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

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ABSTRACT

Objective: 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 C_{18} (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. **Results:** 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%. **Conclusion:** Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of CDM and MCZ in tablets.

Key words: Clindamycin, Miconazole, RP-HPLC, Simultaneous estimation, Tablets.

INTRODUCTION

Clindamycin (CDM) (Figure 1a) is 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.

Miconazole (MCZ) (Figure 1b) is 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. CDM alone or in combination with other drugs is reported to be estimated by spectrophotometric method[1] and high performance liquid chromatography (HPLC).[2-5] Few analytical methods for determination of MCZ using HPLC[6-11] in pharmaceutical formulation have been reported.

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 guidelines[12-13] which are mandatory also.

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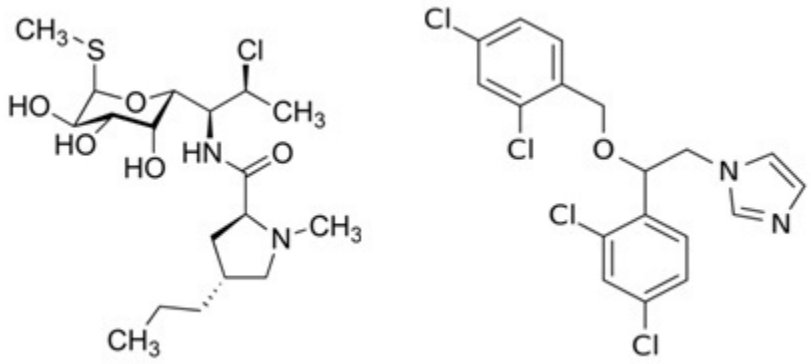

Figure 1: (A)Chemical structure of Clindamycin (B) Chemical structure of Miconazole

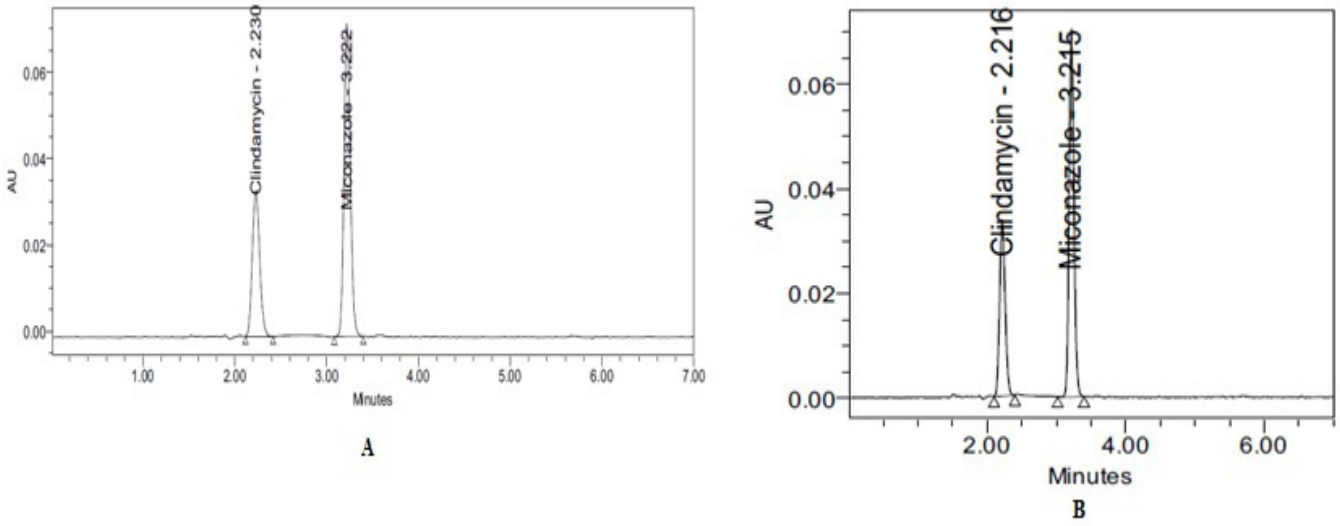

Figure 2: Chromatograph resulting from (A) standard Clindamycin and Miconazole (B) tablet sample Clindamycin and Miconazole

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 Solution

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 C_{18} (250x 4.6 mm, 5 mm) column was used as the

Table 1: System suitability parameters

Parameter	Clindamycin	Miconazole
Retention time (min)	2.2	3.2
No. of theoretical plates	3548	9547
Tailing factor	1.15	1.14
Linearity range (µg/ml)	5-30	10-60

Table 2: Statistical analysis for the calibration curves of Clindamycin and Miconazole

Parameter	Clindamycin	Miconazole
Linearity	5-30 µg/ml	10-60 µg/ml
Correlation Coefficient	0.9995	0.9999
Slope	10191	9151.6
Intercept	491.44	496.44

stationary phase. Detection of the components were carried out at a wavelength of 220 nm.

