

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD FOR CLOTRIMAZOLE LOZENGES FORMULATION

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ABSTRACT

Objective: A simple, sensitive, precise and accurate stability-indicating HPLC method has been developed and validated for determination of Clotrimazole (CLOT) in its lozenges dosage form.

Methods: The mobile phase consists of 0.1% Tri-ethylamine in water (pH 3.00±0.05 adjusted by using Ortho-phosphoric acid) and Methanol in ratio of (25:75 v/v %) with isocratic programming, Gracemart C₁₈ (250mm×4.6mm, 5μ) column used as stationary phase with a flow rate of 1.0 mL/minute. The detection wavelength was set at 215 nm.

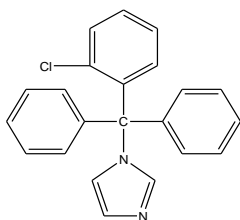
Results: The retention time of clotrimazole was found to be 5.5 ± 0.008 min. Clotrimazole was subjected to different stress testing conditions. The degradation products were well resolved from the drug under the tested conditions. The method was linear (r = 0.9998) at a concentration range of 0.5 to 60 μg/mL. Precision study showed that the percentage relative standard deviation was within acceptable limits, and the mean recovery was found to be 100.75% ± 1.51 for assay of Clotrimazole in lozenges dosage form.

Conclusion: The results demonstrated that the method would have a great value when applied in quality control and stability studies for Clotrimazole.

Keywords: Clotrimazole, HPLC, Stability indicating, Lozenges.

INTRODUCTION

Clotrimazole, 1-[[2-(2-chlorophenyl) (di-phenyl) methyl]-1H-imidazole is an antifungal agent used primarily in the treatment of superficial fungal infections [1]. Clotrimazole lozenges are used for systemic effect with increased bioavailability, reduction of gastric irritation by overcoming the first pass metabolism [2,3].



Chemical structure of Clotrimazole

High performance liquid chromatography (HPLC) is a widely used technique for analysis of drug product and drug substances. Some of analytical methods reported for clotrimazole in classical dosage form like tablets (Canesten Vaginal tablets), pessaries (Canesten 500 mg pessary), creams (Clotrimazole cream USP 1%), spray (Candid Micro), either individually or in combination (betamethasone and clotrimazole in cream formulation and methylparaben, propylparaben, clotrimazole in topical cream) by HPLC method, HPTLC method and spectrophotometric method [4-13]. There is no reported stability indicating assay method for estimation of Clotrimazole lozenges, thus need to develop new simple analytical method for routine analysis.

MATERIALS AND METHODS

Clotrimazole reference standard (claim 99.3%) was provided by Srikem Laboratory Pvt. Limited, Talozha. Lozenges of CLOT (CLENORUSH troche® - 10 mg, Coral Laboratories Limited, Uttarakhand, India) was obtained from the local market, HPLC-grade solvents, laboratory reagents-grade hydrochloric acid, sodium hydroxide, hydrogen peroxide, orthophosphoric acid, and triethylamine were purchased from Merck Laboratories Pvt. Ltd., Mumbai, India. Ultra purified water prepared in the lab using Siemens water purification system was used throughout the work.

Instrumentation

The LC systems of Shimadzu LC 2010 Japan with SIL- 10ADVP auto-injector and column oven of CTO-10ASVP model, SPD M-10AVP photo diode array detector, SCL-10 AVP System controller and LC-solution software were used for the study.

Chromatographic conditions

The chromatographic separation was performed on Gracemart C₁₈ column (250mm × 4.6 mm, i.d., particle size 5μm). The column temperature was kept at 30 ± 2°C. Separations were performed in isocratic mode using a mobile phase consisting of water 0.1% Tri-ethylamine (pH3.00±0.05 adjusted by using Ortho-phosphoric acid): Methanol in ratio of (25:75 v/v %) with a flow rate of 1.0 mL/minute. The detection wavelength was set at 215 nm.

