Spectrophotometric Determination and Commercial Formulation of Tebuconazole Fungicide after Derivatization

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ABSTRACT

A spectrophotometric method has been developed for the determination of tebuconazole fungicide formulation. The method is based on the complexation reaction of ferric chloride with tebuconazole fungicide in acidic media and the purple colored complex was studied at 530 nm. The reaction conditions and other analytical parameters were optimized. A linear calibration curve between absorbance and concentration over the range from 0.2 to 20 µg mL^{-1} was obtained with molar absorptivity of 2.1×10^4 L mol^{-1} cm^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.05 and 0.2 µg mL^{-1} respectively. The proposed method has been successfully applied for the analysis of commercial formulation of tebuconazole fungicide. The recoveries of the method were found to be in the range of 92.16±0.06% to 96.66±0.18%.

Keywords: Ferric Chloride, sodium hydroxide, tebuconazole fungicide, UV / Visible spectrophotometer

1. Introduction

The production of good quality of crops is essential for the existence of human being. Many micro-organisms have spoiled crops which lead to worldwide food shortage. Among these micro-organisms fungi play a critical role in decaying crops. [1-3] Fungicides work in a variety of ways, but most of them damage fungal cell membranes or interfere with energy production within fungal cell. [4, 5] Fungicides have changed the nature of agriculture. Researchers all around the world are involved in the synthesis of fungicides of new formulation in order to meet the modern world needs. [4 - 6] Tebuconazole is a fungicide commonly used in agriculture. Tebuconazole {1-(4-Chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol} is a triazole fungicide. The triazole (tebuconazole) fungicide have increased the persistence of disease control, an effect that leads to prolonged green leaf retention and extended grain fill compared to traditional fungicides. [7 - 9] Tebuconazole has novel mode of action, and are very safe from environmental point of view. Due to its fungicidal action the inhibition of mitochondrial respiration in fungi occurs and stopping their energy supply.[10-11] The plants for which tebuconazole are more effective are cereals, vines, fruits and vegetables, rice, turf and ornamentals. [2, 3] Various studies on tebuconazole have been investigated by

using the HPLC/MS (Boudina, A. *et al.*, 2007), gas chromatography in combination with electron capture detection (Aguilera, A. *et al*., 2012), gas chromatography (Beltran *et al.*, 1997), micellar electro kinetic chromatography (Huertas-Perez *et al.,* 2006; Martinez et al., 2003), spectrophotometric methods reported for the metribuzin determination is based on its complexation with copper (Shah *et al.,* 2009). The present method is based on spectrophotometric determination of tebuconazole fungicide by reacting with ferric chloride in alkaline media through complexation reaction.

2. Experimental

2.1. Apparatus

UV/Vis Spectrophotometer (Model SP-1800 plus, Optima Tokyo Japan), with matched 1cm quartz cells.

2.2. Reagents

All chemicals used were of analytical reagent grade (NaOH and HCl (37%), Ferric chloride (99.98%) [Merck, Germany], Methanol (99.98%) [Merck Darmstadt, Germany], Commercial formulated teboconazole 70% w/w of active ingredient (Roots Internatinal Agro, Division) was purchased from local Market, Ferric Chloride (0.04ml/L) solution was freshly prepared by dissolving 0.6g in 100ml of methanol. Teboconazole stock solution (1000µg/ml) was prepared by dissolving 0.1mg of authentic standard reagent in 50ml methanol and diluted upto 100ml. Working standard of 100 and 10 µg/ml solutions were prepared from stock solution by diluting with methanol. Sodium hydroxide solution of 0.87mol/L was used.

2.3. Procedure

Working of standard solution containing tebuconazole in the range of 0.2-20 µg/ml were

transfered quantitavely into reaction flask followed by the addition of 0.4ml Ferric chloride 5% and 0.4ml of NaOH (5M) solutions. The absorbance of the coloured product was measured at 530 nm against reagent blank.

2.4. Determination of Teboconazole in Commercial formulation

Known amount of commercial formulation containing tebuconazole was taken and diluted with methanol upto 50 mL and was filtered to remove any insoluble impurities. The solutions were then analyzed by the proposed method.

