IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY
SPECTROPHOTOMETRIC DETERMINATION OF BIFENOX AND IN ITS COMMERCIAL FORMULATIONS

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ABSTRACT
A simple, precise, rapid, sensitive and accurate spectrophotometric methods have been developed for the estimation of Bifenox in pure form and its formulations and Spiked vegetables and water samples. This method is based on oxidative coupling of Bifenox with MBTH in the presence of ferric chloride to form green coloured product with maximum of 615 nm. The product obeyed Beer's law in the concentration range   0.5-3 ml (5-30 µgml^{-1}) with molar absorptivity of 1.001×10^4. Sandells sensitivity 0.004945. The assay of results was found to be good agreement with label claim.

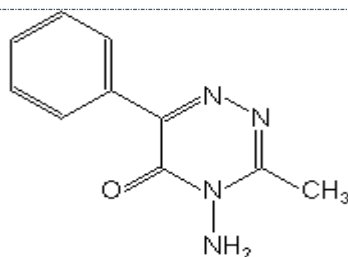**KEYWORDS**: Bifenox, MBTH, UV/Vis., Spectrophotometry, validation.

INTRODUCTION
A survey of the literature revealed that different analytical techniques for the assay of MTM have been reported. Volta metric detection of the herbicide Bifenox at a bismuth film electrode in non-de aerated solution[1]. Electro analysis of Bifenox and metribuzen on lignin by adsorption[2] ,Electrochemical reduction of Bifenox[3], Identification of different products obtained by electrochemical and photochemical reduction of the Bifenox[4] . Volta metric determination of Bifenox with an electro generated molecularity imprinted polymer microsencer[5]. Electrochemical determination of the effect of lead (II) on the photochemical degradation of the pesticide Bifenox[6] .Volta metric determination of herbicide Bifenox using Mercury and silver solid amalgam electrode[7] Preconcentration and volta metric determination of the herbicide Bifenox with a silica modified carbon paste electrode[8]. Determination and method validation of Bifenox in soil by RP-HPLC[9] Electrochemical determination of the effect of Copper (II) on the photochemical degradation of the pesticide Bifenox[10] .

There is however no reported UV-Visible spectrophotometric method for the analysis of Mitamitron in its technical grade and formulations. This describes a validated ible spectrophotometric method for the quantitative determination of Mitamitron. Functional group used for color development of Mitamitron was primary amine group. The results obtained in this method was based on complex formation reaction of Mitamitron with oxidative coupling reaction with MBTH / ferric chloride.

The author has developed ible spectrophotometric method based on the use of method, without use of any interferences. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in the various guidelines.

This is describes a validated UV/Vis method for the quantitative determination of Bifenox. The empirical formula for Bifenox is $C_{10}H_{10}N_4O$ and the molecular weight is 342.128 g/mol . Bifenox has the following structure.

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ISSN: 2277-9655

[Sekhar* *et al.,* 5.(4): April, 2016]
IC™ Value: 3.00

Impact Factor: 4.116
CODEN: IJESS7

BIFENOX STRUCTURE

EXPERIMENTAL
Solvent
Methanol was used as a solvent in the present experiments.
1.   Preparation of standard stock solution
Accurately weighed 100 mg of Bifenox was dissolved in 40 ml of methanol in 100 ml volumetric flask and volume was made up to the mark. i.e. 1000 µg ml^{-1} (stock solution A). From the above stock solution A 10 ml of solution was pipette out into 100 ml volumetric flask and the volume was made up to the mark with methanol obtain the final concentration of 100 µg ml^{-1} (stock solution B).

2.   Preparation of calibration curve
Fresh aliquots of Bifenox ranging from 0.5 to 3 ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 5 to 30 µg ml^{-1}. To each flask 1ml of (0.2%) MBTH solution was added followed by 1ml of (0.7%) ferric chloride solution and resulting solution was heated for 15 min and finally 1ml (0.5N) HCl solution was added. The solutions were cooled at room temperature and made up to mark with methanol. The absorbance of green colored chromogen was measured at   615 nm against the reagent blank. The color species was stable for 24 hrs. The amount of Bifenox present in the sample solution was computed from its calibration curve.