Preparation of standard solutions

10 mg of CLOT is weighed in 10 ml (1mg/ml) volumetric flask and dissolved in methanol. Subsequent dilutions for 0.5, 1.0, 2.0, 4.0, 8.0, 15.0, 25.0, 40.0, and 60.0 μg/mL were prepared by using mobile phase as diluent. Mobile phase is used as blank solution.

Sample preparation

Lozenges sample prepared by weighing powder equivalent to 10mg of CLOT, was dissolved in 10 mL of methanol. It was sonicated for 10 minutes. This solution was filtered. Required volumes were diluted to get a final concentration of 10 μg/mL.

Method Validation

Specificity study

Mobile phase along with placebo were injected to check the interference at the retention time of CLOT in the established chromatographic condition and no interference were observed at the designated Retention Time which was established by peak purity of the chromatogram by PDA detection.

Stress studies

Acid, Alkaline Oxidative and thermal degradation studies were conducted and CLOT lozenges was subjected to this condition. 0.1N

HCl, 0.1N NaOH, 30% hydrogen peroxide and at temperature of 80 °C for 24 hours were used for stress testing studies. The samples are neutralized before injecting into the system for acid and alkaline samples. Oxidative and thermal samples were injected after proper dilution as such. Placebo and mobile phase were also subjected to same treatment as sample to check for interferences.

Linearity

Nine point linearity plot was constructed in order to accommodate wide range from 0.5 µg^{-ml} to 60 µg^{-ml}, so that CLOT with its formulation can be analyzed easily with $r^2 = 0.9998$ for the above plot.

Precision

ICH describes precision as closeness of individual measure of analytes when the procedure is applied repeatedly to multiple times. Interday and intraday precision has been established in the method.

Accuracy

It's was evaluated at three levels of 80%, 100% and 120% of test concentration by adding known amount of drug to placebo and extracting the sample. Three sets were prepared and analyzed.

Solution stability

CLOT API and its formulation stability was carried out for a period of 72 hours at auto sampler at 25°C temperature. (Table 2)

Robustness

Varying conditions of flow rate, buffer pH, column temperature, and wavelength were carried out as per ICH guidelines to estimate the effects on the method.

RESULTS AND DISCUSSIONS

Method development and optimization

Actual chromatographic conditions were established after number of preliminary experiments for selecting the proper mobile phase system. Different mobile phase systems were tested, and selection of the proper system depended on its ability to give good separation between the pure drug and its possible degradation products. Acceptable separation was achieved on Gracemart C₁₈ column (250mm × 4.6 mm, i.d., particle size 5µm) using a mobile phase composed of 0.1% Tri-ethylamine in water (pH3.00 ± 0.05 adjusted by using Ortho-phosphoric acid) and Methanol in ratio of (25:75 v/v %) pumped with a flow rate of 1.0 mL/min. the column temperature was kept constant at 30 ± 2°C. under these chromatographic conditions, the run time sample was 10 min, and the retention time of clotrimazole was 5.5 ± 0.008 min. The representative chromatogram is shown as figure 1.

System suitability

System suitability parameters like theoretical plates per meter, tailing factor, percentage relative standard deviation of area and retention time of six injections were carried out and the values are well within the limits as shown in Table.1.

Linearity and sensitivity

A linear calibration plot of CLOT was constructed at nine point concentration levels 0.5 µg^{-ml} to 60 µg^{-ml} in duplicate. Average peak area of CLOT was plotted against respective concentrations and linear regression analysis was performed. Correlation coefficient was found to be 0.9998.

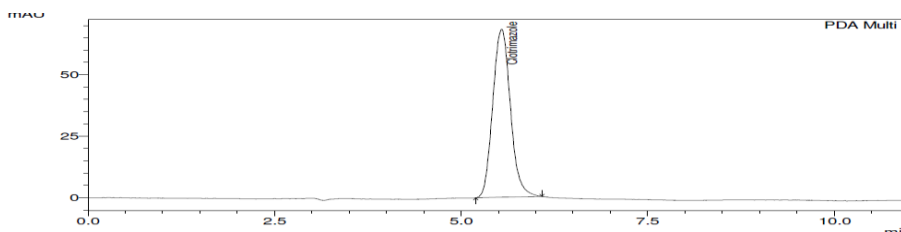


Fig. 1: Representative chromatogram of 10 µg/mL of standard solution of Clotrimazole.

