

## Research Article

## Visible Spectrophotometric Method for Determination of Ciclopirox Using Coupling Reagent

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### ABSTRACT

Ciclopirox is an antifungal drug for the treatment of cutaneous candidiasis infections. A simple, precise, accurate visual spectrophotometric method has been developed for the estimation of Ciclopirox in the presence of ferric chloride (FeCl<sub>3</sub>) and 3-methyl-2-benzothiazolinone hydrazone (MBTH). The method is based on the oxidation followed by coupling of MBTH with Ciclopirox in the presence of ferric chloride to form a green colored chromogen exhibiting absorption maximum at 662 nm. The linearity was found in the concentration range of 2- 12µg/ml and the correlation coefficient was 0.999. The result of analysis of the method has been validated statistically and by recovery studies.

**Key words:** Ciclopirox, colorimetry, ferric chloride, coupling reagent, antifungal.

### INTRODUCTION

Drug analysis involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter. The purpose of chemical analysis is to gather and interpret chemical information that will be of value in a wide range of contexts. Qualitative method yields information about the identity of the atomic or molecular species or the functional groups in the sample. A quantitative method, in contrast provides numerical information as to the relative amount of one or more of these compounds<sup>1</sup>. The scope of analytical chemistry is very broad and embraces a wide range of manual, chemical and instrumental techniques and procedures. It is not always necessary to apply advanced instrumental procedures to carry out accurate analyses, there may be many times when a simple rapid analysis may actually be more desirable than a complicated and time consuming process.<sup>1</sup> Photometric techniques are amongst the most important instrumental techniques available to the pharmaceutical analysts. The basis of all these instrumental techniques is that they measure the interaction of electromagnetic radiation with matter in quantised energy

levels<sup>2</sup>. Spectrometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy<sup>3</sup>. Colorimetric assays generally consists of adding a reagent to the assay preparation or to the substance being tested, to produce a colour that is compared with that of a standard preparation that has been prepared simultaneously and contains approximate quantity of the reference standard.<sup>4</sup> In general in analysis the first step is to determine the nature of the sample that is complete qualitative information and then further proceed for quantitative information by accuracy, LOD, LOQ etc<sup>5</sup>. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use<sup>6</sup>. The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature<sup>7</sup>. The work carried out on Ciclopirox includes RP-HPLC, chromatography etc<sup>8-14</sup>. Ciclopirox (trade name Stieprox) is an antifungal. Unlike antifungals such as itraconazole and terbinafine, which affect sterol synthesis, ciclopirox is thought to act through the chelation of polyvalent metal

cations, such as  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ . These cations inhibit many enzymes, including cytochromes, thus disrupting cellular activities such as mitochondrial electron transport processes and energy production. Ciclopirox also appears to modify the plasma membrane of fungi, resulting in the disorganization of internal structures. The anti-inflammatory action of ciclopirox is most likely due to inhibition of 5-lipoxygenase and cyclooxygenase. ciclopirox may exert its effect by disrupting DNA repair, cell division signals and structures (mitotic spindles) as well as some elements of intracellular transport<sup>15</sup>.

#### IUPAC Name

6-cyclohexyl-1-hydroxy-4-methyl-1,2-dihydropyridin-2-one.

#### Empirical formula

$\text{C}_{12}\text{H}_{17}\text{NO}_2$ .

#### Physical state

White, odourless, crystalline powder.

#### Molecular weight

207.269.

#### Solubility

Sparingly soluble in water, very soluble in alcohol and in methylene chloride, slightly soluble in ethyl acetate, practically insoluble in cyclohexane.<sup>15</sup>

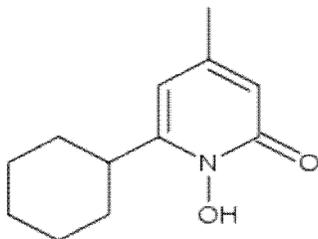


Fig.1: Chemical structure

## EXPERIMENTAL

### Apparatus

A UV/VIS spectrophotometer (Jasco V-630 spectrophotometer) with 1.0 cm matched quartz cells was used for absorbance measurements.