Preparationof 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.

Preparation of Working Standard Solutions

Aliquots of 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.

Samplepreparation

20 tablets were weighed and calculated the average weight of each tablet then the accurately weighed powder sample equivalent to 100 mg of CDM and 200 mg MCZ were transferred to 500 ml of volumetric flask, 300 ml of diluents was added and sonicated for 30 min, further the volume made up to 500 ml with the diluent and filtered. From the filtered stock solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with the diluent which gives 20 µg/ml of CDM and 40 µg/ml of MCZ respectively.

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 C_{18} 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

System suitability parameters such as number of theoretical plates, retention time and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for CDM and MCZwere 3548 and 9547, respectively.

Linearity

CDM and MCZ showed a linearity of response between 5-30 and 10-60 µg/ml, respectively. The linearity was

Table 3: Result of recovery studies

Preanalysed amount (µg/ml)		Spiked Amount (µg/ml)		% Recovered	
Clindamycin	Miconazole	Clindamycin	Miconazole	Clindamycin	Miconazole
20	40	10	20	99.80	100.82
20	40	10	20	99.24	101.07
20	40	10	20	99.93	100.46
20	40	20	40	99.94	100.06
20	40	20	40	99.94	99.23
20	40	20	40	100.72	100.18
20	40	30	60	99.32	100.50
20	40	30	60	98.05	99.84
20	40	30	60	100.64	99.62
			MEAN	99.73	100.20
			SD	0.80	0.58
			%RSD	0.8	0.6

Table 4: Result of precision

	Clindamycin			Miconazole		
S.No.	Area	USP Plate Count	USP Tailing	Area	USP Plate Count	USP Tailing
1	204622	3504	1.14	358736	9600	1.12
2	206760	3380	1.17	363846	9219	1.14
3	208421	3572	1.14	359896	9345	1.13
4	206744	3464	1.12	360012	9078	1.13
5	206239	3476	1.14	359582	9340	1.12
6	208566	3301	1.15	362036	9670	1.13
Mean	206892	-	-	360685	-	-
Std. Dev.	1466.88	-	-	1892.50	-	-
% RSD	0.7	-	-	0.5	-	-

Table 5: Stability data of CDM and MCZ

Drug	%Assay at 0 hr	%Assay at 24 hr	Deviation
Clindamycin	100.42	99.56	0.61
Miconazole	99.60	99.24	0.25

Tablet 6: Result of marketed tablet analysis

Drug Name	Amount injected (µg/ml)	Amount found (µg/ml)	% Assay ± SD
Clindamycin	20	20.08	100.40 ± 0.71
Miconazole	40	39.84	99.60 ± 0.52

represented by a linear regression equation as follows. The results of statistical analysis were shown in Table 2.

$$Y\ (CDM)=10191\ conc + 491.44\ (r^2=0.9995)$$

$$Y\ (MCZ)=9151.6\ conc + 496.44\ (r^2=0.9999)$$

where Y is area under curve and r^2 is correlation coefficient.

Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 3). The mean percentage recoveries obtained for CDM and MCZ were 99.73 ± 0.8 and 100.20 ± 0.58%, respectively.

Repeatability

Six replicates injections in same concentration were analyzed in same day for repeatability and results were found within acceptable limits (relative standard deviation, RSD‹2) as shown in Table 4.

Intermediate precision

Six replicate injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for CDM and MCZ is found to be 1.5 and 1.1 respectively and it is within acceptable limit of ≤2.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method capacity to remain unaffected. The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean R_t and %RSD is within limit of ≤2 .The tailing factor, resolution factor and no. of theoretical plates were found to be acceptable limits for both CDM and MCZ.

Stability of sample solution

The sample solution injected after 24 hr do not show any appreciable change. Results are shown in Table 5.

Tablet analysis

Content of CDM and MCZ found in the tablets by the proposed method are shown in Table 6. The low values of RSD indicate that the method is precise and accurate.

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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