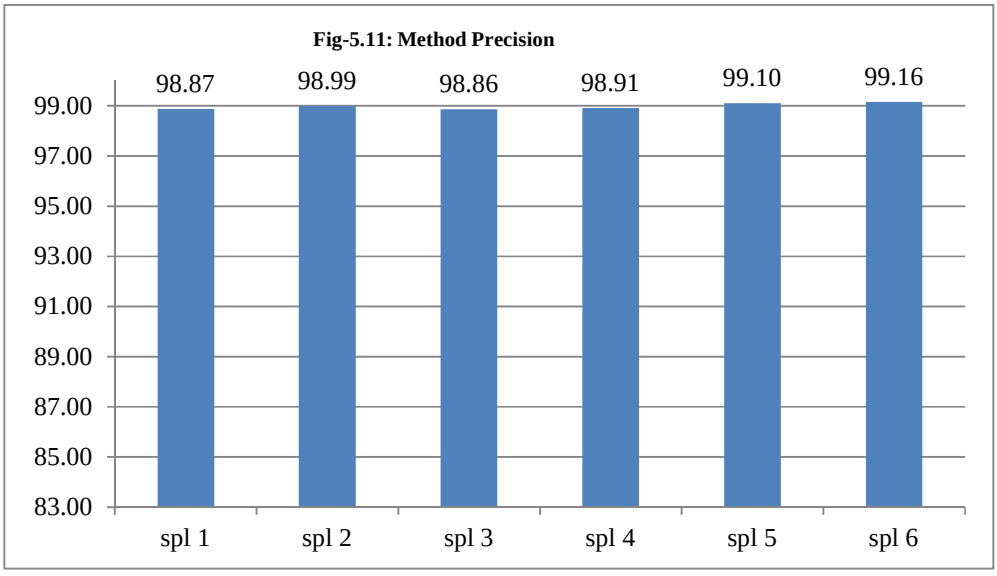
Table2: Results of linearity of standard

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1587.72	**0.999**
Level – 2	75	75	2452.97	
Level – 3	100	100	3267.17	
Level – 4	125	125	4131.99	
Level – 5	150	150	5018.33	

4. **Precision**

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of sample. The percent of Relative Standard deviation calculated for Bifenox and are presented in Tables. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of sample solution was calculated in terms of coefficient of variation (CV)

