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**SPECTROPHOTOMETRIC DETERMINATION OF AMETRIDIONE AND IN ITS  
COMMERCIAL FORMULATIONS**

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**ABSTRACT**

A simple, précis, rapid sensitive and accurate spectrophotometric methods have been developed for the estimation of Ametridione in pure form and its formulations and Spiked vegetables and water samples. This method is based on oxidative coupling of Ametridione with MBTH in the presence of Ferric chloride to form green colored product with Maximum of 615nm. The product obeyed beers law in the concentration range 0.5-3 ml (5-30 µgml<sup>-1</sup>) with molar absorptivity of 1.001x10<sup>4</sup>. Sandel's sensitivity 0.004945. The assay of results was found to be good agreement with label claim.

**Keywords:** Ametridione. UV. Validation.

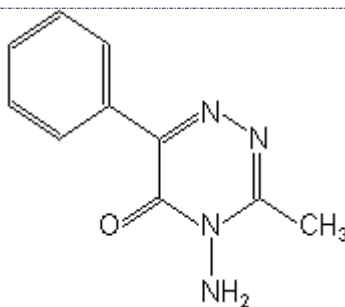
**I. INTRODUCTION**

A survey of the literature revealed that different analytical techniques for the assay of MTM have been reported. Voltametric detection of the herbicide Ametridione at a bismuth film electrode in non deaerated solution<sup>1</sup> Electroanalysis of Ametridione and metribuzen on lignin By Adsorption<sup>2</sup> ,Electrochemical reduction of Ametridione<sup>3</sup> , Identification of different products obtained by electrochemical and photochemical reduction of the Ametridione<sup>4</sup> Voltametric determination of Ametridione with an elctrogenerated molecularity imprinted polymer microsencer<sup>5</sup> Electrochemical determination of the effect of lead (II) on the photochemical degradation of the pesticide Ametridione<sup>6</sup> Voltametric determination of Herbicide Ametridione using Mercury and silver solid amalgam electrode<sup>7</sup> Preconcentration and voltametric determination of the herbicide Ametridione with a silica modified carbon paste electrode<sup>8</sup> Determination and method validation of Ametridione in soil by RP-HPLC<sup>9</sup> Electrochemical determination of the effect of Copper (II) on the photochemical degradation of the pesticide Ametridione<sup>10</sup> .

There is however no reported UV- Visible spectrophotometric method for the analysis of Mitamitron in its technical grade and formulations. This describes a validated UV- visible 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 were based on complex formation reaction of Mitamitron with Oxidative coupling reaction with MBTH / Ferric chloride.

The author has developed UV- Visible 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-Visible method for the quantitative determination of Ametridione. The empirical formula for Ametridione is C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>O and the molecular weight is 202.2 grams. It has the following structure.



## II. EXPERIMENTAL

### 1. Solvent

Methanol was used as Solvent.

### 2. Preparation of standard stock solution

Accurately weighed 100 mg of Ametridione was dissolved in 40 ml of Methanol in 100 ml volumetric flask and volume was made up to the mark. i.e. 1000  $\mu\text{g ml}^{-1}$  (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  $\mu\text{g ml}^{-1}$  (Stock solution B)

### 3. Preparation of calibration curve

Fresh aliquots of Ametridione 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  $\mu\text{g ml}^{-1}$ . 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) Hydrochloric acid 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 24h. The amount of Ametridione present in the sample solution was computed from its calibration curve.

### 4. Procedure for formulations

Twenty tablets containing Ametridione were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Ametridione 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 100ml volumetric flask and the volume was made up to the mark with Methanol to obtain the final concentration of 100  $\mu\text{g ml}^{-1}$  (Stock solution).

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

### 5. Recovery of Ametridione 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 Ametridione in Methanol and blended for 3 min. Chloroform was filtered into 250 ml Standard flask through whatman No.1 filter paper and the residue was retained. The residue was washed twice with 10ml of chloroform and blended for 2 min. 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 tabulate in table 1.7.

