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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF CLIMBAZOLE

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ABSTRACT: Climbazole is a topical antifungal agent commonly used to treat human fungal skin infections such as dandruff and eczema. The goal of research work was to develop and validate a simple, precise, quick, and highly efficient UV spectrophotometric approach for quantifying Climbazole in bulk and shampoo dosage form. The quantification was completed using a twin beam UV spectrophotometer at 222 nm and ethanol (99.9%) as the solvent for the estimate. The Climbazole calibration curve exhibit strong correlation coefficient (R^2=0.9938) and high linearity in the range of 5–25 µg/ml concentrations. The accuracy was found to be between 98.21%- 99.83%. The accuracy of the approach was proved by the percent relative standard deviation being less than 2.0%. It was observed that the intraday and interday precision found within acceptable ranges. The method's sensitivity was assessed using the detection limit and quantification limit, which were discovered to be 2.37 µg/ml and 7.20 µg/ml, respectively. In this paper, we introduce a UV-spectrophotometric technique for investigating Climbazole using ethanol as solvent. The suggested method demonstrated excellent selectivity, specificity, and linearity in accordance with ICH Q2 (R1) requirements. Also, marketed pharmaceutical formulations were used to show the developed approach's efficiency and a high recovery rate. It is obvious that the suggested method will serve as a normative approach for the regular testing of Climbazole in pharmaceutical formulations and bulk dosage forms.

INTRODUCTION: Seborrheic dermatitis is a superficial fungal skin condition that mostly affects regions with a lots of sebaceous glands. Malassezia yeasts and sebum production may be associated, according to some [1]. Most antifungals used to treat seborrheic dermatitis belong to the azole class. Treatment with antifungal agents lowers the number of Malassezia on the skin.

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Chemically, Climbazole is identified as 1-(4-chlorophenoxy) – 1 - (1H – imidazol – 1 - yl) - 3, 3 – dimethylbutan – 2 - one **Fig. 1**. Its chemical composition is $C_{15}H_{17}ClN_2O_2$, and its molecular weight is 292.76 g/mol.

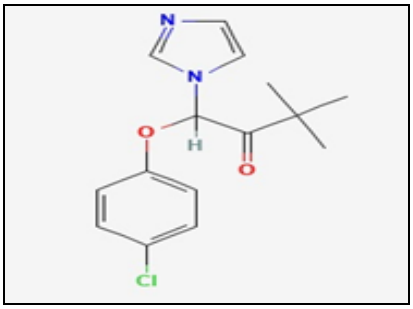

FIG. 1: STRUCTURE OF CLIMBAZOLE

An imidazole antifungal drug called Climbazole (CBZ) treats dandruff in products like shampoos and conditioners. It prevents the enzyme lanosterol 14-alpha-demethylase from converting lanosterol in fungal cell membranes to ergosterol. Lack of ergosterol enables the membrane to become more permeable, which causes cell lysis and death [2]. Along with having antibacterial activity, Climbazole also has antidandruff activity.

Climbazole has been Estimated by using UV Spectrophotometric Techniques with a Methanol: water (50:50) solvent solution [3]. Though methanol is hazardous, it won't be incorporated in pharmaceutical preparations for topical use.

There is no proof that UV Spectrophotometry detected the substance in an ethanol solvent. Therefore, the current study aims to create a straightforward, exact, and specific UV Spectrophotometric approach for the quantitative analysis of Climbazole in ethanol solvent. To validate the established technique, the International Conference on Harmonization (ICH) guidelines under section Q2 (R1) were applied [4].

MATERIALS AND METHODS:

Materials: Climbazole API was procured from Tokyo Chemical Industry Japan with 98% w/w assay value and was used without further purification. Ethanol (99.9%) was purchased from Changshu Hongsheng Fine Chemicals. For the planned study, only analytical-grade compounds were used.

Instruments: The spectroscopic examination used a double beam UV-Visible Spectrophotometer (1800, Shimadzu Japan) with matching quartz cells with 10 mm path length.

Methods:

Selection of Solvent: Ethanol was selected as the ideal solvent for spectrophotometric analysis of Climbazole [5].

Standard Stock Solution Preparation: A stock solution was made by dissolving precisely weighed Climbazole (10mg) in ethanol (10 ml). The final Climbazole concentration (100 µg/ml) was prepared further by pipetting (1 ml) diluting to 10 ml ethanol [6].

