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*Research Article*

# DEVELOPMENT AND VALIDATION OF EXTRACTIVE SPECTROPHOTOMETRIC METHOD FOR CLOTRIMAZOLE USING BROMOCRESOL GREEN

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**ABSTRACT**

Rapid, simple, sensitive and accurate spectrophotometric method has been developed for the determination of Clotrimazole in pure and pharmaceutical formulations. This method is based on the formation of chloroform soluble ion pair complex of Clotrimazole with bromocresol green in buffer of pH 3.5 with absorption maximum at 420 nm. Reaction conditions were optimised to obtain the maximum colour intensity. The absorbance was found to increase linearly with increase in concentration of Clotrimazole. Calibration graph was plotted and the correlation coefficient was found to be 0.998. Beer's law was obeyed in the concentration range of 10-60 µg/ml. Various analytical parameters have been evaluated and the results have been validated by statistical data. This method has been successfully applied for the assay of drug Clotrimazole in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. The proposed method is novel, precise and suitable for quality control applications.

**Keywords**: Clotrimazole, Extractive spectrophotometry, Ion pair complex, Chloroform, Validation.

## INTRODUCTION

Clotrimazole is a broad spectrum antifungal agent[1] used for the treatment of a wide variety of fungal skin and vaginal infections[2]. Clotrimazole acts primarily by damaging the permeability barrier in the cell membrane of fungi. Clotrimazole causes inhibition of ergosterol biosynthesis, an essential constituent of fungal cell membranes[3]. Chemically Clotrimazole is 1-((2-Chlorophenyl) diphenyl methyl)-1*H*-imidazole[4] (Figure 1). Literature survey reported that various spectrophotometric[5-9], HPLC[10,11], HPTLC[12] and polarographic[13] methods were developed for estimation of Clotrimazole, but till now there is no simple extractive spectrophotometric method using bromocresol green (BCG) has not been developed. The aim of the present study is to develop and validate a simple, precise, accurate and reproducible extractive spectrophotometric method for analysis of Clotrimazole in the presence of bromocresol green as per ICH guidelines[14].

## MATERIALS AND METHODS

### Instruments

The analysis was performed in 10 mm quartz cells using T60U UV-Visible spectrophotometer (PG Instruments Ltd., England) with a fixed 2 nm spectral bandwidth and UV-Win 5 software v5.1.1 was used for all absorbance measurements. Shimadzu BL 2208 electronic balance was used for weighing the sample.

### Preparation of buffer solution

Dissolve 3.4 gm of potassium dihydrogen phosphate in water and dilute to 50 ml with the same solvent. Adjust the pH to 3.5 with orthophosphoric acid.

**Preparation of 0.1% bromocresol green**
Weigh accurately 0.1 mg of BCG, dilute in 10 ml of water and make up to 100 ml with same solvent to get the final concentration of 0.1%.

**Preparation of stock solution of Clotrimazole**
1 mg/ml solution of Clotrimazole was prepared by dissolving 100 mg of Clotrimazole in 100 ml of distilled water. From this solution 1.0 ml was taken into a 10 ml volumetric flask and diluted to the volume with distilled water to produce a concentration of 100 µg/ml.

**Optimization of the reaction conditions**
The optimum conditions for quantitative estimation of the drug Clotrimazole were established via a number of preliminary experiments.

**Choice of organic solvent**
A number of organic solvents such as chloroform, carbon tetrachloride, dichloromethane, benzene and toluene were examined for the extraction of ion pair complex in order to provide an applicable extraction procedure. Chloroform was preferred for its selective extraction of ion pair complex from the aqueous solution. Shaking time of 0.5-4 min provided a constant absorbance and hence 2 min was used as an optimum shaking time throughout the experiment.

**Selection of pH of buffer**
The drug reagent complex extracted into the organic solvent should be stable. In order to determine the pH in which the complex formed is stable, various trials were carried out using phosphate buffers of pH 2, 3, 4, 5, 7. The maximum stability was observed with pH 3.5 buffer as evidenced by its maximum absorbance. Hence pH 3.5 buffer was selected to develop the method and a volume of 1.0 ml was used for the ion pair formation.

**Effect of dye concentration**
The effect of dye concentration on the intensity of the colour developed at the selected wavelength and constant Clotrimazole concentration was critically examined using different milliliters of reagent. The results indicated that the maximum absorbance was found with 1.0 ml of reagent and beyond which the absorbance became constant. Therefore 1.0 ml of dye stuff was used for ion pair formation throughout the experiment.