### 6. Recovery of Ametridione from Fortified water samples

After collection of the water samples (Tap and Distilled water minimum volume one liter) the pH of the water samples were adjusted below 4 with 20% sulphuric acid. Then fortified with different concentrations of Ametridione dissolved in methanol. Extract each sample in a 250ml separating funnel with 100ml Chloroform. The chloroform extract was transferred into a funnel and re extracted the aqueous phase twice with further 50ml of chloroform. The second chloroform extracts was added to the first and washed the combined extract with 0.1M K<sub>2</sub>CO<sub>3</sub> then dried the chloroform by passing it through anhydrous Sodium sulphate in a filter funnel and

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collected the extracts in a 250ml flask. The chloroform extracts was reduced to 100ml amount was determined spectrophotometrically. The results obtained were presented in table 1.8.

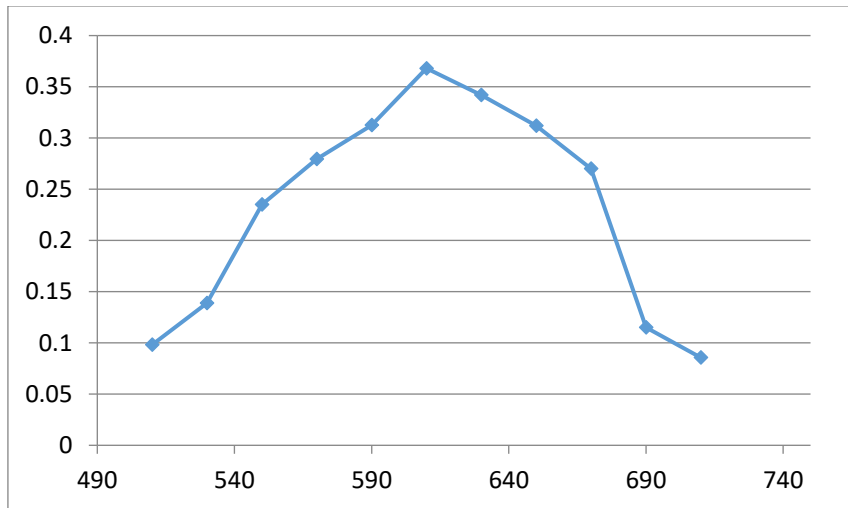


Fig-1.1: Absorption spectrum of Ametridione with MBTH/FeCl<sub>3</sub>

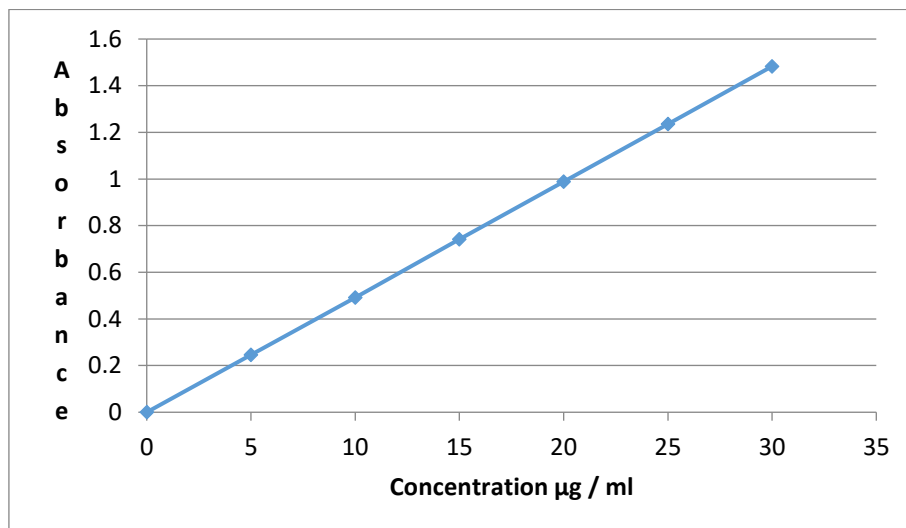


Fig-1.2: Beer's law plot of Ametridione with MBTH/FeCl<sub>3</sub>

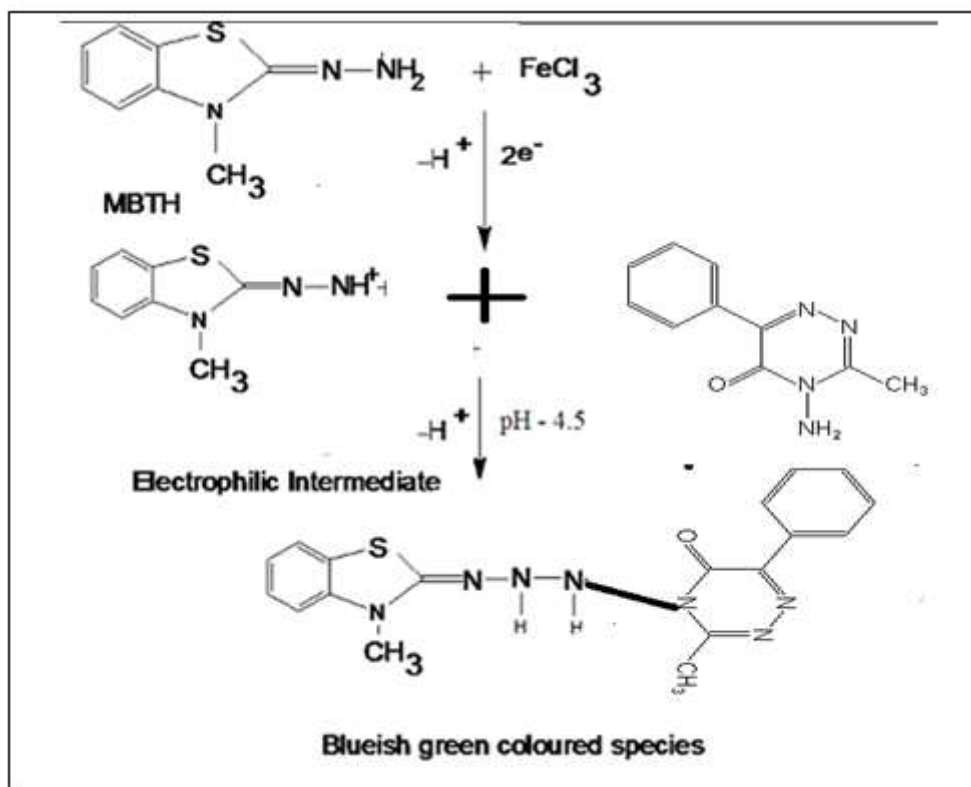


Fig-1.3: A Schematic reaction Mechanism of Ametridione with MBTH

Table-1.1: Optical characteristics and precision by MBTH

Parameter	Visible method
Color	Bluish green
