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Spectrophotometric determination of Ametoctradin and in its commercial formulations

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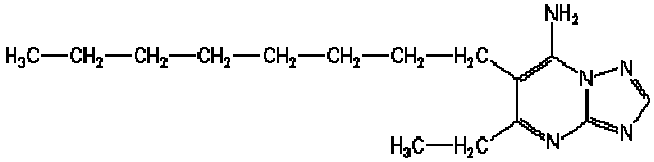
ABSTRACT

A simple, précis, rapid sensitive and accurate spectrophoptometric methods have been developed for the estimation of Ametoctradin in pure form and its formulations and spiked vegetables and water samples. This method is based on oxidative coupling of Ametoctradin with MBTH in the presence of Ferric chloride to form green colored product with Maximum of 610 nm. The product obeyed beers law in the concentration range 0. 4 - 2.4 ml (4-24 µgml-1) with molar absorptivity of 0.9917X10^4. Sandells sensitivity 0.0027777. The assay of results was found to be good agreement with label claim.

Keywords: Ametoctradin, UV Spectrophotometry, Validation

INTRODUCTION

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



Mol. Formula: $C_{15}H_{25}N_5$ Mol.Wt:275.40grams

Ametoctradin having the and IUPAC name is [1,2,4 Triazolo [1,5-a] pyrimidin-7-amine, 5-ethyl-6-octyl. is a Triazolopyramidine derivative.

Ametoctradin is a novel fungicidal compound. It is a non- systemic and preventative compound used for foliar applications to manage plant diseases caused by water moulds. It acts on pathogen cells by interfering with their normal respiration process. Fungicides are used on Brassica leafy vegetables, Bulb vegetables, cucurbit vegetables and fruiting vegetables.

The UV-Visible method was applied for the determination of AMCD by oxidative coupling reaction with MBTH/$FeCl_3$ and an ion association complex reaction with BTB/$CHCl_3$.

There is however no reported UV- Visible spectrophotometric method for the analysis of Ametoctradin in its technical grade and formulations. This describes a validated UV- visible spectrophotometric method for the quantitative determination of Ametoctradin. Functional group used for color development of Ametoctradin was primary amine group.

The author has developed UV- Visible spectrophotometric method based on the use of method, without use of any interference. 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.

MATERIALS AND METHODS

Experimental conditions:

1. Solvent

Methanol was used as a Solvent.

2. Preparation of standard stock solution

Accurately weighed 100 mg of Ametoctradin 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)

3. Preparation of calibration curve

Fresh aliquots of Ametoctradin ranging from 0.5 to 3ml 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) 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 Ametoctradin present in the sample solution was computed from its calibration curve.

4. Procedure for formulations

An accurately weighed portion of the powder equivalent to 100 mg of Ametoctradin 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 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.

5.Recovery of Ametoctradin from Spiked vegetables

100 gm of each vegetable (Potatoes and tomatoes) were spiked with 200ml chloroform for 5 min. The samples were fortified with different concentration of Ametoctradin in Methanol and blunted for 3 min. Chloroform was filtered into 250ml Standard flask through whatmanNo.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 color development after evaporating chloroform on steam

bath. The residue was dissolved in methanol and the amount was determined spectrophotometricaly and the results were presented in tabulated in table-6.10..

6. Recovery of Ametoctradin 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% sulphiuric acid. Then fortified with different concentrations of Ametoctradin 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_2CO_3then dried the chloroform by passing it through anhydrous Sodium sulphate in a filter funnel and collected the extracts in a 250ml flask. The chloroform extracts was reduced to 100ml amount was determined spectrophotometricaly. The results obtained were presented in table 6.11.

RESULTS AND DISCUSSION

1. Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV spectrophotometric method of the colored species formed in each specified amount of Ametoctradin 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- 5.1)

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 Ametoctradin with MBTH, Ferric chloride and Orthophosphoric acid to form green colored chromogen that exhibited maximum absorption at 610 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 Ametoctradin UV 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 Ametoctradin 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 & Sandals sensitivity for Ametoctradin with each of mentioned reagents was calculated. The optical characteristics were present in the table-1.4.