**Extraction procedure**
Into a series of 50 ml separating funnel appropriate volume of Clotrimazole solution was placed followed by 1.0 ml of buffer and 1.0 ml of BCG reagent and shake well. Then 10 ml of chloroform was added to each funnel. The contents were shaken for 2 min and the two layers were allowed to separate. Extract was scanned between 400 to 800 nm. The λ max of Clotrimazole was found to be 420 nm. The spectrum was shown in Figure 2.

**Procedure for calibration curve**
The calibration curve was constructed by considering the absorbance measured at 7 concentrations levels. The amount of drug was computed either from calibration curve or from regression equation. The results were furnished in Table 1. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Figure 3. Results of regression analysis were given in Table 2.

**Validation**
Validation is one of the most important steps in method development for analytical determinations. The main validation parameters such as linearity and range, accuracy and precision, LOD, LOQ, ruggedness were evaluated for the developed method.

## RESULTS AND DISCUSSION

**Linearity and range**
Calibration graph was constructed by measuring the absorbance at seven concentration levels, which showed linear response of absorbance in relation to concentration of Clotrimazole over the range of 10-70 µg/ml. Regression analysis of calibration graph indicated linear relationship.

**Accuracy**
Accuracy was studied through recovery experiments at the level of 3 concentrations 40 µg/ml, 50 µg/ml and 60 µg/ml. All the 3 concentrations were prepared and subjected to the extraction procedure. % recovery was calculated for the 3 concentrations. The results were tabulated in Table 3.

**Precision- Repeatability**
Six individual preparations of Clotrimazole drug substance were prepared with a target concentration of about 40 µg/ml by following the extraction procedure and their absorbances were measured and their % RSD was calculated. The results were displayed in Table 4.

**Specificity**
The specificity of the method was demonstrated by establishing a lack of interference from the diluent blank. Blank solution was prepared and its absorbance was measured. No interference was observed with the blank.

**LOD & LOQ**
LOD & LOQ were calculated by using the relationship $\frac{3.3\sigma}{s}$ and $\frac{10\sigma}{s}$ respectively. Where σ is the standard error of estimate, s is slope. Calculated values of LOD & LOQ for Clotrimazole were found to be 0.21 and 0.60 µg/ml respectively.

**Ruggedness**
Analyst variation and instrument variation were observed by taking 50 µg/ml and their % RSD was calculated. Results were tabulated in Table 5 and 6.

**Robustness**
Wavelength variation was checked by taking 60 µg/ml solution at 3 different wavelengths such as 419, 420 and 421 nm and their % RSD was calculated. Results were furnished in Table 7.

**Procedure for assay of drugs in dosage forms**
Ten tablets of commercial tablets of Clotrimazole were accurately weighed and powdered. An amount of powder equivalent to 50 mg was weighed separately and made upto 50 ml with distilled water. The solution was filtered and subjected to recommended procedure for the determination. The results were displayed in Table 8.

The detection and quantitation limits were found to be 0.21 and 0.60 µg/ml respectively. The repeatability of the proposed procedure was checked by performing six replicate determinations of Clotrimazole. The percent relative standard deviation (% RSD) and recoveries were found to vary over the range of 0.523% and 98.03-99.99% respectively. The accuracy of the proposed method was demonstrated by recovery experiments, which were carried out by taking a fixed amount of pure drug to the sample matrix. The summary of all the results were tabulated in Table 9.

**CONCLUSION**
In the present study the maximum colour development of Clotrimazole with BCG ion-pair complex was instantaneous. In terms of simplicity, rapidity, sensitivity and free from interferences the method could be considered superior. The results indicate that the proposed methods are precise, accurate and linear. The method validation criteria for all parameters evaluated in line with ICH guidelines and it has found to be reliable, simple and rapid. So, it can carry out for routine analysis in pharmaceutical formulations. The method validation criteria for all parameters evaluated as per ICH guidelines it has found to be reliable.

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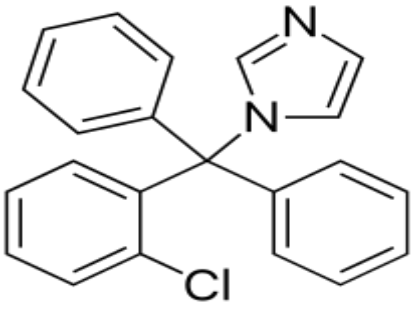