Absorption maxima (nm)	615
Beer's law limits ( $\mu\text{g ml}^{-1}$ )	5-30
Molar absorptivity ( $\text{l mol}^{-1}\text{cm}^{-1}$ )	$1.001 \times 10^4$
Sandell's Sensitivity ( $\mu\text{g cm}^{-2}$ )	0.004945
Regression equation ( $Y^*$ )	
Slope (b)	0.04945
Intercept(a)	0.00014
Standard deviation(SD)	1.074006
Correlation coefficient ( $r^2$ )	0.9999
%RSD (Relative Standard deviation)*	1.243
Range of errors	
Confidence limits with 0.05 level	0.8593
Confidence limits with 0.01 level	1.1293
Limits of detection (LOD)( $\mu\text{g ml}^{-1}$ )	0.06522
Limits of quantification (LOQ) ( $\mu\text{g ml}^{-1}$ )	0.2174

\*RSD of six independent determinations

*Table-1.2: Assay results of Ametrudione 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	249.25 T= 0.0029 F=3.1851	247.0	99.08
Sample -2	250	249.30 T= 0.0028 F=3.1650	248.00	99.47

\*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 Ametrudione*

Amount of AMD in formulation (mg)	Amount of Standard AMD added (mg)	Total amount found (mg)	% Recovery
249.25	200	448.65	99.70
249.31	200	448.75	99.72
249.62	200	449.31	99.84
249.25	250	498.50	99.70
249.50	250	499.00	99.80
249.75	250	499.50	99.90
249.25	300	548.35	99.70
249.54	300	548.98	99.81
249.70	300	549.34	99.88

*Table-1.4: Statistical data for accuracy determination*

Total amount found (mean)	Standard deviation	% RSD
249.39	0.1985	0.07959
249.50	0.25	0.1002
249.49	0.228	0.09138

