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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

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, gas chromatography in combination with electron capture detection, gas chromatography [12,13], micellar electro kinetic chromatography [14,15], spectrophotometric methods reported for the metribuzin determination is based on its complexation with copper [16]. The present method is based on spectrophotometric determination of tebuconazole fungicide by reacting with ferric chloride in alkaline media through complexation reaction.

Apparatus

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

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 (0.04 ml/L) solution was freshly prepared by dissolving 0.6 g in 100 ml of methanol. Teboconazole stock solution (1000 μg/ml) was prepared by dissolving 0.1 mg of authentic standared reagent in 50 ml methanol and diluted upto 100 ml. Working standared of 100 and 10 μg/ml solutions were prepared from stock solution by diluting with methanol. Sodium hydroxide solution of 0.87 mol/L was used.

Procedure

Working of standared solution containing tebuconazole in the range of 0.2-20 μg/ml were transfered quantitavely into reaction flask followed by the addition of 0.4 ml Ferric chloride 5% and 0.4 ml of NaOH (5M) solutions. The absorbance of the coloured product was measured at 530 nm against reagent blank.

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.

Results and Discussion

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,

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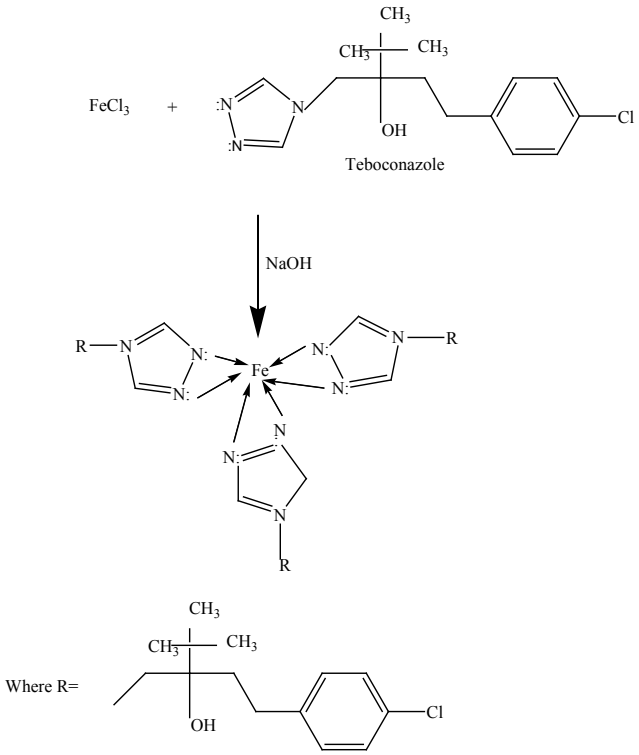

Scanning λ_{max} for tebuconazole

The purple colored complex was scanned for various wavelengths (400-680 nm). As can be seen from Figure 1 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.

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 530 nm, the absorbance gradually increases and reaches the maximum value at 5 mol/L beyond it the absorbance decreases. As the Figure 2, shows that the solution of drug having 5 mol/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. [16] 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 Jan et al. [17] for absorption vs. concentration of pesticide carbofuran.

Volume optimization of ferric chloride

For the optimization of volume of ferric chloride seven solutions of drug was prepared in each sample various quantity (0.1-0.7 ml) of ferric chloride was added. The absorbance of these various complex solutions were measured at 530 nm, the solution having 0.5 ml of ferric chloride showed maximum absorbance as shown in Figure 3.

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 up to 2 mol/L as shown in Figure 4, 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

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- 0.7 ml were added to tebuconazole. In Figure 5 shows the optimum volume of sodium hydroxide (0.4 ml) at which tebuconazole exhibits maximum absorbance. The complex at this alkaline media shows maximum absorbance as this optimum volume provides most favorable condition for complexation [16].