Fig-5.11: Method Precision



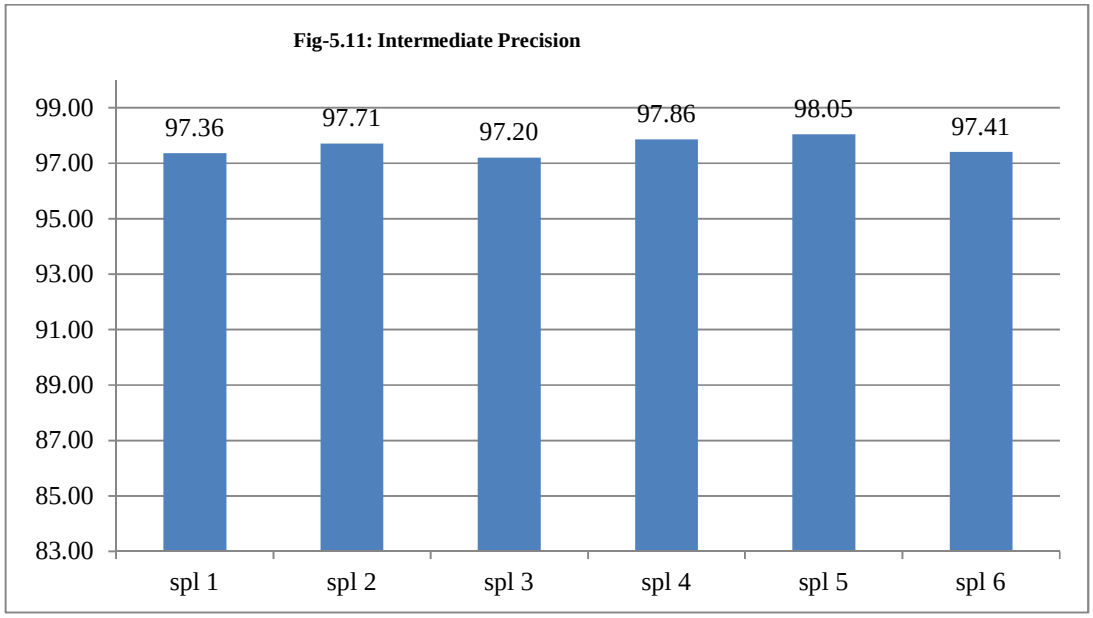


Table3: Performance calculations, detection characteristics precision and accuracy of the proposed method for Metamitron

Parameter	HPLC Method
Wavelength (nm)	300
Retention time (t) min	3.367
Linearity range (µg ml^{-1})	50-150
LOD(µg ml^{-1})	0.00167
LOQ(µg ml^{-1})	0.005585
Regression equation (y=bc+a)	
Slope (b)	3416
Intercept (a)	124.4
Standard deviation (SD)	1.908
Correlation coefficient(r^2)	0.9996
Relative Standard deviation (%RSD)*	0.0579
Intermediate Precision (%RSD)	0.56
Range of errors	
Confidence limits with 0.05 level	3.739
Confidence limits with 0.01 level	4.914

**RSD of five independent determinations*

Table -4: System precision

Sr. No.	Area of Bifenox
1	3103.88
2	3133.72
3	3109.51
4	3112.21
5	3149.09
6	3128.57
7	3105.47
8	3137.34
9	3147.36
10	3141.08
Mean	**3126.82**
Standard Deviation (±)	**17.57**
(%) Relative Standard Deviation	**0.56**

Method Precision:

Table -5: System suitability - Method precision

Analyst – 1 **HPLC No.: EH/R&D/HPLC-024**

Sr. No.	Area of Bifenox
1	3235.97
2	3254.34
3	3278.01
4	3224.18
5	3256.48
Mean	**3249.79**
Standard Deviation (±)	**20.67**
(%) Relative Standard Deviation	**0.64**

Table -6: Results of Method precision

Test Solution	% Assay of Bifenox
1	98.87
2	98.99
3	98.86
4	98.91
5	99.10
6	99.16
Mean	**98.93**
Standard Deviation (±)	**0.13**
(%) Relative Standard Deviation	**0.14**

Intermediate Precision:

Table -7. System suitability - Intermediate precision

Analyst – 2 **HPLC No.: EH/R&D/HPLC-023**

Sr. No.	Area of Bifenox
1	3188.39
2	3195.47
3	3157.09
4	3159.58
5	3154.75
Mean	**3171.06**
Standard Deviation (±)	**19.29**
(%) Relative Standard Deviation	**0.61**

Table – 8 Results of Intermediate precision

Test Solution	% Assay of Bifenox
1	97.36
2	97.71
3	97.20
4	97.86
5	98.05
6	97.41
Mean	**97.60**
Standard Deviation (±)	**0.33**
(%) Relative Standard Deviation	**0.34**

Table – 9: Results of twelve test solutions of Bifenox in FOX 480 g/l (six of Method precision & six of intermediate precision)

Analysis performed during Method precision study	
Same column	**% Assay of Bifenox**
1	98.87
2	98.99
3	98.86
4	98.91
5	99.10
6	99.16
Analysis performed during intermediate precision study	
Column sr. no.	**015337030136 01**
Test Solution	**% Assay of Bifenox**
7	97.36
8	97.71
9	97.20
10	97.86
11	98.05
12	97.41
Mean of twelve samples	**98.29**
Standard Deviation (±)	**0.76**
(%) Relative Standard Deviation	**0.77**

5. Interference Studies

The effect of wide range of excipients and other additives usually present in the formulations of Bifenox in the determinations under optimum conditions were investigated. The common excipients such as colloidal Silicon dioxide, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium state, microcrystalline cellulose provide have been added to the sample solutions and injected. They have not disturbed the elution or quantification of pesticide. In fact many have no absorption at this λ_{max}

6. Analysis of Formulation

To find out the stability of the proposed methods for the assay of formulations containing Bifenox was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision from reference method. The results are recorded in Table.