Determination of Wavelength of Maximum Absorption (λ_{max}): Adequate volume of Climbazole (2 ml) from standard stock solution (100 µg/ml) was added into volumetric flask (10 ml) and diluted with ethanol leading to a concentration of 20 µg/ml. Further, the final solution was exposed to ultraviolet light in the range of 200 nm to 400 nm to realize the maximum absorption wavelength [7, 8]. Climbazole's maximum absorbance wavelength was discovered to be 222 nm **Fig. 2.**

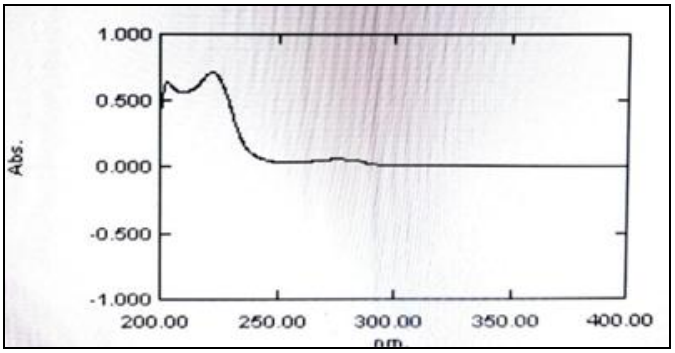

FIG. 2: SPECTRUM OF CLIMBAZOLE IN THE RANGE OF 200 TO 400 NM

Analytical Method Validation: UV method validation was accomplished through the use of ICH Q2(R1) guidelines for quantifying Climbazole. We reassessed the linearity, accuracy, precision, robustness, detection limit (LOD) and quantification limit (LOQ) [9].

Preparation of Calibration Curve: A five-diverse calibration standard reflecting strengths of 5, 10, 15, 20, and 25 µg/ml was created using a stock solution of 100 µg/ml to create the calibration curve. The maximum absorbance of each calibration standard was calculated using the fixed wavelength measuring mode at 222 nm. The calibration curve depicting absorbance vs. concentration were plotted in Microsoft Excel 2016 [10].

Linearity: The linearity was demonstrated by examining 5 different levels of the calibration curve in the 5–30 µg/ml range. At 222 nm, each solution's absorption against ethanol was measured. Based on the examination of calibration standards, calibration curves containing absorbance *vs*. concentration were plotted and linear least square regression analysis was performed.

Correlation coefficient square value was thought to be a key aspect in proving the suggested method's linearity [5, 10].

Range: According to reports, the range of the suggested UV method is the region between the higher and lower concentration limits with preferable linearity [10].

Accuracy: A definite quantity of standard stock solution was transferred to the previously tested sample solutions at 80%, 100%, and 120%. The proposed method was used to re-examine the solutions.[7]An analysis of recovery data at the three-level of percentage addition was used to determine the suggested method's accuracy [4].

Precision: The intraday and interday changes in the method's precision were observed. The intraday precision was ascertained by analysing the 10 µg/ml of Climbazole solution thrice on the same day. By examining the 10 µg/ml of Climbazole solution daily for three days a week, interday precision was calculated [3, 7]. The standard deviation (SD) and relative standard deviation (RSD) were calculated.

Limit of Detection (LOD): In order to assess the limit of detection (LOD), solutions with varying concentrations between 5-10 µg/ml were prepared.

The formula for the limit of detection:

$$LOD = 3.3\ \sigma / S$$

Where, σ = standard deviation of regression line and S = slope of the calibration curve.

Limit of Quantification (LOQ): The formula for calculating the limit of quantification is:

$$LOQ = 10\ \sigma / S$$

Where, σ = standard deviation of the regression line and S = slope of the calibration curve.

Robustness: The ability of an analytical technique to stay unaffected by a slight change in the method parameters is a measure of its robustness, which also reveals its stability throughout the normal stage. Wavelength variations accomplish the determination of robustness. The absorbance readings for Climbazole were 10 µg/ml at 220 nm, 222 nm, and 224 nm [9].

Ruggedness: Two separate analysts examined A sample of Climbazole using the UV Spectrophotometry method. The outcome was given as a % RSD [10].

Assay:

Preparation of Marketed Sample Solution: Ciola shampoo containing 0.5% Climbazole was taken for examination of commercial formulations. The shampoo containing 5 mg of Climbazole was precisely weighed, and 20 ml of ethanol was transferred before the solution was sonicated for 60 minutes. Filter the solution using whatmann filter paper. Pipette out 0.5 ml of the above solution, then diluent it up to 10 ml. At a wavelength of 222 nm, the absorbance of this marketed sample solution and standard 5 µg/ml solution was measured and recorded [11, 12].

RESULTS AND DISCUSSION: The newly established analytical technique was developed, optimized approved and used for the quantitative evaluation of Climbazole as a pure drug.

Linearity: Fig. 3 displays Climbazole's UV spectra and calibration curve into the ethanol at 222 nm. Climbazole's linearity was observed between 5-30 µg/ml, having a correlation coefficient of 0.9938. The linearity of Climbazole was depicted in **Table 1** [13].