3. Results and discusion

In the present study spectrophotometric determination of tebuconazole fungicide was carried out. For this purpose tebuconazole is first converted into a purple colored complex by reacting it with ferric chloride (III) in alkaline media. The proposed reaction mechanism is given as,

Where R=
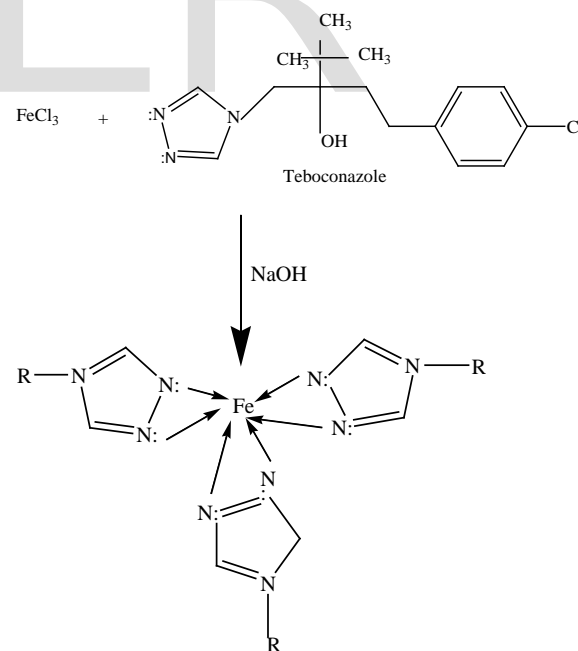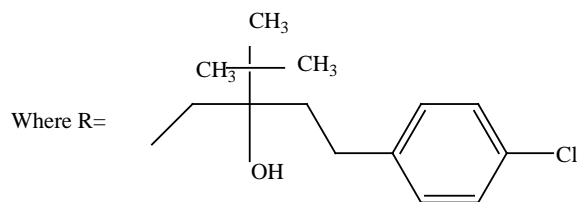
3.1. Scanning λ_{max} for Tebuconazole

The purple colored complex was scanned for various wavelength (400-680 nm). As can be seen from Fig. 01 the colored complex shows maximum absorbance at 530 nm. The obtained maximum wavelength (λ_{max}) of the complex was used for further analysis of the tebuconazole fungicide.
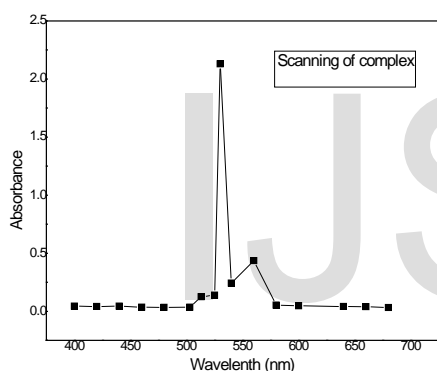

Figure 01. Determination of λ_{max} for teboconazole complex.

3.2. Optimization of Ferric (III) chloride

For the concentration optimization of ferric chloride six solutions of drug were prepared each contain different concentration of ferric chloride and their absorbance were measured at 530nm, the absorbance gradually increases and reaches the maximum value at 5mol/L beyond it the absorbance decreases. As the Fig. 02, shows that the solution of drug having 5mol/L concentration of ferric chloride have high degree of capability to convert maximum amount of drug into complex which gives maximum absorbance. It is worth mentioning that; identical plot

was also obtained by Shah *et. al.* [12] in their study, concerning the optimization of ferric (III) chloride; although they applied Fenoxaprop-p-ethyl under similar experimental conditions. However, similar work is also reported by M.R. Jan et al. [13] for absorption vs. concentration of pesticide carbofuran.
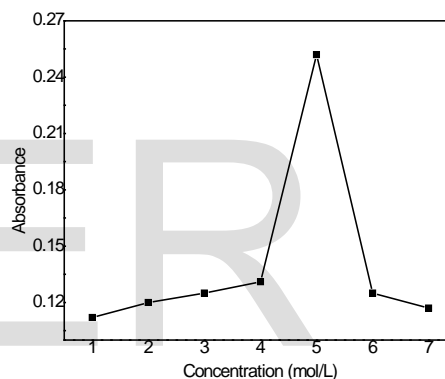

Figure 02. Concentration optimization of Ferric chloride.

3.3. Volume optimization of ferric chloride

For the optimization of volume of ferric chloride seven solutions of drug was prepared in each sample various quantity (0.1-0.7ml) of ferric chloride was added. The absorbance of these various complex solutions were measured at 530nm, the solution having 0.5ml of ferric chloride showed maximum absorbance as shown in Fig. 03.
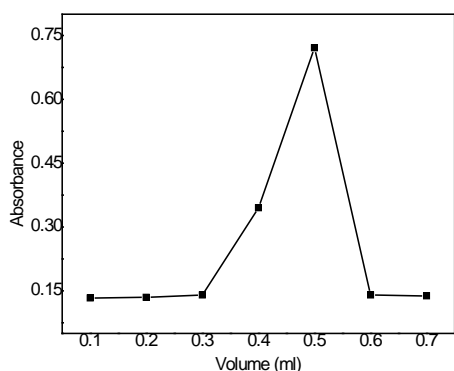
Figure 03. Volume optimization of Ferric chloride.