### REAGENTS AND CHEMICALS

1. Working standard stock solution (100 $\mu\text{g}/\text{ml}$ )
2. 0.3% MBTH
3. 0.8%  $\text{FeCl}_3$
4. Distilled water
5. Ethanol

### Preparation of the standard stock solution of the pure drug

100mg of the pure drug was weighed accurately on the analytical balance and transferred carefully to a 100ml volumetric flask. The pure drug in the volumetric flask was then dissolved in ethanol up to the 100ml mark to obtain a 1000 $\mu\text{g}/\text{ml}$  solution to further prepared the working standard solution. (Stock solution A).

### Preparation of the working standard stock solution

From the above standard stock solution A 10ml was pipetted using a 10ml volumetric pipette. This pipetted solution was transferred carefully to another 100ml volumetric flask and dissolved further with ethanol up to the 100ml mark to obtain a 100 $\mu\text{g}/\text{ml}$  solution (Stock solution B). This solution was used for further work.

### Preparation of 0.3% MBTH

The solution was prepared by dissolving 0.3gms of MBTH in 100 ml of distilled water.

### Preparation of 0.8% $\text{FeCl}_3$

0.8g of ferric chloride was dissolved in 100 ml of distilled water.

### Preparation of Standard curve

Standard curve was prepared by using pure Ciclopirox in the concentration range of 2-12  $\mu\text{g}/\text{ml}$  and selecting the absorbance maximum at 662nm.

### Procedure

A series of concentration of 2-12 $\mu\text{g}/\text{ml}$  were prepared of the drug solution from stock solution B in 10 ml volumetric flask. To each appropriately labeled flask 1ml of 0.8%  $\text{FeCl}_3$  was added and kept aside for 15 minutes. Then 1ml of 0.3% MBTH was added. Finally the volume was made upto the mark with ethanol. The absorbance was taken at 662nm against reagent blank. The graph and the absorbance for the above are given in **Table 1** and **Fig No: 3**.

### Method Validation

#### Linearity

Calibration curve was plotted over a concentration range of 2–12  $\mu\text{g}/\text{ml}$  at 662 nm. The graph was plotted of absorbance vs. concentration to give calibration curve, and regression equation and correlation coefficient was calculated and presented in **Table 1** and **Fig No 3**.

**%Recovery (Accuracy)**

Recovery studies by the standard addition method were performed to study the accuracy of the proposed method. Preanalysed samples of Ciclopirox (10 µg/ml) were spiked with 80, 100 and 120 % extra Ciclopirox standard and the mixture were analysed with the proposed method. Accuracy was assessed as the % Recovery at each concentration level. The results were reported in **Table 2**.

**Method precision (% Repeatability)**

Method precision was determined by using sample solution of drug concentrations 2, 4, 6, 8, 10, and 12 µg/ml and it was analyzed six times in a day by the same analyst. The results were reported in **Table 2**.

**Intermediate precision**

Intraday precision was determined by using three different levels of drug concentrations (2, 4, 6 µg/ml) and each level was analysed three times in a day. Same procedure was followed for three different days to study the inter-day precision. The results were reported in **Table 2**.

**Ruggedness**

To establish ruggedness of the proposed method, assays for two different concentrations of Ciclopirox were performed by two different analysts. The results of assays are represented as % Recovery with SD and % RSD showing the ruggedness of the proposed method are illustrated in **Table 2**.

**Robustness**

The absorbance readings of 2 µg/ml were measured at different laboratories using different spectrophotometer by another analyst and the %RSD values obtained to verify their robustness given in **Table 2**.

**Limit of detection and Limit of quantification**

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived

by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:  $LOD = 3.3 \times \sigma/S$  and  $LOQ = 10 \times \sigma/S$

Where,  $\sigma$  = the standard deviation of the response,  $S$  = slope of the calibration curve. The results were displayed in **Table 2**.

**Determination of Ciclopirox from dosage form****Analysis of marketed formulation**

A liquid marketed formulation, **Stieprox(GSK Pharma)** was obtained for all analytical study. Solution equivalent to 1000 µg/ml was pipetted from the marketed formulation and added to a 100ml volumetric flask. The volume was made up to 100 ml using ethanol. The flask was shaken and volume was made up to the mark with ethanol to give a solution of 1000 µg/ml (Stock Solution A). The above solution was filtered. From the above filtrate 10ml was pipetted into a 100ml volumetric flask and made up to the mark with ethanol to obtain 100 µg/ml, (Stock Solution B).