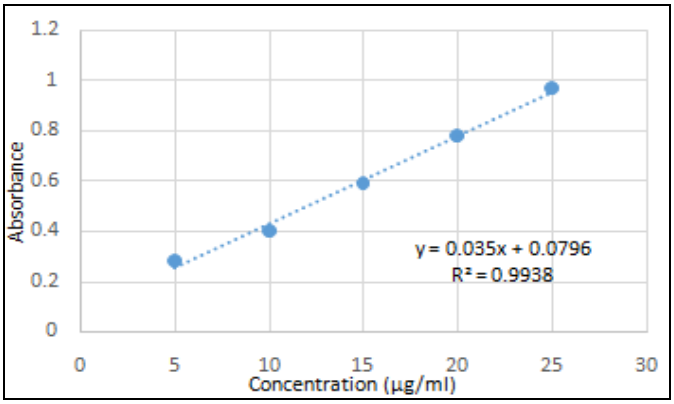

FIG. 3: CALIBRATION CURVE OF CLIMBAZOLE

TABLE 1: LINEARITY OF CLIMBAZOLE

Concentration (µg/ml)	Absorbance value
5	0.282
10	0.401
15	0.591
20	0.779
25	0.967
Regression equation	y = 0.035x + 0.0796
R^2	0.9938

Range: The linearity has been observed in the 5-30 µg/ml range.

Accuracy: Recovery studies were used to determine accuracy for the UV Climbazole technique. Mean recovery of Climbazole was reported to be 98.21% at 80% standard addition, 99.83% at 100% standard addition, and 98.93% at 120% standard addition. According to the Climbazole recovery study, the % RSD was observed to be less than 2, as indicated in **Table 2**. The UV method was shownto be quite effective based on the findings of accuracy studies, with a percent recovery range of 98.21 to 99.83% and an RSD considerably below 2% [10].

TABLE 2: ESTIMATION OF ACCURACY BY % RECOVERY METHOD

Sr. no.	Concentration (%)	Sample conc. (µg/ml)	Amount added (µg/ml)	% Recovery	Statistical analysis
1	80	5	4	98.08	
2	80	5	4	98.40	% RSD =
3	80	5	4	98.15	98.21%
4	100	5	5	99.50	
5	100	5	5	101.04	% RSD =
6	100	5	5	98.60	99.83%
7	120	5	6	99	
8	120	5	6	99.80	% RSD
9	120	5	6	98.01	=98.93%

Conc.: Concentration, RSD: Relative standard deviation.

Precision: The proposed approach was demonstrated to be accurate as the average %RSD values for the intraday and interday precision studies were found to be 0.50% and 0.88%, respectively **Table 3** and **Table 4** [14].

TABLE 3: INTRADAY PRECISION STUDIES OF CLIMBAZOLE

Sample no.	Concentration (µg/ml)	Absorbance			% RSD	Average % RSD
		Morning	Afternoon	Evening		
1	10	0.470	0.470	0.472	0.24%	
2	10	0.475	0.478	0.482	0.73%	0.50%
3	10	0.480	0.482	0.485	1.35%	

RSD: Relative standard deviation.

TABLE 4: INTERDAY PRECISION STUDIES OF CLIMBAZOLE

Sample no.	Concentration (µg/ml)	Absorbance			% RSD	Average % RSD
		Day 1	Day 2	Day 3		
1	10	0.431	0.435	0.440	1.03%	
2	10	0.452	0.455	0.460	0.88%	0.88%
3	10	0.482	0.484	0.489	0.74%	

RSD: Relative standard deviation.

Limit of Detection and Limit of Quantitation: According to **Table 5**, the LOD and LOQ of the established UV technique were determined to be 2.37 and 7.20 µg/ml, respectively.

TABLE 5: EVALUATION DATA OF LOD AND LOQ

Drug	LOD (µg/ml)	LOQ (µg/ml)
Climbazole	2.37	7.20

LOD: Limit of detection, LOQ: Limit of quantitation.

Robustness: The analysis of the Climbazole solution in ethanol at various wavelengths (±2 nm) demonstrates the robustness of the suggested approach by demonstrating a non-significant influence of the absorption level. **Table 6** displays the robustness results of a study. % RSD was determined [15, 16].

TABLE 6: ROBUSTNESS STUDY

Sample no.	Concentration (µg/ml)	Wavelength			% RSD	Average % RSD
		220 nm	222 nm	224 nm		
1	10	0.429	0.430	0.439	1.27%	
2	10	0.427	0.430	0.467	0.48%	1.05%
3	10	0.467	0.476	0.472	1.40%	

Ruggedness: Table 7 demonstrates that changing analysts did not significantly alter the outcome, confirming the robustness of the presented study.

