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RESEARCH ARTICLE

Development and Validation of a Rapid High Performance Liquid Chromatography Method for Simultaneous Quantification of Ornidazole and Miconazole from Cream Formulations

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ABSTRACT

High temperature and humidity typically describe the tropical regions in which Indian sub-continent lies. These conditions are the breeding grounds required for opportunistic fungal and other infections. The common treatment for fungal infections are azole antibiotics. However, combination of two drugs provides better effect. For an analytical chemist this is a challenge as many of the times the active ingredients (analytes) have distinct chemical and physical properties leading difficulty in developing a single method which can be used for the quantitation of these drugs. In the current research a rapid analytical method employing HPLC has been developed and validated for simultaneous quantification of the active ingredients Ornidazole and Miconazole from the cream formulation. The analytes were extracted from cream base and filtered. An ODS column enabled chromatographic is used for the separation of the analytes. The method involves simple isocratic chromatography and UV detection. Validation of the method showed response was a linear function of concentration in the range 50-150 $\mu g\ mL^{-1}$ for both Ornidazole and Miconazole. The method was suitably validated and was found to be precise and robust, with recoveries for both the analytes being consistent and complete. The method has been successfully applied for the analysis of samples from marketed cream formulations.

KEYWORDS

Ornidazole, Miconazole, HPLC, Formulation Analysis

INTRODUCTION

In a tropical region like the Indian subcontinent, the high temperature and humidity and the conditions most suitable for fungal infections to proliferate. With the rampant and uncontrolled use of antibiotics and other drugs used in the treatment of infections the incidences of the infection causing agents developing resistance to the various drugs is on the rise. One of the solutions to tackle this problem is the use of a combination drug regimen.

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For many drugs originally used as standalone therapies, combination drug formulations are being developed and marketed for a faster and better control of the infections. For the analytical chemist this presents with a new set of challenges. Many times the components of this combination therapy have significantly differing physical (solubility, melting point etc) and chemical (pKa, UV absorption maxima, stability in solvent etc.) properties.

In addition to this, there are the excipients and preservatives which also need to be separated from the analytes of interest for the quantification of the active drugs in the formulation. The easiest solution to this problem

is to develop separate methods of quantification for the two drugs. However, this means more efforts for the chemist and less output in terms of number of samples analysed. For a quality control laboratory such situations are best avoided. Efforts are now being put in developing a common analytical method for multicomponent formulation analysis.

Ornidazole is an antifungal agent of the 5-nitro imidazole class of compounds. Ornidazole has a molecular formula $C_7H_{10}ClN_3O_3$ and its molecular weight is 219.625. It is soluble in chloroform and methanol. It is available commercially in the form of tablets, creams etc. Various analytical methods have been developed for the pharmaceutical analysis of Ornidazole alone or in combination with other drugs using HPLC[1-3], HPTLC[4-6], GC[7], Derivative spectroscopy[8-11] method of analysis etc.

Miconazole is an imidazole antifungal agent. Miconazole has a molecular formula $C_{18}H_{14}Cl_4N_2O$ and its molecular weight is 416.127. It is soluble in ethanol, methanol, acetone and chloroform. It is marketed as injection, tablet, cream etc. Various analytical methods have been developed for the pharmaceutical analysis of Miconazole alone or in combination with other drugs using HPLC[12-15], HPTLC[15-17], GC[18], Derivative spectroscopy[19] method of analysis etc.

In spite of all the work done on the pharmaceutical analysis on the combination formulation of Ornidazole and Miconazole, no work has been done in developing a simultaneous method of quantification of these drugs.

There is hence a need for developing an analytical method suitable for quantification of Ornidazole and Miconazole for routine quality control analysis. The current research involves development and validation of a new HPLC-UV method to quantify the drugs from marketed cream formulations as per the ICH Q2 (R1) guidelines[20].

MATERIALS AND METHODS

Chemical and Reagents

The working standards of Ornidazole (99.85%) were provided by Endoc Lifecare Pvt. Ltd., Indiaand Miconazolenitrate (99.70%) were obtained from Cipla Ltd., India. Analytical standards of methyl paraben and propyl paraben were providing by Cipla Ltd., India. High purity water was prepared in house using Milli-Q water purification system. HPLC grade methanol, acetonitrile, o-phosphoric acid and triethyl amine were used.