3.   Procedure for formulations
Twenty tablets containing Bifenox were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Bifenox was dissolved in a 100 ml of methanol and mixed for about 5 min and then filtered. The methanol was evaporated to dryness. The remaining portion of solution was diluted in a 100 ml volumetric flask to the volume with methanol up to 100 ml to get the stock solution A. 10 ml of aliquots was pipette out into 100 ml volumetric flask and the volume was made up to the mark with methanol to obtained the final concentration of   100 µg ml^{-1} (stock solution).

Subsequent dilutions of this solution were made with methanol to get concentration of   5 to 30 µg ml^{-1} and were prepared as above and analyzed at the selected wavelength, 615 nm and the results were statistically validated.

4.   Recovery of Bifenox from spiked vegetables
100 gm of each vegetable (potatoes and tomatoes) were spiked with 200 ml chloroform for 5 min. The samples were fortified with different concentration of Bifenox in Methanol and blended for 3 min. Chloroform was filtered into 250ml Standard flask through whatmanNo.42 filter paper and the residue was retained. The residue was washed twice with 10 ml of chloroform and blended for 2 minutes. Chloroform extracts were combined and made up to the mark. Known aliquots of the chloroform extracts were used for colour development after evaporating chloroform on steam bath. The residue was dissolved in methanol and the amount was determined spectrophotometrically and the results were presented in tabulated in table 1.7.

5.   Recovery of Bifenox from fortified water samples
After collection of the water samples (tap and distilled water minimum volume one litre) the pH of the water samples were adjusted below 4 with 20% sulphuric acid, then fortified with different concentrations of Bifenox dissolved in methanol. Extract each sample in a 250 ml separating funnel with 100 ml chloroform. The chloroform extract was transferred into a funnel and re extracted the aqueous phase twice with further 50 ml of chloroform. The second chloroform extracts was added to the first and washed the combined extract with 0.1 M K_2CO_3 then dried the chloroform by passing it through anhydrous sodium sulfate in a filter funnel and collected the extracts in a 250 ml flask. The chloroform extracts was reduced to 100 ml amount was determined spectrophotometrically. The results obtained were represented in table 1.8.
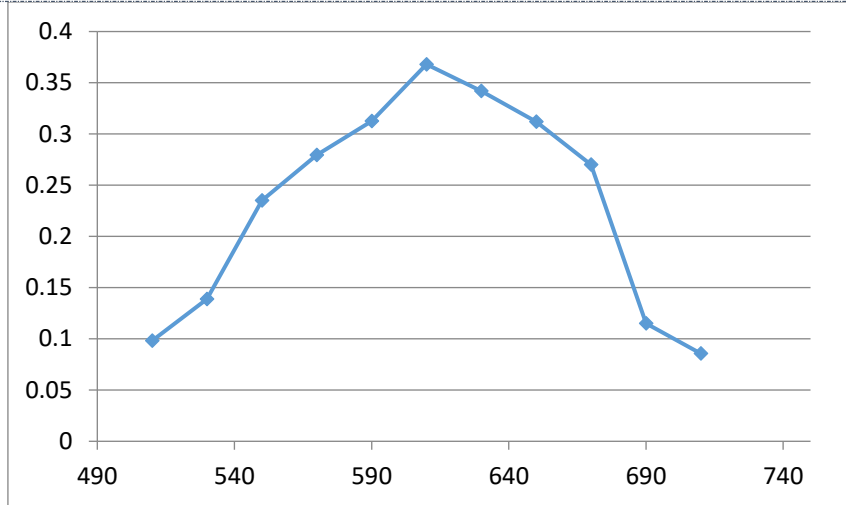
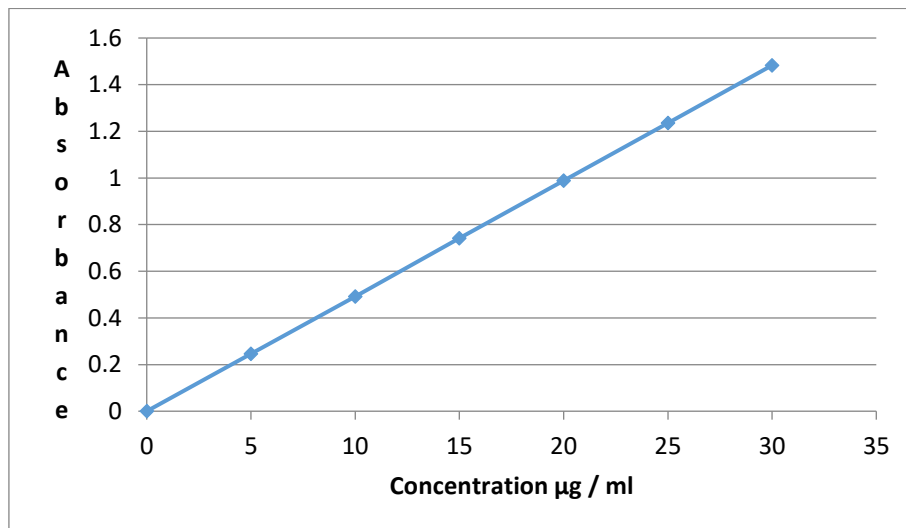*Fig-1.1: Absorption spectrum of Bifenox with MBTH /FeCl₃*