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

*Table-1.5: Repeatability data for AMD at 615 nm*

Conc. ( $\mu\text{g ml}^{-1}$ )	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%)RSD*
5	0.246	0.246	0.246	0.246	0.0005	0.2032
10	0.493	0.493	0.492	0.492	0.0005	0.1016
15	0.740	0.740	0.740	0.74	0.0005	0.06756
20	0.988	0.987	0.989	0.988	0.001	0.1012
25	1.235	1.235	1.234	1.234	0.0005	0.0405
30	1.482	1.481	1.482	1.481	0.0005	0.0337

\*RSD of six independent determinations

Table-1.6: Color stability data for MBTH Method

Conc. in $\mu\text{g ml}^{-1}$	Time in Hours							
	4	8	12	16	20	24	28	32
20	0.986	0.987	0.988	0.987	0.987	0.987	0.897	0.867

Table-1.7: Recoveries of Ametrudione from spiked Vegetables (Potatos and Tmatoes)

S.No	Amount of AMD added $\mu\text{g ml}^{-1}$	Average amount found $\mu\text{g ml}^{-1}$		% Recover		SD		%RSD	
		Potatos	Tomatos	Potatos	Tomatos	Potatos	Tomatos	Potatos	Tomatos
1	0.3	0.251	0.273	74.90	91.00	0.6350	0.001	0.227	0.3676
2	0.6	0.504	0.523	84.00	87.16	0.00057	0.0011	0.11330	0.2107
3	0.9	0.809	0.816	89.88	90.66	0.00057	0.001	0.0705	0.1226
4	1.2	1.112	1.104	92.66	92.00	0.00115	0.0005	0.099	0.0453
5	1.5	1.352	1.397	90.13	93.133	0.001	0.00057	0.074	0.0408

Average of Five determinations

Table-1.8: Recoveries of Ametrudione from fortified water samples (Tap and Distilled water)

S.No	Fortification level ( $\mu\text{g ml}^{-1}$ )	Tap water				Distilled water			
		amount found $\mu\text{g ml}^{-1}$	% Recover	SD	%RSD	amount found $\mu\text{g ml}^{-1}$	% Recover	SD	%RSD
1	0.3	0.251	83.66	0.0005	0.1992	0.262	87.33	0.0005	0.1908
2	0.6	0.584	97.33	0.0005	0.0856	0.591	98.50	0.0005	0.0846
3	0.9	0.893	99.22	0.001	0.1119	0.890	98.88	0.0005	0.0561
4	1.2	1.173	97.75	0.0005	0.0426	1.182	98.50	0.0005	0.0423
5	1.5	1.426	95.06	0.001	0.0701	1.452	96.80	0.0005	0.0344

Average of Five determinations 99.960.0005

### III. RESULTS AND DISCUSSIONS

#### 1. Optical parameters

In order to ascertain the optimum wavelength of maximum absorption ( $\lambda_{\text{max}}$ ) formed in UV spectrophotometric method of the colored species formed in each specified amount of Ametrudione in final solution  $5 \mu\text{g ml}^{-1}$  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 reagent 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.

### Method

The results obtained in this method were based on oxidation followed by coupling reaction of Ametrudione 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 Ametrudione 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 Ametrudione and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank.

The beers 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 Ametrudione with each of mentioned reagents was 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 Ametrudione 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 Ametrudione with each of mentioned reagents were calculated. The optical characteristics are presented in the Tables -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 Ametrudione in, 5 µg ml<sup>-1</sup> 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 Tables-1.1,

### 5. Analysis of Samples

Commercial formulations of Ametrudione 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 Standare addition method to sample present in formulations for the known amount of Ametrudione the recovery studies were carried . By applying the same method to Samples **spiked Vegetables and water samples** to which known amount of Ametrudione correspond to Formulations.. At each level of recovery five determinations were performed and present 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 with in 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

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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 Ametrudione 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 Ametrudione 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 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in Table -1.6.

## IV. 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 sumery of validation parameters of proposed UV- Visible method is given.

The simple, accurate and precise UV- Visible method for the determination of Ametrudione as bulk, Comercial 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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