Stability of colored complex

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

Analytical characteristics

The absorbance concentration curve was found linear over the concentration of 0.2-20 µg 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 standared 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.

Verification of Beer Lambert law

For the verification of Beer's Lambert law a calibration curve between concentration and absorbance was plotted in Figure 6. 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} (Table 1).

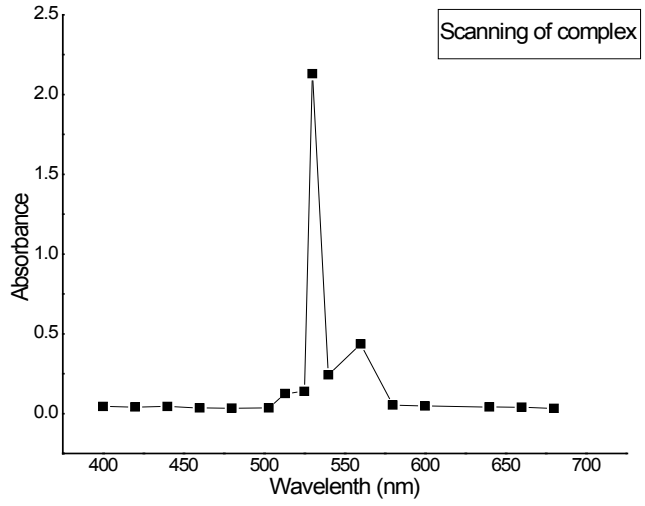

Figure 1: Determination of λ_{max} for teboconazole complex.

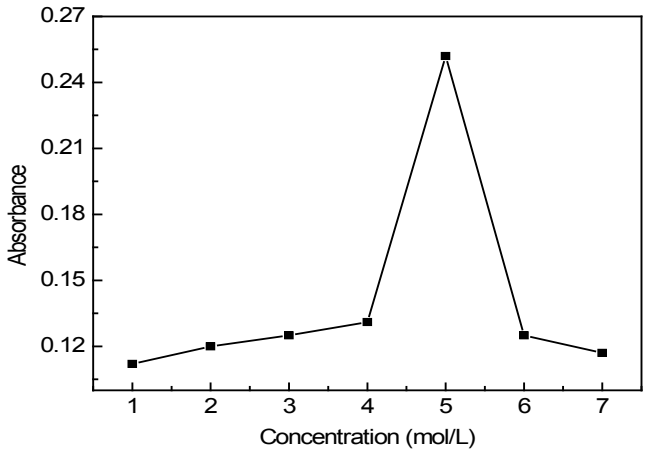

Figure 2: Concentration optimization of Ferric chloride.

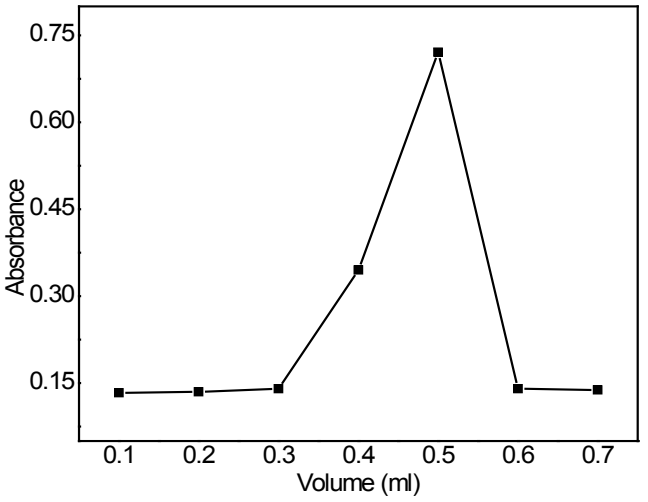

Figure 3: Volume optimization of Ferric chloride.