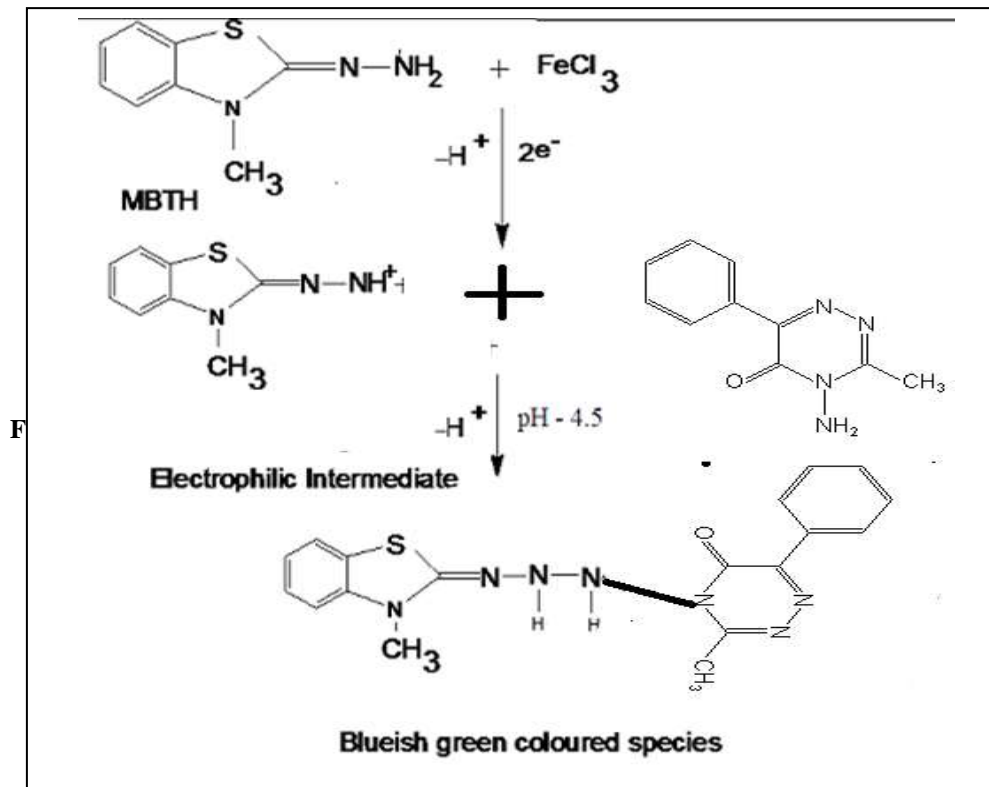*Fig-1.2: Beer's law plot of Bifenox with MBTH/FeCl₃*

Table-1.1: Optical characteristics and precision by MBTH

Parameter	Visible method
Color	Bluish green
Absorption maxima (nm)	615
Beer's law limits ($\mu g\ ml^{-1}$)	5-30
Molar absorptivity ($l\ mol^{-1}cm^{-1}$)	$0.9917*10^4$
Sandal's Sensitivity ($\mu g\ cm^{-2}$)	0.0027
Regression equation (Y*)	----
Slope (b)	0.0421
Intercept(a)	0.000204
Standard deviation(SD)	0.9997
Correlation coefficient (r^2)	0.9999

