Development and Validation of UV Spectroscopic Method for Estimation of Climbazole in Climbazole Shampoo

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ABSTRACT

To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Climbazole in Climbazole shampoo. The drug is freely soluble in organic solvents such as Chloroform and Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on Melting Point Apparatus of Equiptronics. It showed absorption maxima were determined in Methanol: Water (50:50). The drug obeyed the Beer’s law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Climbazole in shampoo dosage form and also validated as per ICH guidelines. The drug is freely soluble in organic solvents such as Methanol, Chloroform. So, the Analytical Grade Methanol: Water is used as a diluent in equal proportion for method. The melting point of Climbazole was found to be 93-94˚C (uncorrected). It showed absorption maxima 256 nm in Methanol: Water (50:50). On the basis of absorption spectrum the working concentration was set on 6µg/ml (PPM). The linearity was observed between 2-10 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 98.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.47%. A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Climbazole in shampoo dosage form. The method could be considered for the determination of Climbazole in quality control laboratories.

Keywords: Climbazole, Development, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

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INTRODUCTION

Emerging pollutants such as personal care products can reach the environment via effluents of waste water treatment plants (WWTP) and digested sludge. Only recently, the anti-dandruff agent and antimycotic Climbazole were detected for the first time in a WWTP effluent with concentrations up to 0.5µg/L [1, 2]. Despite its mode of action as C14-demethylase inhibitor (DMI) fungicide and thus its high efficacy against fungi, knowledge on its potential environmental impact is lacking. Chemically 1-(4-chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-dimethylbutan-2-one is a ketone that is butan-2-one substituted by a 4-chlorophenoxy and a 1H-imidazol-1-yl group at position 1 and 2 methyl groups at position 3 [3]. It is a member of mono chloro benzenes, a member of imidazoles, aromatic ether, a ketone and hemiaminal ether. Climbazole is white solid powder having an imidazole antifungal agent that can provide anti-dandruff benefits when incorporate into shampoo [4]. Climbazole is a topical antifungal agent commonly used in the treatment of human fungal skin infections such as dandruff and eczema. Climbazole has shown a high in vitro and in vivo efficacy against Malassezia spp. that appears to play an important role in the pathogenesis of dandruff [5]. Its chemical structure and properties are similar to other fungicides such as ketoconazole and miconazole [6]. It is most commonly found as an active ingredient in OTC anti-dandruff and anti-fungal products, including shampoos, lotions and conditioners. It may be accompanied by other active ingredients such as zinc pyrithione or triclosan [7, 8].

Figure 1: Chemical Structure of Climbazole

Major side effect of Climbazole may cause localized irritation of the skin with symptoms including redness, rashes and itching [9].

From literature review it’s found that no method was reported on estimation of Climbazole on any dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Climbazole in any dosage forms. So, the work has to be decided.

MATERIALS AND METHOD

Instruments:

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair
Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

**Materials:**
Climbazole was obtained as a gift sample. Climbazole shampoos were procured from local pharmacy. Milli-Q water and Analytical grade Methanol was used throughout the experiment. Freshly prepared solutions were employed.

**Diluent:**
Analytical Grade Methanol and Milli-Q Water in equal proportion are used as a diluent.

**Method development** \[^{10,11}\]:

**Determination of \(\lambda\) max (7 PPM)**
20 mg weighed amount of Climbazole was dissolved into 200 ml of volumetric flask with diluent. Pipette out 7 ml and added in 100 ml of volumetric flask, dissolved and diluted up to the mark with diluent. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

![Figure 2: Calibration Curve](image)

**Preparation of Working concentration**

**Preparation of Standard stock solution:**
Standard stock was prepared by dissolving 20 mg of Climbazole in 200 ml of diluent to get concentration of 100\(\mu\)g/ml (PPM).

**Preparation of Standard solution:**
Pipette out 6 ml from standard stock solution and diluted up to 100 ml with diluent to get concentration of 6\(\mu\)g/ml (PPM).

