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RP-HPLC Method Development and Validation for the Simultaneous Determination of Clindamycin and Miconazole in Pharmaceutical Dosage Forms

Mr. Saurabh A. Dhumane, Ms.Aishwarya P. Walke, Mr. Shreyash P. Vyavahare, Mr. Pranav S. Pawar, Mr. Shubham U. Gholap
Samarth College of Pharmacy, Belhe, Pune, Maharashtra, India

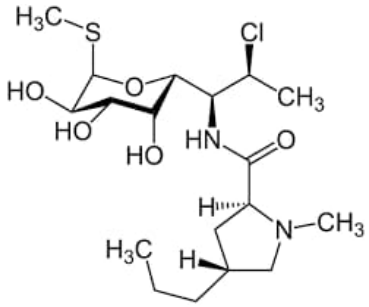
Abstract: *A simple, precise, reliable, rapid and reproducible reversed phase–high-performance liquid chromatography method was developed and validated for the simultaneous estimation of Clindamycin (CDM) and Miconazole (MCZ) present in tablet dosage forms... Method: Chromatographic separation achieved isocratically on Inertsil ODS C18 (250x4.6 mm, 5 mm) column and buffer (pH 3.5) and acetonitrile (65:35 v/v) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 220 nm. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The retention times for CDM and MCZ was found to be 2.2 and 3.2 min, respectively. Linearity for CDM and MCZ was in the range of 5-30 µg/ml and 10-60 µg/ml, respectively. The mean recoveries obtained for CDM and MCZ were 99.73 ± 0.8 and 100.2 ± 0.58%, respectively, and Relative standard deviation (RSD) was less than 2. The correlation coefficients for all components are close to 1. The RSDs for three replicate measurements in three concentrations of samples in tablets are always less than 2%.: Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of CDM and MCZ in tablets.*

Keywords: Clindamycin, Miconazole, RP-HPLC, Simultaneous estimation, Tablets.

I. INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance [7-10], separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products.

Clindamycinis an antibiotic of the lincosamide class, which blocks the ribosomes of microorganisms. It is not only used to treat infections caused by anaerobic bacteria, but also used to treat protozoal diseases, such as malaria. It is a common topical treatment for acne and can be useful against some methicillin-resistant Staphylococcus aureus (MRSA) infections. The most severe common adverse effect of Clindamycin is Clostridium difficileassociated diarrhea (the most frequent cause of Pseudomembranous colitis). Although this side effect occurs with almost all antibiotics, including beta-lactam antibiotics, it is classically linked to Clindamycin use



(Fig.01): Chemical structure of Clindamycin

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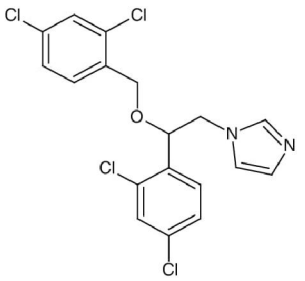
Drug profile of Clindamycin:

It's IUPAC name is methyl 7-chloro-6,7,8-trideoxy-6-{[(4R)-1-methyl-4-propyl-L-prolyl]amino}-1-thio-L-threo-α-D-galacto-octopyranoside(Fig.01). Clindamycin is a medication used for the treatment of numerous infections, including but not limited to septicemia, intra-abdominal infections, lower respiratory infections, gynecological infections, bone and joint infections, and skin and skin structure infections.Clindamycin hydrochloride is freely soluble in water, in dimethylformamide, and in methyl alcohol.

Miconazoleis an imidazole antifungal agent, developed by Janssen Pharmaceutical, commonly applied topically to the skin or to mucous membranes to cure fungal infections. It works by inhibiting the synthesis of ergosterol, a critical component of fungal cell membranes. It can also be used against certain species of Leishmania protozoa which are a type of unicellular parasite that also contain ergosterol in their cell membranes. In addition to its antifungal and antiparasitic actions, it also has some antibacterial properties. It is marketed in various formulations under various brand names. Miconazole is also used in Ektachrome film developing in the final rinse of the Kodak E-6 process and similar Fuji CR-56 process, replacing formaldehyde. Fuji Hunt also includes Miconazole as a final rinse additive in their formulation of the C-41RA rapid access color negative developing process.