From Stock solution B 1ml was pipetted out into a 10ml volumetric flask. To this 1ml of 0.8% FeCl<sub>3</sub> was added and kept aside for 15 minutes. Then 1ml of 0.3% MBTH was added. Finally the volume was made up to the mark with ethanol. The absorbance was taken at 662nm against reagent blank. The data obtained is given in **Table No: 3**

**RESULT AND DISCUSSION**

This method is based on oxidation followed by coupling of MBTH with Ciclopirox in the presence of ferric chloride to form a green colored chromogen. This is a catalyzed oxidative coupling reaction of MBTH with the drug. Under reaction conditions, on oxidation MBTH loses two electrons and one proton forming an electrophilic intermediate which is the active coupling agent. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The reaction scheme has been shown in **Figure no 4**.

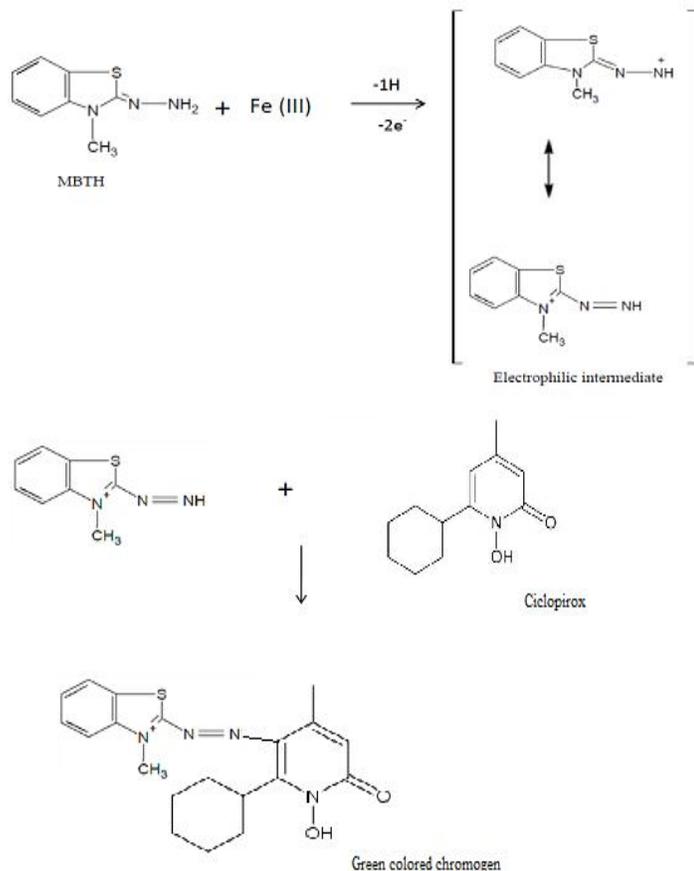


Fig. 4: Reaction scheme for the current method

#### CONCLUSION

The proposed method is simple, selective and reproducible and can be used in the routine analysis of Cyclopirox in bulk drug and formulations with reasonable accuracy and precision.

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Table 1: Absorbance of different concentrations of Cyclopirox at 662 nm