Preparation of Solutions

Two separate stock solutions each of Ornidazole (OZ) and Miconazole (MZ) nitrate were prepared for the calibration curve and precision and accuracy experiment for the method validation exercise.

The stock solutions of OZ and MZ were prepared in Methanol and stored at 2-8°C. The stock concentration for OZ and MZ were 1000µg/ ml respectively by dissolving about 50 mg of each standard in 50 ml of methanol. Subsequent dilutions of the stock solutions were prepared from stock solutions by dilution with Methanol. For identification purpose, solutions of 5 and 0.5 µg/ ml respectively of methyl paraben and propyl paraben were prepared. The working standard solutions thus prepared were used to prepare the solutions used in the validation experiment.

For Ornidazole and Miconazole a seven-point standard curve was prepared. The calibration curve ranged from 50 - 150µg/mL with concentration levels as 50, 60, 80, 100, 120, 140 and 150 µg/mL for both OZ and MZ.

Sample Preparation Procedure

The cream samples of about 0.5 gms were weighed in a clean dried 100 ml volumetric flask. Care was taken so that the cream sample does not stick to the neck of the flask. 30 ml of methanol is added to the flasks and sonicated for 60 seconds to disperse the cream in the methanol.

The flasks after sonication are allowed to come to the room temperature and then diluted to volume with methanol. The samples were further filtered with syringe filters into HPLC vials for analysis.

High Performance Liquid Chromatography Conditions

Chromatographic separation was carried out using a Shimadzu 2010 HPLC with a Phenomenex Luna C8 (250 x 4.6mm, 5µ) column. A mobile phase consisting of Acetonitrile: Distilled water: O-phosphoric acid: triethyl amine (50:50:0.2:0.2 v/v) was delivered with a flow rate of 1.5 mL/min. The column oven was maintained at 30 ºC. The total run time for each sample analysis was 7.0 min. The detection was done using a UV detector maintained at 237 nm.

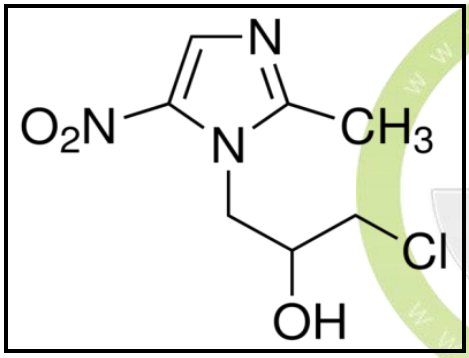

Figure 1: Chemical structure of Ornidazole

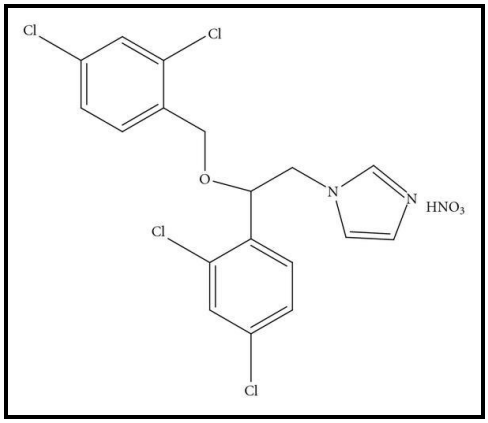

Figure 2: Chemical structure of Miconazole

Method Validation

The analytical method for quantification of OZ and MZ from cream formulation has been validated for selectivity, linearity, precision, accuracy, solution stability, ruggedness and robustness following appropriate recommendations of the ICH Q2(R1)regulatory guidelines recommendations[20].

RESULTS AND DISCUSSION

Specificity

Specificity was performed by chromatographing the individual working level solutions of Ornidazole, Miconazole, methyl paraben and propyl paraben. OZ and MZ were solutions of 100 ppm each and 5 ppm solution of methyl paraben and 0.5 ppm solution of propyl paraben were injected in the chromatographic system. Propyl paraben when injected at higher concentrations was found to elute just before the miconazole peak, however at working level no peak of propyl paraben could be seen at the retention time.