Table - 1: Optical characteristics and precision by MBTH

Parameter	Visible method
Color	Bluish green
Absorption maxima(nm)	610
Beer's law limits (µg ml^{-1})	4-24
Molar absorptivity (l $mol^{-1}cm^{-1}$)	$0.9917X10^4$
Sandell's Sensitivity (µg cm^{-2})	27777
Regression equation (Y*)	
Slope (b)	0.0361
Intercept(a)	0.000206
Standard deviation(SD)	0.8669
Correlation coefficient (r^2)	0.9999
%RSD (Relative Standard deviation)*	172345
Range of errors	
Confidence limits with 0.05 level	1.69909
Confidence limits with 0.01 level	2.2329
Limits of detection (LOD)(µg ml^{-1})	72041
Limits of quantification (LOQ) (µg ml^{-1})	24013

**RSD of six independent determinations*

Table - 2: Assay results of Ametoctradin 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	246.87 t=0.002964 f=6.6313	231.00	93.12
Sample -2	250	248.12 t=0.002954 f=6.6321	230.00	92.12

**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 - 3: Determination of accuracy of Ametoctradin

Amount of AMCD in formulation(mg)	Amount of Standard AMCD added (mg)	Total amount found (mg)	% Recovery
246.87	200	444.36	98.74
247.18	200	444.92	98.87
248.43	200	447.17	99.37
246.87	250	4963.74	98.74
247.0	250	494.00	98.80
247.25	250	494.5	98.90
246.87	300	543.11	98.74
247.7	300	544.94	99.08
249.58	300	549.07	99.83

Table - 4: Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
247.49	0.8258	0.3502
247.04	0.1931	0.3509
248.05	1.388	0.3494

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

Table - 5: Repeatability data for AMCD at 615 nm

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%)RSD*
0.4	0.0143	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	0.0432	0.0431	0.0430	0.0431	0.0001	0.2320
1.6	0.0573	0.0572	0.0571	0.0572	0.0001	0.1748
2.0	0.0718	0.0717	0.0716	0.0717	0.0001	0.1394
2.4	0.0867	0.0866	0.0865	0.0866	0.0001	0.1154

**RSD of six independent determinations*

Table - 6: Color stability data for MBTH Method

Conc. In µg ml^{-1}	Time in Hours							
1.2	4	8	12	16	20	24	28	32
	0.0432	0.0432	0.0432	0.0432	0.0432	0.0432	0.0412	0.0389

Table - 7: Recoveries of Ametoctradin from spiked Vegetables (Potatos and Tmatoes)

S.No	Amount of AMCD Added µg ml^{-1}	Average amount found µg ml^{-1}		% Recover		SD		%RSD	
		Potatos	Tomatos	Potatos	Tomatos	Potatos	Tomatos	Potatos	Tomatos
1	1.2	1.189	1.190	99.08	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	98.33	0.02309	0.02319	0.4886	0.4846
5	6.0	5.812	5.801	96.86	696.68	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

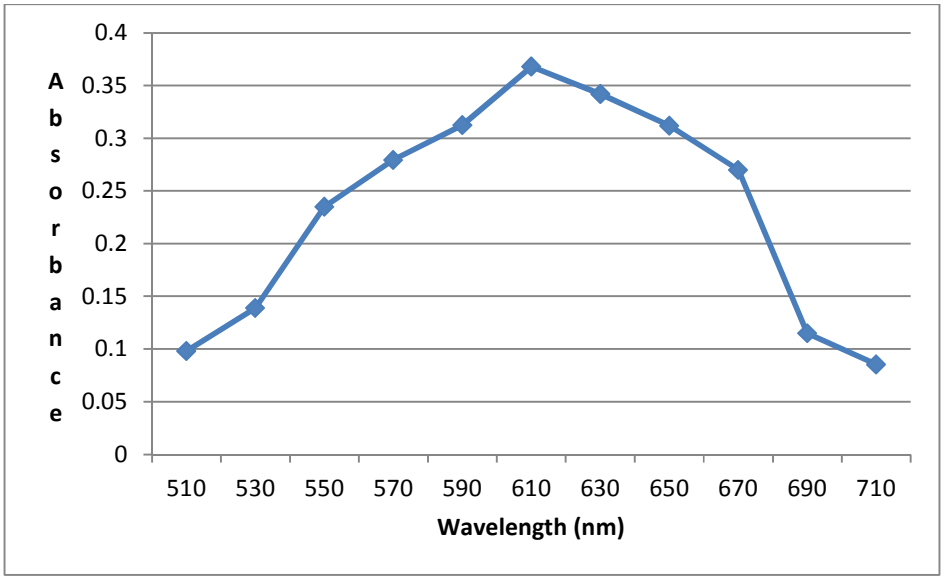
Average of Five determinations

Table - 8: Recoveries of Ametoctradin from fortified water samples (Tap and Distilled water)