TABLE 7: RUGGEDNESS STUDY

Analysts	Sample no.	Concentration (µg/ml)	Absorbance	Statistical analysis
Analyst 1	1	10	0.421	Mean ± SD = 0.4215±0.0007 % RSD = 0.167
	2	10	0.422	
Analyst 2	1	10	0.452	Mean ± SD = 0.4535±0.0021 % RSD = 0.467

Assay: The assay of the commercially available product was observed to be 98% **Table 8.**

TABLE 8: ANALYSIS OF MARKETED FORMULATION

Sr. no.	Sample	Absorbance	% Assay
1	Standard solution	0.254	98%
2	Marketed solution	0.249	

CONCLUSION: The determination of Climbazole using UV spectroscopic analysis has been established and validated, as stated in section Q2 (R1) of the International Conference on Harmonization (ICH) guidelines. The technique was discovered to be straightforward, dependable, more accurate, and precise with lower detection limits, more exact quantification, and higher sensitivity. The techniques were discovered to be quick and easy. The %RSD in the validation parameters of both approaches was not more than 2%. The accuracy of existing procedures was checked by performing accuracy parameters that revealed results within the range. Using intraday and interday precision tests, the precision of current procedures was verified. Test findings show that UV spectroscopy is one of the best methods for quantifying Climbazole.

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CONFILICTS OF INTEREST: Nil

REFERENCES:

1. Gupta AK, Nicon K and Batra R: Role of antifungal agents in the treatment of seborrheic dermatitis. American Journal of Clinical Dermatology 2004; 5(6): 417-22.
2. Gupta AK, Madzia SE and Batra R: Etiology and management of seborrheic dermatitis. Dermatology 2004; 208(2): 89-93.
3. Patil RN, Patil PA, Patil SR and Sawale A: Development and validation of UV spectroscopic method for estimation of Climbazole in climbazole shampoo. American Journal of Pharmtech Research 2020; 10(1): 241-49.
4. Shrivastava S and Kaur CD: Development and validation of novel UV spectrophotometric method for the determination of Mebendazole in pharmaceutical formulation. International Journal of Pharmaceutical Sciences 2021; 12(4): 2317-22.
5. Shinde M, Jondhale V, Pingale AP, Dhikale GK, Derle DV and Wagh MP: Development and validation of RP-HPLC method for simultaneous estimation of Amlodipine and Valsartan in its bulk and tablet dosage form by using the quality-by-design approach. International Journal of Pharmaceutical Sciences and Research 2023; 14(5): 2409-16.
6. Gandhi SV, Waghmare AD and Kadam AV: Chemometrics-assisted UV spectrophotometric method for determination of Cefixime and Ornidazole in pharmaceutical formulation. International Journal of Pharmaceutical Research and Analysis 2017; 7(2): 41-46.
7. Prasad AR and Thireesha B: UV Spectrophotometric method development and validation for the determination of Lornoxicam in microsponges. International Journal of Applied Pharmaceutics 2018; 10(1): 74-78.
8. Chanda I, Bordoloi R, Chakraborty DD, Chakraborty P and Das SRC: Development and validation of UV-Spectroscopic method for estimation of Niacin in bulk and pharmaceutical dosage form. Journal of Applied Pharmaceutical Science 2017; 7(9): 81-84.
9. Gala PS, Wais M and Ghadge O: Development and validation of UV spectrometric method of Econazole nitrate co-crystal loaded in gel formulation. International Journal of Pharmaceutical Sciences and Research 2022; 13(12): 5057-67.
10. Peerzade MV, Memon S, Bhise K and Aamer AI: Development and validation of UV-Visible spectrophotometric method for estimation of Ritonavir in bulk and formulation. Pharma Innovation 2019; 8(4): 30-34.
11. Ahmad S, Usman R, Shaikh T, Imran and Akhtar R: Development and validation of UV spectrophotometric method for estimation of Saxagliptin and Dapagliflozin in bulk and dosage form. International Journal of Pharmaceutical Sciences and Research 2021; 12(4): 2185-92.
12. Zadbuke N, Shahi S, Jadhav A and Borde S: Development and validation of UV-Visible spectroscopic method for estimation of carbamazepine in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2016; 8(2): 234-38.
13. Dulange V and Gajeli GB: Development and validation of UV spectroscopy method for the estimation of Dolutegravir in bulk and pharmaceutical dosage form 2021; 11(3): 188-90.

14. Tegeli V, Birajdar A and Matole V: UV Spectrophotometric method development and validation of Darunavir in bulk and solid dosage form 2021; 14(6): 3262-64.
15. Popovska O, Kavrakpvski Z and Rafajlovskav: Development and validation of UV spectroscopic method for determination of ketoconazole in pharmaceutical formulations. Inter J of Pharmaceutics 2014; 4(4): 95-101.
16. Chhajed SS, Sonawane S, Pingle AP, Dashputre N, Sakshi and Kshirsagar SJ: Development and Validation of UV Spectrophotometric Method for Estimation of Pregabalin 2019; 9(1): 15-18.

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