%RSD (Relative Standard deviation)*	1.7234
Range of errors	-----
Confidence limits with 0.05 level	1.6780
Confidence limits with 0.01 level	2.222
Limits of detection (LOD)(µg ml^{-1})	0.07204
Limits of quantification (LOQ) (µg ml^{-1})	0.02401

*RSD of six independent determinations

Table-1.2: Assay results of Bifenox in formulations by visible method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method (mg)	% Recovery
Sample -1	250	249.00	232.5	92.90
Sample -2	250	249.37	234.5	93.65

*t and F- values refer to comparison of the proposed method with reference method.
*Theoretical values at 95% confidence limits t= 0.00152 and F= 2.1985.

Table-1.3: Determination of accuracy of Bifenox

Amount of MET in formulation (mg)	Amount of Standard MET added (mg)	Total amount found (mg)	% Recovery
249.00	200	448.20	99.60
249.03	200	448.25	99.61
249.31	200	448.75	99.72
249.00	250	498.00	99.60
248.75	250	497.5	99.50
248.5	250	497.0	99.4
249.00	300	547.80	99.6
249.16	300	548.15	99.66
249.58	300	549.07	99.83

Table-1.4: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
249.11	0.1986	0.07958
248.75	0.24	0.1001
249.24	0.226	0.09136

The results are the mean of five readings at each level of recovery.

Table-1.5: Repeatability data for MET at 615 nm

Conc. ($\mu g\ ml^{-1}$)	Abs 1	Abs 2	Abs 3	Mean	Std. deviation	(%)RSD*
0.2	0.0141	0.0142	0.0141	0.0142	0.0001	0.7042
0.8	0.0287	0.0286	0.0285	0.0286	0.0001	0.3496
1.2	0.0413	0.0431	0.04031	0.0431	0.0001	0.2320
1.6	0.0573	0.0572	0.0572	0.0572	0.0001	0.1748
2.0	0.0718	0.0717	0.0717	0.0717	0.0001	0.1394
2.4	0.0867	0.0866	0.0866	0.0866	0.0001	0.1154

*RSD of six independent determinations

Table-1.6: Color stability data for MBTH Method

Conc. in $\mu g\ ml^{-1}$	Time in hrs							
	4	**8**	**12**	**16**	**20**	**24**	**28**	**32**
20	0.984	0.986	0.984	0.984	0.984	0.984	0.914	0.984

Table-1.7: Recoveries of Bifenox from spiked vegetables (Potatoes and Tomatoes)

S. N o	Amount of MET added $\mu g\ ml^{-1}$	Average amount found $\mu g\ ml^{-1}$		% Recover		SD		%RSD*	
		Potatoes	Tomatoes	Potatoes	Tomatoes	Potatoes	Tomatoes	Potatoes	Tomatoes
1	1.2	1.189	1.190	90.08	99.16	0.00057	0.00058	0.0479	0.0478
2	2.4	2.395	2.391	99.79	99.62	0.0023	0.0024	0.1002	0.1012
3	3.6	3.584	3.575	99.55	99.30	0.0051	0.0052	0.1452	0.1442
4	4.8	4.76	4.72	99.16	99.33	0.02309	0.02319	0.4886	0.4846
5	6.0	5.812	5.801	99.86	96.68	0.0063	0.0043	0.074	0.064
6	7.2	7.192	7.181	99.88	99.73	0.0063	0.0053	0.0737	0.0537

*Average of six determinations

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[Sekhar* et al., 5.(4): April, 2016]
IC™ Value: 3.00

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

Table-1.8: Recoveries of Bifenox from fortified water samples (tap and distilled water)