S.No	Fortification level (µg ml^{-1})	Tap water				Distilled water			
		amount found µg ml^{-1}	% Recover	SD	%RSD	amount found µg ml^{-1}	% Recover	SD	%RSD
1	0.2	0.195	97.50	0.00288	0.1503	0.190	95.00	0.00268	0.1513
2	0.4	0.387	96.75	0.00288	0.7378	0.392	98.00	0.00298	0.7358
3	0.6	0.579	96.50	0.0063	0.1074	0.590	98.33	0.0053	0.1174
4	0.8	0.791	98.87	0.0225	0.0172	0.752	94.00	0.0215	0.0152
5	1.0	0.990	99.00	0.0296	0.3075	0.956	95.60	0.0276	0.3065
6	1.2	1.191	99.25	0.0023	0.1777	1.195	99.25	0.0025	0.1677

Average of Five determination

Fig-1.1: Absorption spectrum of Ametoctradin with MBTH /$FeCl_3$



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 Ametoctradin 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 –1.4) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits, molar absorptivity , Sandals sensitivity for Ametoctradin with each of mentioned reagents were calculated. The optical characteristics are presented in the Tables -1.4.

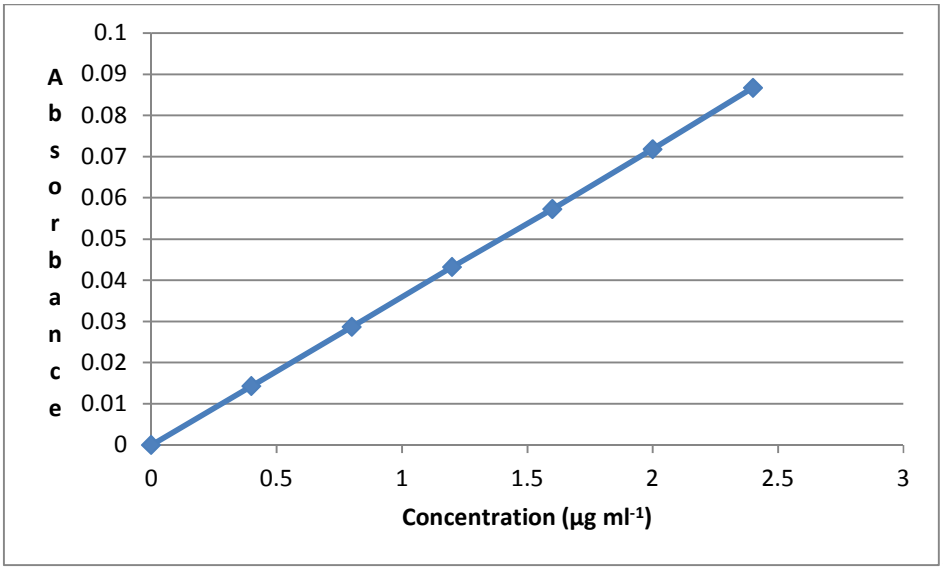
4. Precision

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 Ametoctradin in, 5µg ml-1in 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-1.4.

5. Analysis of Samples

Commercial formulations of Ametoctradin 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. The proposed methods also applied for Samples spiked Vegetables and water samples for good recoveries are obtained which were recorded in Tables.

Fig1.2: Beer's law plot of Ametoctradin with MBTH/$FeCl_3$



6. Accuracy

Recovery studies were carried by applying the Standard addition method to sample present in formulations for the known amount of Ametoctradin the recovery studies were carried. By applying the same method to Samples spiked Vegetables and water samples to which known amount of Ametoctradin correspond to Formulations. At each level of recovery five determinations were performed and present in Tables The results obtain were compared with expected results and were statistically validated in Tables.