3.4. Optimization of sodium hydroxide

For the optimization of sodium hydroxide various concentrations ranging from 1-6 mol/L were prepared. Each of this sodium hydroxide was added to six different samples of tebuconazole complex. Initially as the concentration of sodium hydroxide raises the absorbance of the complex increases upto 2 mol/L as shown in Fig. 04, followed by decreases in absorbance significantly. This is optimum concentration of sodium hydroxide at which complete complexation of tebuconazole occurs and maximum absorbance was observed.
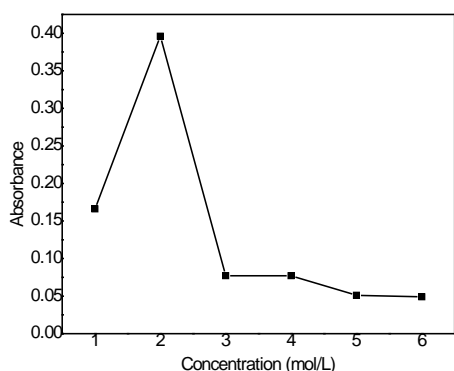

Figure 04.   Concentration optimization of sodium hydroxide.

3.5. Volume optimization of sodium hydroxide

For investigation of the optimum volume of sodium hydroxide at which maximum complexation takes place, various volume ranging 0.1- 0.7ml were added to tebuconazole . Fig. 05 shows the optimum volume of sodium hydroxide (0.4ml) at which tebuconazole exhibits maximum absorbance. The complex at this alkaline media shows maximium absorbance as this optimum volume provides most favorable condition for complexation. [12]
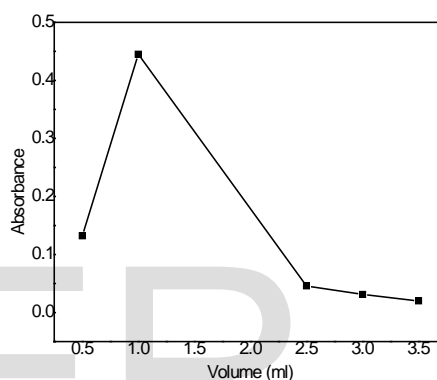

Figure 05. Volume optimization of NaOH.

3.6. Stability of Coloured complex

To check the stability of the final reaction product, the effect of time was studied upto 3 days (72h) with the interval of 2h. The colored reaction product was found to be stable for about 2 days (48h).

3.7. Analytical Characteristics

Table 1. shows the analytical characteristics of tebuconazole. The absorbance concentration curve was found linear over the concentration of 0.2-20μg mL^{-1}. The molar absorbitivity of the resulting yellow coloured product was found to be 2.1×10^4 Lmol^{-1} cm^{-1} with limit of detection (3s/b) and quantification(10s/b) of 0.05 and 0.2 μg mL^{-1} respectively, where s is the slope and b is the

intercept. For more confirmation of the analtical applications of the proposed method, the standered addition method was applied to a commmercial product. The recovery studied were carried out after adding known quantities of authentic standered to the pre-analyzed formulation. The percentage recoveries were in the range of 93.68-96.66%. The value obtained for commericial formulation was comparable with the labeled value.

3.8. Verification of Beer Lambert law

For the verification of Beer's Lambert law a calibration curve between concentration and absorbance was plotted in Fig. 06. The figure shows a linear relation between concentration and absorbance till 20 μg mL^{-1} concentration and beyond that concentration deviation was observed. In other words the Beer's Lamberts law was obeyed up to concentration 20 μg mL^{-1}.
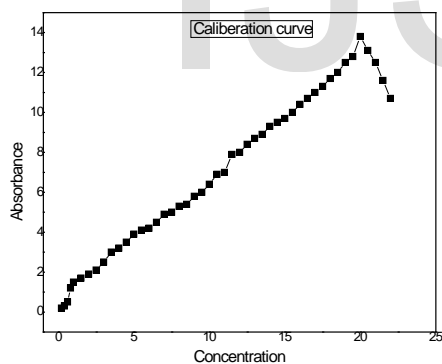

Figure 06. Calibration curve approving Beer lambert's law.

4. Conclusion

The proposed method described for spectrophotometric determination of tebuconazole is simple, rapid and cost-effective as compared to the reported chromatographic methods for determination of tebuconazole. The color of the final product is

stable at room temperature for two days and the limit of quantification shows that the developed method

Characteristics	Analysis
λ_{max} (nm)	530
colour	purple
Caliberation Curve Range ($\mu g/mL^{-1}$)	0.2-20
Slope	0.0287
Limit of detection ($\mu g/mL^{-1}$)	0.05
Limit of quantification ($\mu g/mL^{-1}$)	0.2
Molar absorbtivity ($Lmol^{-1}cm^{-1}$)	2.1×10^4

can be used to determine tebuconazole at lower concentration in different samples. The optimum conditions for the highest absorption due to maximum colored product formation were investigated.

Table 1. Analytical Parameters for spectrophotometric determination of Tebuconazole.

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