S. No	Fortification level ($\mu g\ ml^{-1}$)	Tap water				Distilled water			
		amount found $\mu g\ ml^{-1}$	% Recover	SD	%RSD	amount found $\mu g\ ml^{-1}$	% Recover	SD	%RSD*
1	1.5	0.193	97.5	0.00287	0.1502	0.180	95.00	0.0025	0.1512
2	3	0.385	96.75	0.00285	0.7375	0.391	88.00	0.00281	0.7320
3	4.5	0.577	96.5	0.0062	0.1073	0.500	98.33	0.0052	0.1172
4	6	0.790	98.85	0.0022	0.0171	0.751	94.20	0.0210	0.052
5	7.5	0.990	99.00	0.0295	0.3072	0.954	95.60	0.2550	0.3065
6	9	1.190	99.23	0.0021	0.1752	1.192	99.25	0.0023	0.1672

*Average of six determinations= 99.960.0005

RESULTS AND DISCUSSIONS

1. Optical parameters
In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV/Vis spectrophotometric method of the colored species formed in each specified amount of Bifenox in final solution 5 µg ml^{-1} was taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 380-800 nm against corresponding reagent blanks. The regent blank absorption spectrum of each method was also recorded against distilled water /methanol. The results are graphically represented in fig- 1.1.

2. Parameters fixation
In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development reproducibility and reasonable period of stability of final colored species formed. The following studies were conducted in the present investigation.

Method :
The results obtained in this method were based on oxidation followed by coupling reaction of Bifenox with MBTH, Ferric chloride and Orthophosphoric acid to form green colored chromogen that exhibited maximum absorption at 615 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of Bifenox with MBTH reagent was shown in (fig-1.3). The effect of various parameters such as concentration and volume of MBTH and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameters at a time.

3. Optical Characteristics
The reference method adhere to Beer's law the absorbance at appropriate wave length of a set of solutions contains different amounts of Bifenox and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank.
The Beer's law plot of the system illustrated graphically (fig:1.2) least square regression analysis was carried out for the slope. Intercept and Correlation Coefficient.Beer's law limits, Molar absorptivity & Sandells sensitivity for Bifenox with each of mentioned reagents were calculated. The optical characteristics were present in the table-1.1.
In order to test whether the colored species formed in the method adhere the Beer's law the absorbance at appropriate wavelength of a set of solutions contain different amounts of Bifenox and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The Beers law plots of the system illustrated graphically (fig – 1.1) least square regression

analysis was carried out for the slope, intercept and correlation coefficient, Beer's law limits molar absorptivity, Sandells sensitivity for Bifenox with each of mentioned reagents were calculated. The optical characteristics are presented in the table -1.1.

4. Precision
The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtain by actual determination of a fixed amount of Bifenox in 5 µg ml⁻ in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods and presented in table-1.1.

5. Analysis of samples
Commercial formulations of Bifenox were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the t and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in tables-1.7&1.8,. The proposed methods also applied for samples spiked vegetables and water samples for good recoveries are obtained which were recorded in tables-1.7&1.8.

6. Accuracy
Recovery studies were carried by applying the standard addition method to sample present in formulations for the known amount of Bifenox the recovery studies were carried. By applying the same method to samples spiked vegetables and water samples to which known amount of Bifenox correspond to formulations.. At each level of recovery five determinations were performed and presented in tables -1.6&1.7. The results obtain were compared with expected results and were statistically validated in tables - 1.6,&1.7.

7. Linearity and range
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

8. Specificity and Selectivity
Specificity is a procedure to detect quantitatively the analyze in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyze qualitatively in presence of components that may be expected to present in the sample matrix. The excipient in formulations was spiked in a pre weighed quantity of drugs and then absorbance was measured and calculations were done to determine the quantity of the samples.

9. Repeatability
Standard solutions of Bifenox were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure five times and standard deviation was calculated and presented in tables-1.5.

10. Interferences Studies
The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Bifenox under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in samples.

11. Solution Stability
The stability of the solutions under study was established by keeping the solution at room temperature for 24 hrs. The results indicate no significant change in assay values indicating stability of drug in the solvent used during analysis. The results are recorded in tables -1.6.

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 UV/Vis method is given.

The simple, accurate and precise UV/Vis method for the determination of Bifenox as bulk , commercial samples and spiked vegetables and water samples has been developed. The method may be recommended for routine and quality control analysis of the investigated pure in bulk and 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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