The limit of detection (LOD) and limit of quantitation (LOQ) were based on the signal-to-noise ratio. The LOD and LOQ values were 0.0451µg/mL and 0.1368 µg/mL, respectively.

Precision

The precision of the assay method was evaluated for repeatability and intermediate precision. For intra-day precision and inter-day precision, the percentage relative standard deviation of CLOT was found to be 0.10% and 0.20% respectively. These values were well within the acceptable limit of 2%, as per USP. Result is given in (Table 2).

Accuracy

Known amount of standard was spiked in 80%, 100%, 120% concentration in triplicate to test solution and recovery of drug was calculated. The accuracy of method was established at three concentration levels at 8, 10 and 12 µg^{-ml} of CLOT standard. The recoveries at three different concentrations were found to be within the range of 98 to 102 % as per ICH guidelines. Mean % recovery (mean ± SD) was found to be 100.75±1.51. The results indicated that the recovery of CLOT in three different concentrations (Table 3).

Robustness

The robustness of assay method was studied by incorporating small but deliberate changes in the analytical method (variations in flow rate, column temperature, mobile phase composition, pH of buffer,) and also by observing the stability of the drugs for 24 hours at room temperature in the dilution solvent. In all the varied chromatographic conditions, there was no significant change in chromatographic parameters. Result is given in (Table 4).

Stress studies

Acid and alkali hydrolysis

25mg of the clotrimazole working standard was weighed accurately and transferred to 25ml volumetric flask containing 0.1M HCl or 0.1M NaOH and kept at room temperature. The concentration was 1000µg/ml. The sample was taken initially and at different time intervals and the pH was neutralized to 7.0 using 0.1M NaOH or 0.1M HCl and the final dilution (10 µg/mL) were done with the mobile phase and loaded into HPLC system.

Oxidation degradation

25mg of the drug substance was weighed accurately and transferred to 25ml volumetric flask containing 15%w/v H₂O₂ and kept at room temperature. The concentration was 1000µg/ml. The samples were taken initially and at different time intervals and the final dilution were done with the mobile phase and loaded into HPLC system.

Thermal degradation

Sufficient amount of clotrimazole powder was transferred into petridish spread evenly for NMT 1mm thickness and kept inside hot air oven at 80°C for 24 hours. Samples were collected at different time intervals and final dilution were done with the mobile phase and loaded into HPLC system.

Applicability of the method

Clotrimazole containing lozenges were subjected to the analysis by the proposed method. The label claim percentage was 98.99 ± 0.07%. this acceptable value indicate the applicable of the method for the routine quality control of Clotrimazole lozenges without interference from the excipients and degradation products.