No interfering peak of endogenous compounds was observed at the retention time of the analytes. The theoretical plates, tailing factor observed for peaks of OZ and MZ are 5444 & 1.50 and 6446 & 1.72 respectively. The resolution between the peaks of OZ and MZ was 14.30. Representative chromatograms of blank diluent, Ornidazole, miconazole and methyl paraben are presented in Figure 3, Figure 4, Figure 5 and Figure 6 respectively. The representative chromatogram for sample preparation is presented in Figure 7.

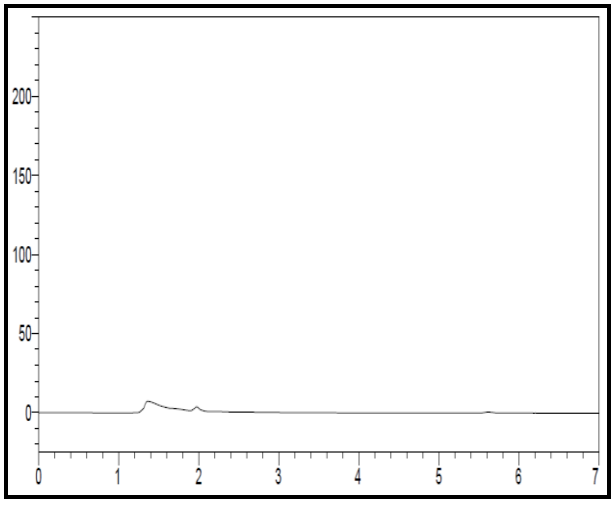

Figure 3: Representative Chromatogram of diluent blank

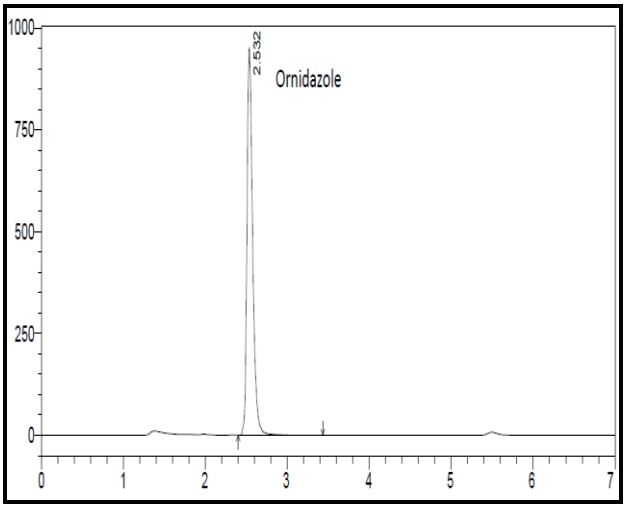

Figure 4: Representative Chromatogram of Ornidazole

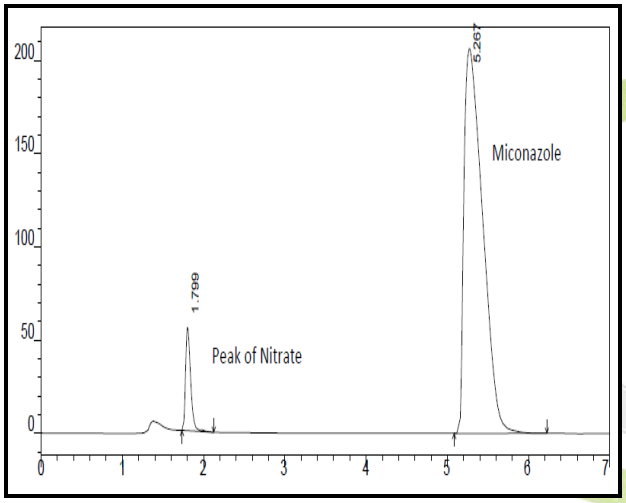

Figure 5: Representative Chromatogram Miconazole nitrate

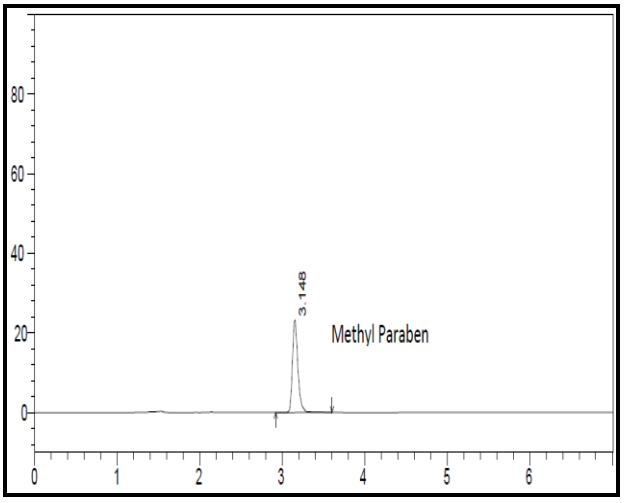

Figure 6: Representative Chromatogram methyl paraben

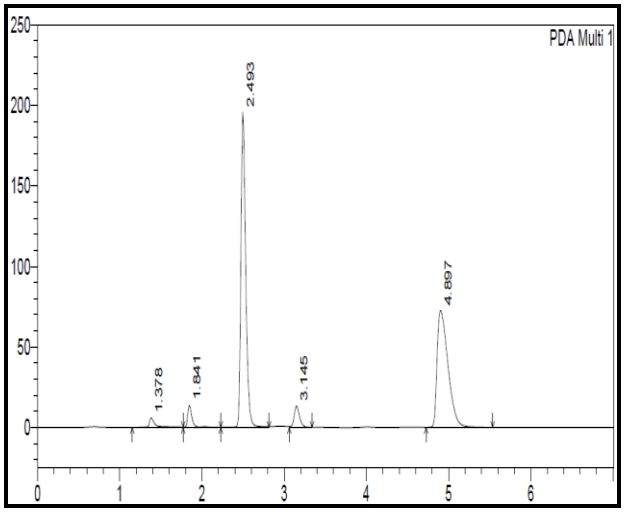

Figure 7: Representative Chromatogram of sample

Precision

System Precision

System suitability was evaluated by injecting six replicates of the mix standard preparation in the chromatographic system. The relative standard deviation (RSD) of the area response and the retention time were evaluated. The RSD values for area response was found to be 0.39 and 0.15 for OZ and MZ respectively. The RSD values for retention time was found to be 0.22 and 0.23 for OZ and MZ respectively.

Method Precision

Method precision was evaluated by injecting six preparations each of the two marketed formulations. The RSD for the back calculated % label claim of the active components was evaluated. The RSD values for the assay of OZ and MZ was found to be 0.11% and 0.29% respectively.

Linearity and Range

The response against concentration relationship was evaluated using a seven point calibration curve. Mixed linearity levels were prepared having concentration of 50, 60, 80 100, 120, 140 and 150 µg/ml for both OZ and MZ. The detector response of a 20 µl injection volume was measured and was plotted against the nominal concentration of each concentration level for both the analytes. The analytical

method was found to be linear between 50 to 150 µg/ml for both Ornidazole and Miconazole. The regression coefficient value for both the analytes was observed to be >0.9998. The linearity plot for OZ and MZ are shown in Figure 8 and Figure 9 respectively.