Drug profile of miconazole nitrate:

It is a 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy] ethyl]imidazole nitric acid (Fig. 2), and it has broad-spectrum antifungal activity against various Candida species including Candida albicans, Candida tropicalis, Candida glabrata, and Candida krusei. It is white to off-white powder which is soluble in methanol and acetonitrile and insoluble in water



(Fig.02)Chemical structure of Miconazole

Extensive literature survey reveals that no sensitive reversed-phase (RP)-HPLC method is reported for simultaneous determination of CDM and MCZ in tablet dosage form. Therefore, an attempt was made to develop a new, rapid and sensitive RP-HPLC method for the simultaneous determination of CDM and MCZ in tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines12-13 which are mandatory also.

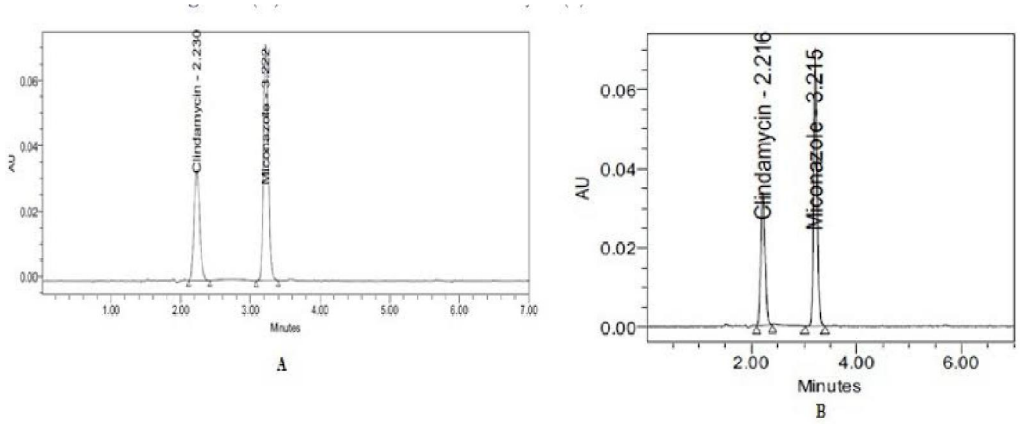


Fig: Chromatograph resulting from (A) standard Clindamycin and Miconazole (B) tablet sample Clindamycin and Miconazole

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II. EXPERIMENTAL INSTRUMENTATION

Liquid chromatographic system from Alliance Waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and 2996 PDA detector to provide a compact and with class Empower-2 software.

Reagents and Chemicals

Analytically pure samples of CDM and MCZ was kindly supplied by Spectrum pharma research solutions, Hyderabad. Acetonitrile, Methanol and all other chemicals of HPLC grade supplied by Merck Ltd., India. The pharmaceutical dosage form used in this study was a Clind M Tablets containing Clindamycin 100 mg and Miconazole 200 mg were purchased from the local pharmacy. HPLC grade water obtained from Milli-Q water purification system was used throughout the study.

Preparation of buffer:

Accurately weighed 1.36 gm of potassium dihyrogen ortho Phosphate in a 1000 ml of volumetric flask, about 900 ml of HPLC grade water was added, sonicated and degassed and finally made up the volume to 1000 ml with water, then pH was adjusted to 3.5 with dilute orthophosphoric acid solution.

Preparation of diluent solution:

Diluent solution was prepared by mixing 500 ml of HPLC grade water with 500 ml of methanol, in a 1000 ml beaker and sonicated for 15 min.

Chromatographic condition:

The isocratic mobile phase consisted of buffer (pH 3.5) and acetonitrile in the ratio of (65:35 v/v), flowing through the column at a constant flow rate of 1.0 ml/min. AInertsil ODS C18 (250x 4.6 mm, 5 mm) column was used as thestationary phase. Detection of the components were carried out at a wavelength of 220 nm.

Preparation of Standard Stock Solution:

Standard stock solutions were prepared by dissolving 10 mg of CDM and 20 mg of MCZ in a clean and dry 50 ml volumetric flask, to that 30 ml of diluent was added, sonicated for 5 minutes and volume was made upto 50 ml with diluents to get stock solutions with concentration of 0.2 mg/ml for CDM and 0.4 mg/ml for MCZ respectively.