**Procedure for UV reading**

**Blank Solution:** (For Auto zero)
Fill the cuvette with Diluent. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Standard Solution:**
Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Sample Solution:**
Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Procedure for sample preparations**[11]
For analysis of commercial formulations; Climbazole shampoo is taken. The shampoo equivalent to 20 mg of Climbazole was accurately weighed and transferred into the 200 ml of volumetric flask, added 150 ml of diluent; the solution was sonicated for 20 min. After sonication cool the flask and diluted up to 200 ml with diluent. Filter the solution through whatmann filter paper, if required. Pipette out 6 ml of the above solution and diluted up to 100 ml with diluent. The absorbance was measured at 256 nm. The absorbance was recorded:

**Table 1: Absorbance of Dosage Form**

| Sr. no. | Sample                              | Absorbance |
|---------|-------------------------------------|------------|
| 1       | Blank Solution (Auto zero)          | 0.0000     |
| 2       | Standard Solution                   | 0.7140     |
| 3       | Sample Solution                     | 0.7112     |

**Table 2: Dosage Form Specifications with % Assay**

| Sr No | Company                  | M.D.   | E.D.   | Batch No.   | Assay (%) |
|-------|--------------------------|--------|--------|-------------|-----------|
| 1     | Surya Pharma Pvt. Ltd     | 02/2019| 03/2022| CKS08764A   | 99.61     |

**Method of validation**[12, 13, 14]
The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

**Linearity:**
The linearity of the proposed assay was studied in the concentration range 2 - 10 PPM at 256nm. The calibration data showed a linear relationship between concentrations.

**Table 3: Linearity Studies**

| Sr. no. | Sample Concentration | Absorbance |
|---------|----------------------|------------|
| 1       | 2 PPM                | 0.2311     |
| 2       | 4 PPM                | 0.4549     |
Accuracy:
To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with analytical grade water and measuring the absorbance at 256nm.

| Spectrophotometric Method | Accuracy (%) | Qty weighed (mg) | Qty found (mg) | Recovery (98-102%) |
|---------------------------|--------------|------------------|----------------|-------------------|
| 80                        | 0.8          | 0.79             |                | 98.75             |
| 100                       | 1            | 0.98             |                | 98.00             |
| 120                       | 1.2          | 1.19             |                | 99.17             |

Precision:
The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

| Sr. No. | Sample Solution       | Absorbance |
|---------|-----------------------|------------|
| 1       | Sample Solution 1     | 0.7142     |
| 2       | Sample Solution 2     | 0.7185     |
| 3       | Sample Solution 3     | 0.7192     |
| 4       | Sample Solution 4     | 0.7110     |
| 5       | Sample Solution 5     | 0.7155     |
| Mean    | 0.7157                |
| SD      | 0.0033                |

| % RSD   |
|---------|
| 0.4659  |

Ruggedness:
Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

| Sr. No. | Analyst   | Results | Mean  | % Assay | % RSD  |
|---------|-----------|---------|-------|---------|--------|
| 1       | Analyst 1 | 0.7052  | 0.7079| 99.14   | 0.4630 |
|         |           | 0.7105  |       |         |        |
| 2       | Analyst 2 | 0.7118  | 0.7125| 99.79   |        |
|         |           | 0.7132  |       |         |        |
RESULTS AND DISCUSSION

Solubility of Climbazole
Solubility test was passed as per criteria.

| Sr. no. | Title       | Result       |
|---------|-------------|--------------|
| 1       | Chloroform  | Freely Soluble|
| 2       | Methanol    | Freely soluble |

Melting point of Climbazole
The Melting Point of Climbazole was found to be 93-94˚C (uncorrected).

Results for linearity for assay method of Climbazole
The linearity of method was determined at concentration level ranging from 2 - 10μg/ml (PPM). The correlation coefficient value was found to be $R^2 = 0.9994$.

![Figure 3: Climbazole Standard Curve](image)

Results for accuracy for assay method of Climbazole
The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

Results for precision for assay method of Climbazole
The % RSD for different sample of precision was found to be 0.4659 and it is within acceptance criteria represented in Table - 5.

Results for ruggedness for assay method of Climbazole
The %RSD for different sample of ruggedness was found to be 0.4630 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Climbazole in Climbazole shampoo has been developed. From the spectrum of Climbazole, it was found that the maximum absorbance was 256 nm in diluent. A good linear relationship was observed in the concentration range of 2-10 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Climbazole in shampoo dosage forms. Hence, the method could be considered for the determination of Climbazole in quality control laboratories for Climbazole Shampoo.

ABBREVIATIONS
1. PPM - Parts per Million
2. nm – Nanometer
3. OTC – Over The Counter
4. WWTP - Waste Water Treatment Plants
5. DMI - Demethylase Inhibitor
6. UV - Ultra violet
7. GC - Gas Chromatography
8. ICH - International Council for Harmonization
9. RSD - Relative Standard Deviation
10. SD - Standard Deviation
11. Qty - Quantity
12. °C - Degree Celsius
13. Fig. – Figure
14. Qty - Quantity
15. % - Percentage
16. M.D. - Manufacturing Date
17. E.D. - Expiry Date

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