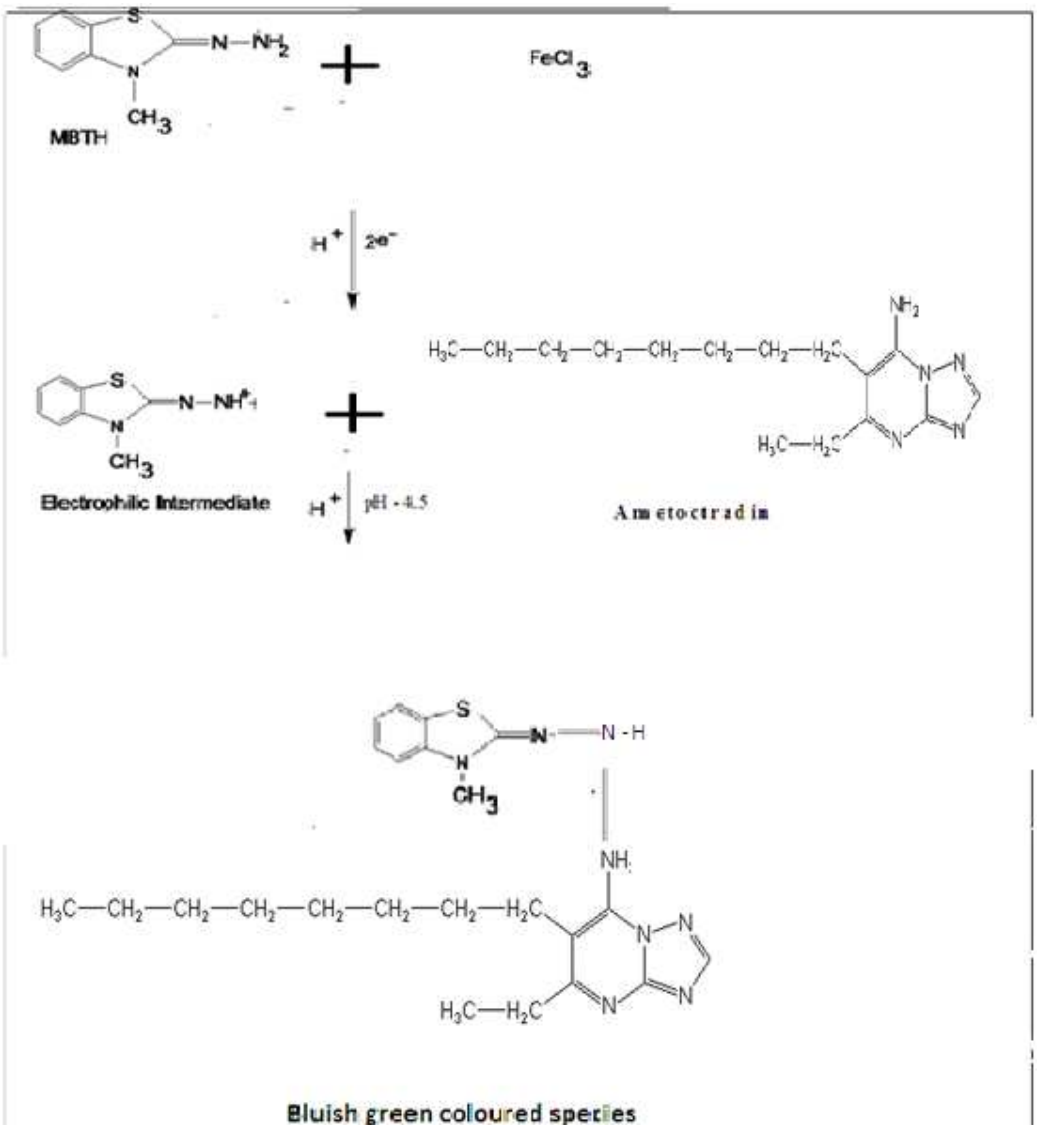
7. Linearity and Range

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 Pesticides and then absorbance was measured and calculations were done to determine the quantity of the samples.

Fig1.3: A Schematic reaction Mechanism of Ametoctradin with MBTH



9. Repeatability

Standard solutions of Ametoctradin were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measured five times and standard deviation was calculated and presented in Table1.

10. Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Ametoctradin 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 Pesticide in the solvent used during analysis. The results are recorded in Table -1.5.

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- Visible method is given.

The simple, accurate and precise UV- Visible method for the determination of Ametoctradin 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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