Linearity:

solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25 and 1.5 ml were pipetted out from the stock solution, transferred into 10 ml volumetric flask and volume was made up to 10 ml with diluent. This gives the solutions of 5, 10, 15, 20, 25 and 30 µg/ml for CDM and 10, 20, 30, 40, 50 and 60 µg/ml for MCZ respectively.

Sample Preparation

20 tablet were weighed and calculate the average weight of each tablet then the tablet powder weight equivalent to 200mg of Miconazole and 100mg of Clindamycin was transferred into a 500ml volumetric flask, 300ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pippete out into a 10 ml volumetric flask and made upto 10ml with diluentwhich gives 20 µg/ml of CDM and 40 µg/ml of MCZ respectively.

Method Validation

All the solutions were prepared according to the procedures given under preparation of standard and sample solutions. The developed method was validated as per ICH guidelines.

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III. RESULTS AND DISCUSSION

Chromatography

The mobile phase was chosen after several trials with methanol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of and buffer (pH 3.5) and acetonitrile (65:35 v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a C18 column, the retention times for CDM and MCZ were observed to be 2.2 and 3.2 min, respectively. Total time of analysis was less than 5 min. Detection wavelength of 220 nm was chosen for the analysis (Figure 2)

System Suitability

All the system suitability parameters(Table-1) are within range and satisfactory as per ICH guidelines

Property	Miconazole	Clindamycin
Retention time (R)	2.161min	2.906min
Theoretical plates (N)	3248 ± 63.48	5729 ± 63.48
Tailing factor (T)	1.59 ± 0.117	1.34± 0.117

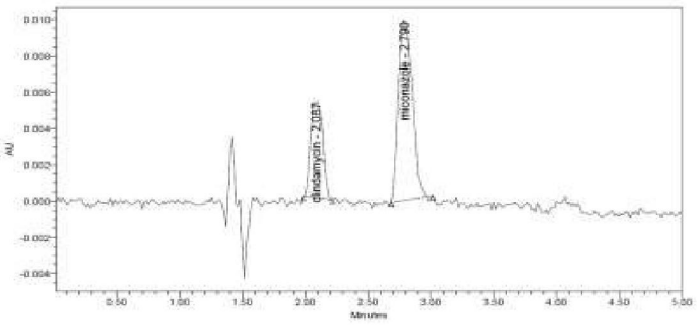
Table-1: System suitability studies of Clindamycin and Miconazole

Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method.The mean percentage recoveries obtained for CDM and MCZ were 99.73 ± 0.8 and 100.20 ± 0.58%, respectively.

LOD

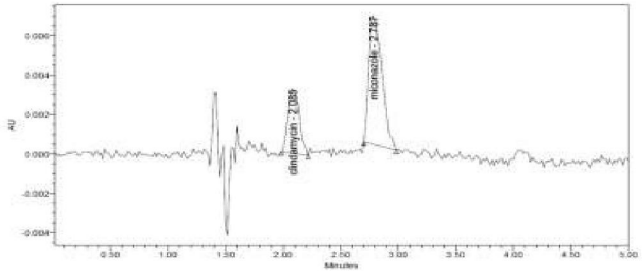
Limit of detection was calculated by standard deviation method of Miconazole and Clindamycin and LOD for Miconazole and Clindamycin were found to be 1.42 and 0.60 respectively.



(Fig.03)LODChromatogram of Clindamycin and Miconazole

LOQ

Limit of Quantification was calculated by standard deviation method of Miconazole and Clindamycin and LOQ for Miconazole and Clindamycin were found to be 4.31 and 1.82 respectively.



(Fig04). LOQ Chromatogram of ofClindamycin and Miconazole.

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Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

IV. CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of CDM and MCZ in tablet dosage form. The developed method is suitable for the quantification of binary combination of CDM and MCZ. A high percentage of recovery shows that the method can be successfully used on a routine basis. Proposed method is simple, fast, accurate, precise and sensitive and could be applied for quality and stability monitoring of CDM and MCZ combination

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