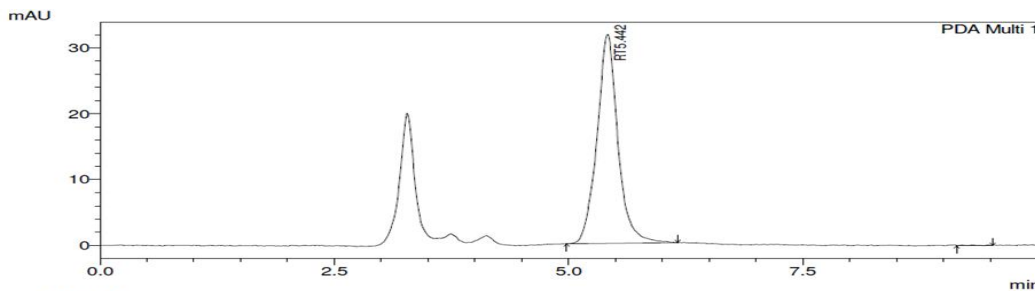


Fig. 2: Chromatogram for Clotrimazole in 0.1N NaOH at room temperature

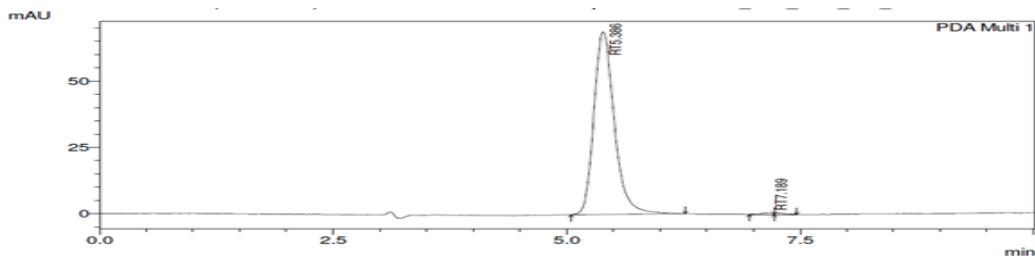


Fig. 3: Chromatogram for Clotrimazole in 0.1N HCl at room temperature.

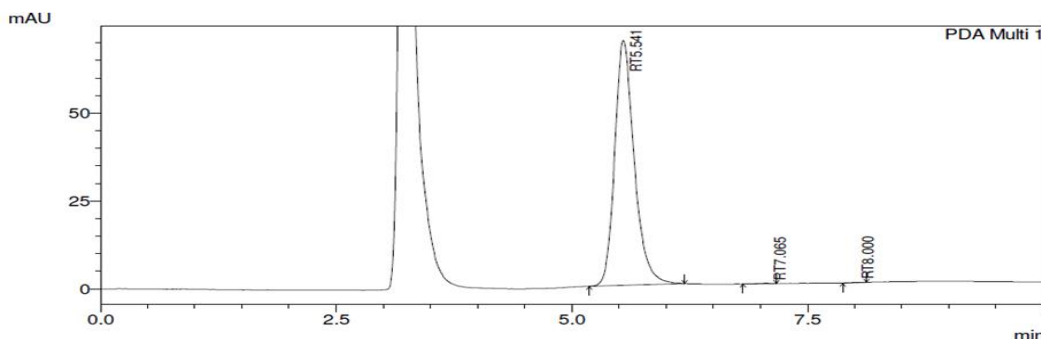


Fig. 4: Chromatogram for Clotrimazole in 15%v/v H₂O₂ at room temperature.

Table 1: System suitability

Parameter	Clotrimazole	Acceptance Criteria
Tailing factor	1.17	not more than 2.0
Theoretical plates	8412	not less than 2000
% RSD of 6 injections (area)	0.10	not more than 1.0
% RSD of 6 injections (retention time)	0.07	not more than 1.0

Table 2: Precision, Solution stability, Linearity (n=6) and Sensitivity

Parameter		% RSD	Limit
Precision	Repeatability	0.10	1.0%
	Intermediate	0.20	2.0%
Solution Stability for 72 hours		0.23	3.0%
Linearity (r ²)		0.9998	0.999
LOD		0.0451 µg/mL	
LOQ		0.1368 µg/mL	

Table 3: Accuracy

Amount added	% Mean Recovery ± SD
0.8 µg/ml	98.611±0.116
1.0 µg/ml	101.91±0.067
1.2 µg/ml	101.744±0.066

Table 4: Method Robustness

Parameter	Acceptance criteria: (% RSD \leq 2.0 %)	(Average Area, n=6)
Flow rate	0.9 ml/min	0.13
	1.1 ml/min	0.11
pH	2.8	0.44
	3.2	0.32
Wavelength	+3nm	0.27
	-3nm	0.33
Temperature	+5°C	0.32
	-5°C	0.23

CONCLUSION

A simple, accurate and precise stability indicating RP-HPLC assay method was developed for estimation of Clotrimazole in bulk drug and lozenges formulation. Statistical analysis proves that method is repeatable, sensitive and selective for the analysis of Clotrimazole in lozenges formulation.

Based on these evidence the method can be stated as highly economical and it is recommended for routine use in quality control laboratories and stability studies.

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