ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Spectrophotometric Determination of Metamitron and In Its Commercial Formulations.

P Padmavathi*, NVS Naidu, K Dhanalakshmi, and P Suguna.

Department of Chemistry, S.V.University, Tirupati-517502, Andhra Pradesh, India.

ABSTRACT

A simple, precis, rapid sensitive and accurate spectrophoptometric methods have been developed for the estimation of Metamitron in pure form and its formulations and spiked vegetables and water samples. This method is based on oxidative coupling of Metamitron with MBTH in the presence of Ferric chloride to form green coloured product with Maximum of 615nm. The product obeyed beers law in the conceentration range 0.5-3 ml (5-30 µgml^{-1}) with molar absorptivity of 1.001x10^4. Sandells sensitivity0.004945. The assay of results was found to be goood agreement with label claim.

Keywords: Metamitron. UV. Validation.

**Corresponding author*

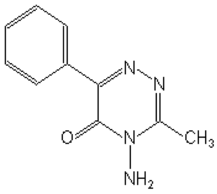
INTRODUCTION

A survey of the literature revealed that different analytical techniques for the assay of MTM have been reported. Voltametric detection of the herbicide Metamitron at a bismuth film electrode in nondeaerated solution [1] Electroanalisis of metamitron and metribuzen on lignin By Adsorption [2], Electrochemical reduction of Metamitron [3], Identification of different products obtained by electrochemical and photochemical reduction of the Metamitron [4] Votametric determination of metamitron with an elctrogenerated molecularity imprinted polymer microsencer [5] Electrochemical determination of the effect of lead (II) on the photochemical degradation of the pesticide metamitron [6] Votametric determination of Herbicide Metamitron using Mercury and silver solid amalgam electrode [7] Preconcentration and votametric determination of the herbicide Metamitron with a silica modified carbon paste electrode [8] Determination and method validation of Metamitron in soil by RP-HPLC [9] Electrochemical determination of the effect of Copper (II) on the photochemical degradation of the pesticide metamitron [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 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 was 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 Metamitron. The empirical formula for Metamitron is $C_{10}H_{10}N_4O$ and the molecular weight is 202.2 grams. It has the following structure.



EXPERIMENTAL

Solvent

Methanol was used as Solvent.

Preparation of standard stock solution

Accurately weighed 100 mg of Metamitron 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)

Preparation of calibration curve

Fresh aliquots of Metamitron 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) 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 Metamitron present in the sample solution was computed from its calibration curve.

Procedure for formulations

Twenty tablets containing Metamitron were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of Metamitron was dissolved in a 100 ml of Methanoland mixed for about 5 min and then filtered. The Methanolwas evaporated to dryness. The remaining portion of solution was diluted in a 100 ml volumetric flask to the volume with Methanolup 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 Methanolto obtained the final concentration of 100 µg ml^{-1} (Stock solution).

Subsequent dilutions of this solution were made with Methanolto 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.

Recovery of Metamitron 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 Metamitron in Methanol and blented for 3 min. Chloroform was filtered into 250ml Standared 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 colour development ofter evoparating chloroform on steam bath. The residue was dissolvd in methanol and the amount was determined spectrophotometricaly and the results were presented in tabellate in table 1.7.

Recovery of Metamitron 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 Metamitron 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 addesd to the first and washed the combined extract with 0.1M K_2CO_3 then 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 reducd to 100ml amount was determined spectrophotometricaly. The results obtained were presented in table 1.8.