**Figure 1: Structure of Clotrimazole**

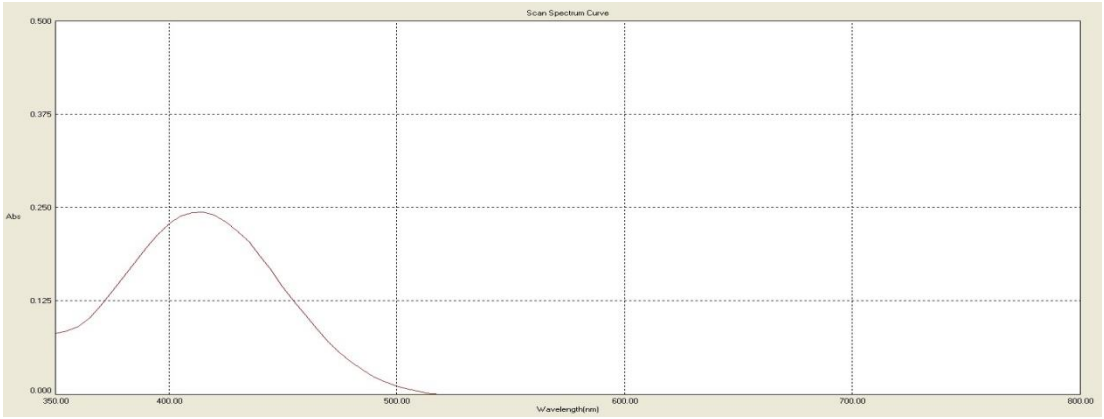


**Figure 2: Spectrum of Clotrimazole**

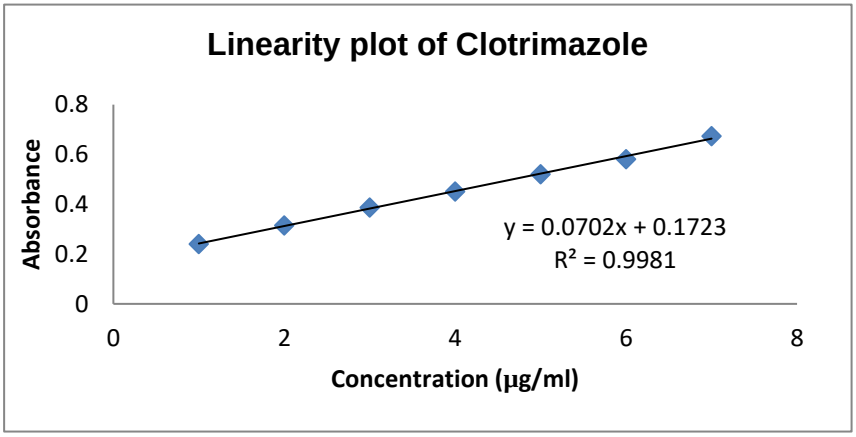


**Figure 3: Standard calibration graph**

**Table 1: Linearity Results**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 10 | 0.241 |
| 20 | 0.315 |
| 30 | 0.387 |
| 40 | 0.451 |
| 50 | 0.521 |
| 60 | 0.582 |
| 70 | 0.623 |

**Table 2: Statistical parameters of calibration graph**

| Parameter | Result |
|---|---|
| Slope | 0.070 |
| Intercept | 0.172 |
| Regression equation | Y= 0.070x + 0.172 |
| Regression coefficient | 0.998 |
| Variance | 0.023026 |
| Beers law limit | 10-70 µg/ml |

**Table 3: Accuracy**

| Concentration level (µg/ml) | Absorbance | % Recovery |
|---|---|---|
| 40 | 0.4465 | 98.03% |
| 50 | 0.5180 | 98.80% |
| 60 | 0.5916 | 99.99% |

**Table 4: Repeatability**

| Concentration (µg/ml) | Absorbance |
|---|---|
| 40 | 0.456 |
| 40 | 0.453 |
| 40 | 0.459 |
| 40 | 0.449 |
| 40 | 0.447 |
| 40 | 0.456 |
| Average | 0.4533 |
| Standard deviation | 0.00459 |
| % RSD | 1.01257 |

**Table 5: Analyst variation**

| Concentration (µg/ml) | Analyst-1 | Analyst-2 |
|---|---|---|
| 50 | 0.521 | 0.534 |
| 50 | 0.532 | 0.536 |
| 50 | 0.528 | 0.549 |
| 50 | 0.527 | 0.538 |
| 50 | 0.518 | 0.54 |
| 50 | 0.524 | 0.539 |
| Average | 0.525 | 0.539333 |
| Standard deviation | 0.00506 | 0.005203 |
| % RSD | 0.963742 | 0.964629 |

**Table 6: Instrument variation**

| Concentration (µg/ml) | Instrument-1 | Instrument-2 |
|---|---|---|
| 50 | 0.521 | 0.531 |
| 50 | 0.532 | 0.526 |
| 50 | 0.528 | 0.528 |
| 50 | 0.527 | 0.539 |
| 50 | 0.518 | 0.524 |
| 50 | 0.524 | 0.539 |
| Average | 0.525 | 0.531167 |
| Standard deviation | 0.00506 | 0.006494 |
| % RSD | 0.963742 | 1.222514 |