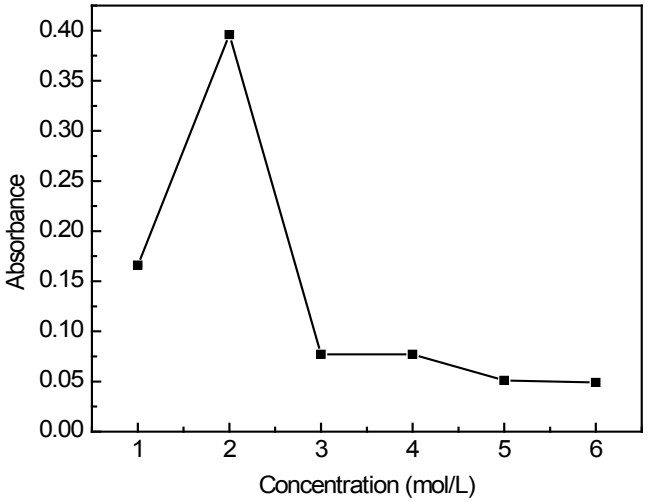

Figure 4: Optimization of sodium hydroxide.

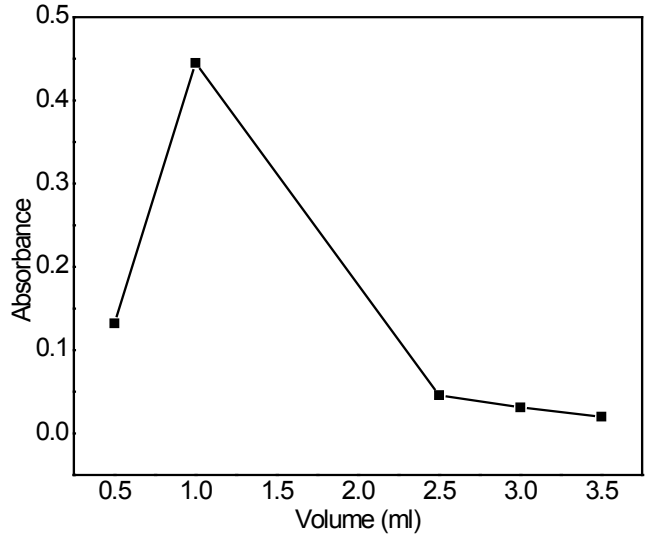

Figure 5: Volume optimization of NaOH.

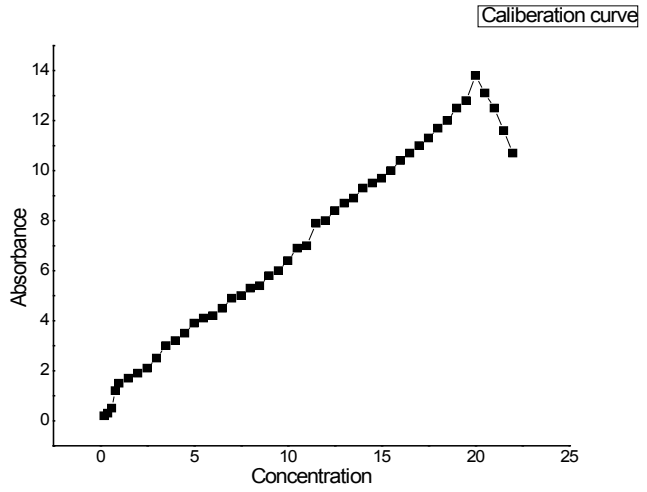

Figure 6: Calibration curve approving Beer lambert's law.

Characteristics	Analysis
λ_{max} (nm)	530
Colour	Purple
Calibration Curve Range (μg/mL^{-1})	0.2-20
Slope	0.0287
Limit of detection (μg/mL^{-1})	0.05
Limit of quantification (μg/mL^{-1})	0.2
Molar absorbtivity (L.mol^{-1}cm^{-1})	2.1 × 10^4

Table 1: The optimum conditions for the highest absorption due to maximum colored product formation were investigated.

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 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.

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