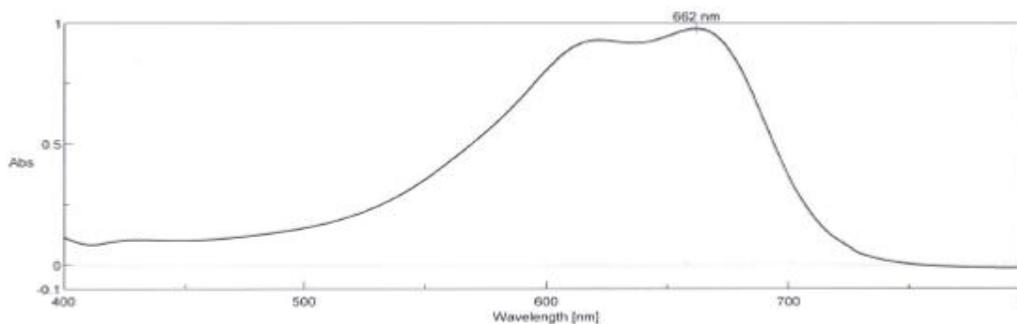
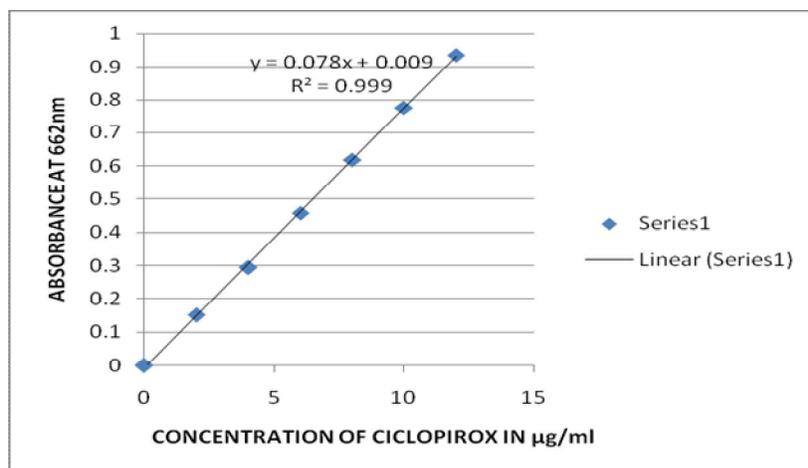
S. No	Volume of working standard of drug (ml)	Concentration in $\mu\text{g/ml}$	Absorbance at 662nm Mean $\pm$ S.D. (n=6)
1	0.2ml	2 $\mu\text{g/ml}$	0.1505 $\pm$ 0.00151
2	0.4ml	4 $\mu\text{g/ml}$	0.2941 $\pm$ 0.001472
3	0.6ml	6 $\mu\text{g/ml}$	0.4570 $\pm$ 0.002191
4	0.8ml	8 $\mu\text{g/ml}$	0.6176 $\pm$ 0.001751
5	1.0ml	10 $\mu\text{g/ml}$	0.7765 $\pm$ 0.001871
6	1.2ml	12 $\mu\text{g/ml}$	0.9630 $\pm$ 0.001414

**Table 2: Statistical data for Ciclopirox**

Parameters	Absorbance of Ciclopirox at 662nm
Linear range ( $\mu\text{g/ml}$ )	2-12 $\mu\text{g/ml}$
Regression equation	$Y=bx+a: 0.078x+0.009$
Slope (b)	0.078
Intercept (a)	0.009
Correlation coefficient ( $R^2$ )	0.999
Limit of detection ( $\mu\text{g/ml}$ )	0.0243
Limit of quantitation ( $\mu\text{g/ml}$ )	0.0661
%Recovery	1)At level-1 (80%)=98.21 $\pm$ 0.190 2)At level-2 (100%)=97.73 $\pm$ 0.472 3)At level-3 (120%)=96.85 $\pm$ 0.220
Repeatability data (%RSD)	0.22%-0.97%
Ruggedness	
Analyst 1	97.91 $\pm$ 0.220- 99.3 $\pm$ 0.360
Analyst 2	98.80 $\pm$ 0.127- 99.73 $\pm$ 0.321
Robustness	
Instrument 1	0.94%
Instrument 2	0.86%
Intermediate precision	
Intraday precision (%RSD)	0.25%- 0.40%
Interday precision (%RSD)	0.22%- 0.76%

**Table 3: Assay results for marketed formulation**

Formulation	Actual concentration of Ciclopirox ( $\mu\text{g/ml}$ )	Amount obtained of Ciclopirox ( $\mu\text{g/ml}$ )	%Ciclopirox
Liquid	10 $\mu\text{g/ml}$	9.68 $\mu\text{g/ml}$	96.8%

**Fig. 2:  $\lambda_{\text{max}}$  for Ciclopirox complex with MBTH****Fig. 3: Standard curve of Ciclopirox at 662nm**

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