**Table 7: Wavelength variation**

| Concentration (µg/ml) | 419 nm | 420 nm | 421 nm |
|---|---|---|---|
| 60 | 0.6126 | 0.591 | 0.6064 |
| 60 | 0.6124 | 0.5824 | 0.5923 |
| 60 | 0.6271 | 0.5874 | 0.5934 |
| Average | 0.617367 | 0.586933 | 0.597367 |
| Standard deviation | 0.00843 | 0.004319 | 0.007842 |
| % RSD | 1.365462 | 0.73585 | 1.31283 |

**Table 8: Assay**

| Label claim (mg) | Amount found (mg) | Assay |
|---|---|---|
| 20 | 20.11 | 100.56%±0.64 |
| 20 | 20,34 | 101.70%±0.93 |

**Table 9: Summary of Results**

| Parameters | Value |
|---|---|
| λmax (nm) | 420 |
| Beer's law limits (µg/ml) | 10-70 |
| Molar absorptivity (g/100ml) | 30029.384 |
| Linear regression equation<br>Intercept (a)<br>Slope (b) | Y=0.070x + 0.172<br>0.172<br>0.070 |
| Correlation coefficient (r) | 0.998 |
| Variance ($S_o^2$) | 0.023026 |
| Relative standard deviation (%) | NMT 2% |
| Recovery (%) | 98-102% |
| LOD | 0.21 µg/ml |
| LOQ | 0.60 µg/ml |

**REFERENCES**


1. Sawyer PR, Brogden RN, Pinder KM, Speight T, Avery GS. Clotrimazole: A review of its antifungal activity and therapeutic efficacy. Drugs. 1975; 9: 424-447.
2. Ritter W. Pharmacokinetic fundamentals of vaginal treatment with Clotrimazole. American Journal of Obstetrics and Gynecology. 1985; 152(7): 945-947.
3. Hashem FM, Shaker DS, Ghorab MK, Nasr M, Ismail A. Formulation, characterization and clinical evaluation of microemulsion containing Clotrimazole for topical delivery. AAPS PharmSciTech. 2011; 12(3): 879-886.
4. Crowley PD, Gallagher HC. Clotrimazole as a pharmaceutical: Past, present and future. Journal of Applied Microbiology. 2014; 117(3): 611-617.
5. Bedair MM, Koranty MA, Elsayed MA, Fahmy OT. Derivative spectrophotometric determination of Clotrimazole in single formulations and in combination with other drugs. Journal of AOAC International. 1989; 72(3): 432-435.
6. Osama HA, Pakinaz YK. Spectrophotometric determination of Clotrimazole in bulk drug and dosage forms. Talanta. 1993; 40(8): 1289-1294.
7. Ramachandra Rao PSNH, Siva Rao T, Viplava Prasad U, Sastry CSP. Extractive spectrophotometric determinations of Clotrimazole in formulations. Asian Journal of Pharmacy. 2002; 14(1): 190-196.
8. Radoslaw JK, Jan K. Determination of azole antifungal medicines using zero-order and derivative UV spectrophotometry. Acta Poloniae

Pharmaceutica. 2009; 66(1): 19-24.
9. Sajid M, Zaheer A, Muhammad A, Fiza N, Abrar H, Naresh K. Method development and validation for the estimation and evaluation of Clotrimazole (an-antifungal drug) in tablet preparation by UV-VIS spectroscopy. International Journal of Pharmaceutical Sciences Review and Research. 2015; 32(2): 55-58.
10. Renata H, Hana S, Ludmila M, Petra S, Petr S. Development and validation of HPLC method for determination of Clotrimazole and its two degradation products in spray formulation. Talanta. 2007; 73(3): 483-489.
11. Prashanth K, Jagadish PC, Muddukrishna, Krishnamurthy B. Development and validation of stability indicating HPLC method for Clotrimazole lozenges formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(1): 121-129.
12. Parul P, Ankita M. Development and validation of HPTLC method for the estimation of Clotrimazole in bulk drug and tablet formulation. Indian Journal of Pharmaceutical Sciences. 2009; 71(4): 451-454.
13. Pereira FC, Zanoni MVB, Guaratini CCI, Fogg AG. Differential pulse polarographic determination of Clotrimazole after derivatization with procion red HE-3B. Journal of Pharmaceutical and Biomedical Analysis. 2002; 27(1-2): 201-208.
14. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: Text and methodology, Q2(R1). International Conference on Harmonization. 2005; 1-13.