5. **Forced degradation**:

There is no interference between the peaks obtained for the chromatograms of degradation preparations. The degradation peaks under forced degradation are well separated from each other. The peak purity for Bifenoxpeak is passing.

Hence, the method is very precise, selective and specific to the estimation of Assay of Bifenoxin in Bifenox SG 700 g/l by HPLC and the same method is stability indicating, as the degraded products are well separated from Bifenoxand as well from each adjacent peaks.

Table -10: System suitability - Selectivity

Sr.No.	**Area of Bifenox**
1	3336.23
2	3354.57
3	3357.19
4	3344.76
5	3359.66
Mean	**3350.48**
Standard Deviation (±)	**9.77**
(%) Relative Standard Deviation	**0.29**

Table –11. System suitability – Forced Degradation

Sr. No.	Area of Bifenox
1	3242.14
2	3238.98
3	3229.80
4	3254.33
5	3218.67
Mean	**3236.78**
Standard Deviation (±)	**13.40**
(%) Relative Standard Deviation	**0.41**

Table -12: Conditions – Forced Degradation

Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60°C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60°C for 10 min on a water bath.
Thermal degradation	105ºC for 12 hours
UV degradation	expose to UV-radiation for 7 days

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Acid Stress	**% Degradation**
Standard	0.195
Sample	0.107
Alkali Stress	**% Degradation**
Standard	0.664
Sample	0.376
Thermal Stress	**% Degradation**
Standard	0.008
Sample	0.107
UV Stress	**% Degradation**
Standard	0.674
Sample	0.115

6. Ruggedness and Robustness

Ruggedness of the proposed method was determined by carrying out the analysis by two different analysts using similar operational i.e. Robustness with Change in Column Lot, Change in Flow rate, Change in wavelength and Change in p^H of the Mobile phase . The results were indicated by % CV in Tables. Robustness of the method was determined by carrying out the analysis at two different wavelengths i.e. at 308 nm and 312 nm and the results were indicated by % CV in Table.

a. Change in Column Lot:

[Normal Experimental Condition: RP - C18, 25mm x 4.6mm x 5µ**)**

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical Method. (Refer to Table - 13 for system suitability results).

Table -13: System suitability - Robustness with Change in Column Lot

Sr. No.	Area of Bifenox	
	Same column	**Diff column**
1	3267.17	3145.30
2	3249.79	3148.31
Mean	**3258.48**	**3146.81**
Standard Deviation (±)	**12.29**	**2.13**
(%) Relative Standard Deviation	**0.38**	**0.07**

Table – 14: Results for Change in Column Lot

Flow rate →	Same column	Diff column
Sample	**% Assay**	
Test solution	98.63	98.35
Average assay result from Method precision	98.93	98.93
Mean	**98.78**	**98.64**
Standard Deviation (±)	**0.21**	**0.41**
(%) Relative Standard Deviation	**0.21**	**0.42**

b. Change in Flow Rate (± 0.2 mL/minute):
(Normal Experimental Condition: 1.0ml/minute)

Table – 15: System suitability - Robustness with change in flow rate

Sr. No.	Area of Bifenox	
	0.8mL/minute	1.2 mL/minute
1	3160.42	3179.65
2	3173.45	3183.76
Mean	**3166.94**	**3181.71**
Standard Deviation (±)	**9.21**	**2.91**
(%) Relative Standard Deviation	**0.29**	**0.09**