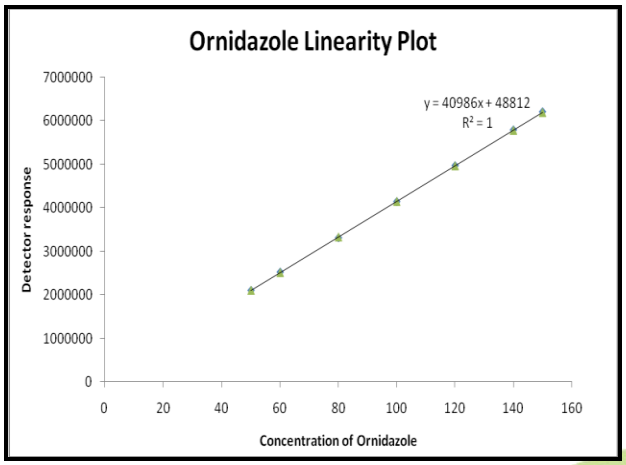

Figure 8: Linearity plot for Ornidazole

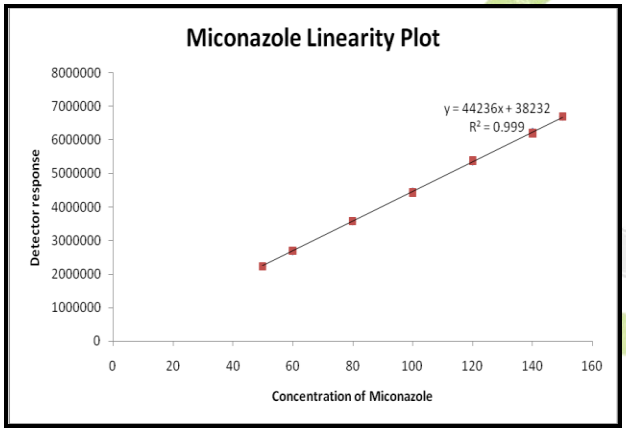

Figure 9: Linearity plot for Miconazole

Accuracy

The Accuracy of the method was evaluated at three levels i.e. 50, 100 and 150 % of the working concentration. Three replicate weighing at each of the three levels were done in the volumetric flask. These samples were then processed as per the sample processing method. The resultant solution was injected into the chromatographic system. The back calculated content of each of the three replicates at an individual level were evaluated. The accuracy was found to be consistent for both OZ and MZ across the three concentration levels. The accuracy for OZ was found to be between 99.46% and 99.88% and for MZ was found to be between 99.67% and 99.94%.

Ruggedness (Intermediate Precision)

For ruggedness experiment the sample preparation and analysis was performed by another analyst using the same method of analysis. Six replicates of each of the marketed formulation were prepared and chromatographed on the next day of the method precision experiment. Intermediate assay method precision was evaluated by injecting six preparations each of the two marketed formulations. The RSD for the back calculated % label claim of the active components was evaluated. The RSD values for the assay of OZ and MZ was found to be 0.19% and 0.35% respectively. The cumulative RSD values for the 12 samples for assay of OZ and MZ was found to be 0.19% and 0.34% respectively.

Solution Stability

The sample solutions prepared for the assay method precision experiment were re-injected after intervals of 12, 24, 36 and 48 hrs after initial injections. The stability of the analytes in the sample solution was evaluated by comparing the back calculated assay values for both OZ and MZ. The analytes were found to be stable in the sample solution for at least 48 hrs. The stability was found to be 99.10% and 98.93% for OZ and MZ respectively.

Ruggedness

As a part of the method validation, minor changes were done the chromatographic parameters to determine their impact on the analysis results. The flow rate was changed from 1.5 ml/min to 1.3 ml/min and 1.7 ml/min. No merging of any placebo peaks with the analytes peaks was observed. The results of the analysis in both the cases were found to be consistent with the precision experiment results. The column oven temperature was changed from 30 ºC to 28 ºC and 32 ºC. No merging of any placebo peaks with the analytes peaks was observed. The results of the analysis in both the cases were found to be consistent with the precision experiment results.

Application of Method to Marketed Formulations

The assay of OZ and MZ was performed on commercial marketed samples of the cream formulation. Purchased samples of Candimale and Candifem were analysed using the analytical method. The assay results were 99.92% and 100.26% for Candimale and 100.14% and 99.99% for Candifem. Assay testing performed on different days showed similar results.

CONCLUSION

Ornidazole and Miconazole have distinct absorption maxima and pKa values thus developing a simultaneous method of analysis a difficult task. The preservatives present in the formulations methyl paraben and propyl paraben are the other components to be resolved from the peaks of OZ and MZ and which can also be quantified.

The HPLC –UV assay method has been developed and validated for quantification of OZ and MZ from cream formulations. The validation data demonstrate good precision and accuracy of the method. The method was robust and did not encounter any variation with minor changes in the method parameters. This method was applied for the analysis of marketed formulations and was found to provide consistent and accurate results.

This assay method for simultaneous quantification of OZ and MZ will be beneficial for the routine and Quality control analysis of cream formulations containing these active ingredients, by saving the time and efforts of the analyst.

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