Figure 1.1: Absorption spectrum of Metamitron with MBTH /$FeCl_3$

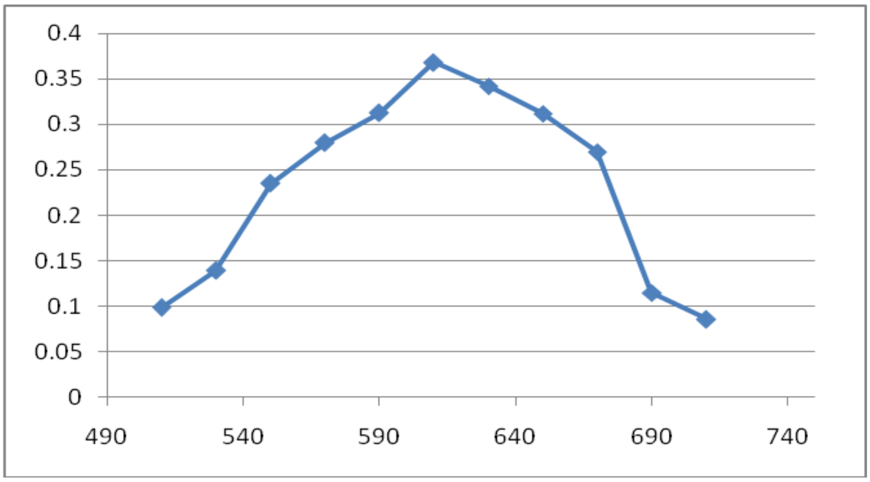


Figure 1.2: Beer's law plot of Metamitron with MBTH/FeCl$_3$

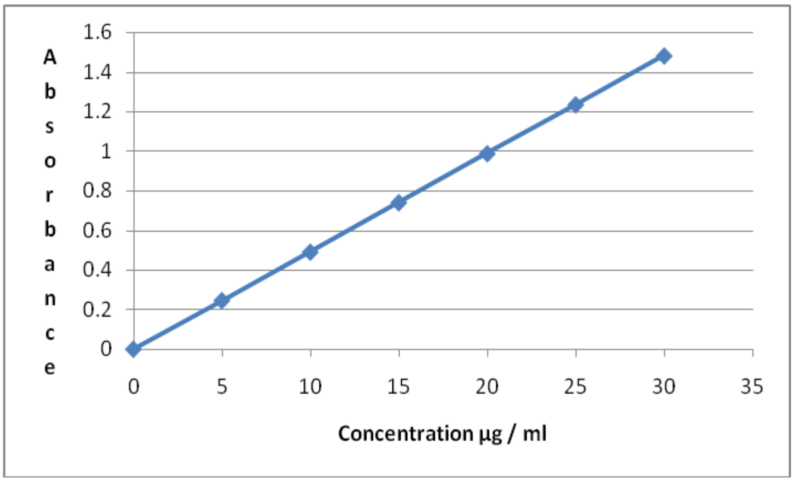


Figure 1.3: A Schematic reaction Mechanism of Metamitron with MBTH

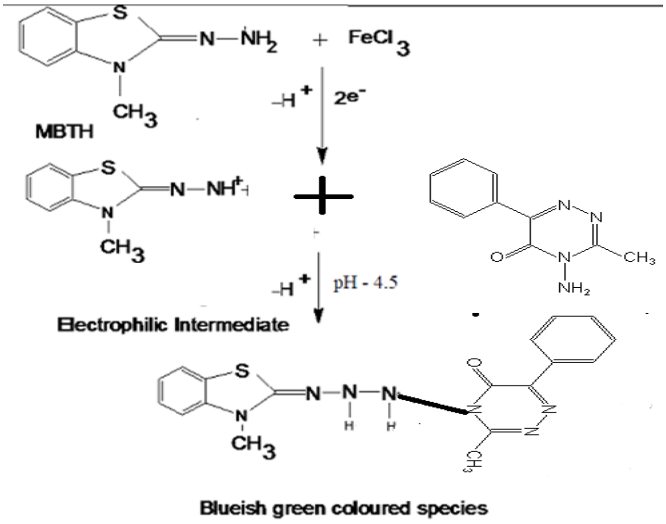


Table 1.1: Optical characteristics and precision by MBTH

Parameter	Visible method
Color	Bluish green
Absorption maxima (nm)	615
Beer's law limits (µg ml^{-1})	5-30
Molar absorptivity (l mol^{-1}cm^{-1})	1.001X10^{4}
Sandell's Sensitivity (µg 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)(µg ml^{-1})	0.06522
Limits of quantification (LOQ) (µg ml^{-1})	0.2174

*RSD of six independent determinations

Table 1.2: Assay results of Metamitron 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 Metamitron

Amount of MET in formulation (mg)	Amount of Standard MET added (mg)	Total amount found (mg)	% Recovery
249.25 249.31 249.62	200 200 200	448.65 448.75 449.31	99.70 99.72 99.84
249.25 249.50 249.75	250 250 250	498.50 499.00 499.50	99.70 99.80 99.90
249.25 249.54 249.70	300 300 300	548.35 548.98 549.34	99.70 99.81 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 MET at 615 nm

Conc. (μg 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-16: Color stability data for MBTH Method

Conc. in μg 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 Metamitron from spiked Vegetables (Potatos and Tmatoes)

S.No	Amount of MET added μg ml^{-1}	Average amount found μg 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 Metamitron 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	0.3	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 determinations99.960.0005

RESULTS AND DISCUSSION

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 Metamitron 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)

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 ware conducted.

Method

The results obtained in this method were based on oxidation followed by coupling reaction of Metamitron 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 Metamitron 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

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 Metamitron 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 Metamitron 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 Metamitron 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 Metamitron with each of mentioned reagents were calculated. The optical characteristics are presented in the Tables -1.1,

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 Metamitron 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 Tables-1.1,

Analysis of Samples

Commercial formulations of Metamitron 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 Tables1.7,1.8.

Accuracy

Recovery studies were carried by applying the Standare addition method to sample present in formulations for the known amount of Metamitron the recovery studies were carried . By applying the same method to Samples spiked Vegetables and water samples to which known amount of Metamitron 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

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.

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.

Repeatability

Standard solutions of Metamitron 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.

Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Metamitron 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.

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.

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 Metamitron 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.

REFERENCES

[1] As.Arribas,E.Berimizo,2006 (Willey online librury)
[2] J.Ludivik,P.Zuman 2000,Elsevier (Microchemical journal)
[3] J.Ludivik ,F.Riedi Journal of electroanalytical chemisry 1998 Elsevier
[4] C.Olmedo,L.deban Electrochemica 1994 Esevier
[5] A.Gomez-caballero 2007 (Willey online librury)
[6] D.Sancho,M.Vega 1999,Taylor &Francis
[7] R,Selesovska,L.Bandzuchova 2004
[8] A.Arranz,SF De Betono 1997,Spriger
[9] S.Kumar,S.Tandon 2014 Spriger
[10] D.Sancho,M.Vega 1999,Taylor &Francis