Table – 16: Results for change in flow rate

Flow rate →	0.8mL/minute	1.2 mL/minute
Sample	**% Assay**	
Test solution	98.82	98.71
Average assay result from Method precision	98.93	98.93
Mean	**98.88**	**98.82**
Standard Deviation (±)	**0.08**	**0.16**
(%) Relative Standard Deviation	**0.08**	**0.16**

c. Change in Wavelength (± 2 nm):
(Normal Experimental Condition: 300nm)

Table – 17: System suitability - Robustness with change in wavelength

Sr. No.	Area of Bifenox	
	298 nm	302 nm
1	3238.98	3230.96
2	3235.97	3227.91
Mean	**3237.47**	**3229.43**
Standard Deviation (±)	**2.13**	**2.15**
(%) Relative Standard Deviation	**0.07**	**0.07**

Table – 18: Results for change in wavelength

Wavelength →	298 nm	302 nm
Sample	**% Assay**	
Test solution	98.97	98.85
Average assay result from Method precision	98.93	98.93
Mean	**98.95**	**98.89**
Standard Deviation (±)	**0.03**	**0.06**
(%) Relative Standard Deviation	**0.03**	**0.06**

d. Change in composition of Mobile Phase (± 50ml):
(Normal Experimental Condition: Acetonitrile: water = 500ml:500Table -5.39: System suitability - Robustness with change in composition of mobile phase

Sr. No.	Area of Bifenox	
	450ml:550ml	550ml:450ml
1	3129.70	3113.54
2	3133.72	3102.37
Mean	**3131.71**	**3107.96**
Standard Deviation (±)	**2.84**	**7.90**

Table – 19: Results for change in composition of mobile phase

Composition of Methanol & water	450:550	550:450
Sample	**% Assay**	
Test solution	98.2	98.82
Average assay result from Method precision	98.93	98.93
Mean	**98.57**	**98.88**
Standard Deviation (±)	**0.52**	**0.08**
(%) Relative Standard Deviation	**0.52**	**0.08**

7. Solution Stability
The stability of the solutions under study was established by keeping the solution at room temperature for 48 Hours. The results indicate no significant change in assay values indicating stability of Pesticide in the solvent used during analysis. The results are recorded in Table.

Table-20: The assay results obtained during solution stability

TIME	Std Area	Avg std area	Spl area	Avg Spl area
0^{th} hr	3226.858	3232.251	3250.045	3249.97
	3237.644		3249.895	
12^{th} hr	3213.234	3209.906	3215.299	3238.226
	3206.577		3261.152	
24 hr	3255.473	3244.042	3247.792	3239.374
	3232.61		3230.955	
36 hr	3235.327	3236.95	3201.502	3215.47
	3238.573		3229.438	
48 hr	3224..013	3271.589	3246.421	3232.25
	3271.589		3218.078	
Mean	**3235.32**	**3238.95**	**3235.06**	**3235.06**
Standard Deviation (±)	**19.78**	**22.27**	**19.06**	**12.67**
(%) Relative Standard Deviation	**0.61**	**0.69**	**0.59**	**0.39**

Table -21: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Bifenox**
0^{th}hr	98.65
12^{th}hr	98.79
24 hr	98.15
36 hr	98.02
48 hr	98.05
Mean	98.33
Standard Deviation (±)	0.36
(%) Relative Standard Deviation	0.37

CONCLUSION

The method was found to be accurate and precise, as indicated by recovery studies close to 100 and % RSD is not more than 2. The summery of validation parameters of proposed HPLC method is given.

The simple, accurate and precise HPLC method for the determination of Bifenox as bulk and form has been developed. The method may be recommended for routine and quality control and analysis of the investigated

pesticide in pure and its formulations and environmental samples. The analytical solution is found to be stable up to 48 Hrs at room temperature. Hence, it is concluded that the analytical method is validated and can be used for routine analysis and for stability study.

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