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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR CLINDAMYCIN AND CLOTRIMAZOLE IN COMBINE DOSAGE FORM BY RP-HPLC METHOD

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Abstract:

New method was established for simultaneous estimation of Clindamycin and Clotrimazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Clindamycin and Clotrimazole by using ACE C18 column (4.6×150mm) 5µ, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol:Phosphate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.344 mins and 3.284 mins. The % purity of Clindamycin and Clotrimazole was found to be 101.27% and 99.97% respectively. The system suitability parameters for Clindamycin and Clotrimazole such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Clindamycin and Clotrimazole was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.Hence the suggested RP-HPLC method can be used for routine analysis of Clindamycin and Clotrimazole in API and Pharmaceutical dosage form.

Keywords: *ACE C18 column, Clindamycin and Clotrimazole, RP-HPLC*

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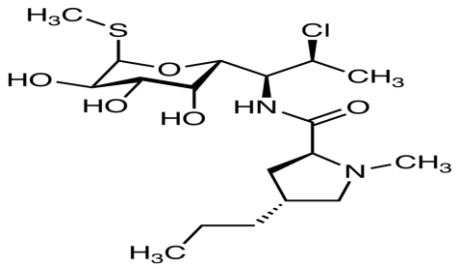
INTRODUCTION:

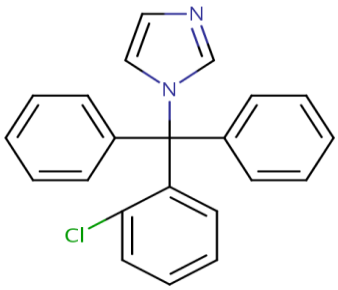
Clindamycin is an antibiotic useful for the treatment of a number of bacterial infections. This includes middle ear infections, bone or joint infections, pelvic inflammatory disease, strep throat, pneumonia, and endocarditis among others. It can be useful against some cases of methicillin-resistant Staphylococcus aureus (MRSA). It may also be used for acne and in addition to quinine for malaria. It is available by mouth, intravenously, and as a cream to be applied to the skin or in the vagina. Common side effects include nausea, diarrhea, rash, and pain at the site of injection. It increases the risk of hospital-acquired Clostridium difficile colitis about fourfold. Other antibiotics may be recommended instead due to this reason. It appears to be generally safe in pregnancy. It is of the lincosamide class and works by blocking bacteria from making protein methyl 7-chloro-6,7,8-trideoxy-6-{[(4R)-1-methyl-4-propyl-L-prolyl]amino}-1-thio-L-threo-α-D-galacto-octopyranoside

CLINDAMYCIN



Clotrimazole, sold under the brand name Canesten among others, is an antifungal medication. It is used to treat vaginal yeast infections, oral thrush, diaper rash, pityriasis versicolor, and types of ringworm including athlete's foot and jock itch. It can be taken by mouth or applied as a cream to the skin or in the vagina. Common side effects when taken by mouth include nausea and itchiness. When applied to the skin common side effects include redness and burning. In pregnancy, use on the skin or in the vagina is believed to be safe. There is no evidence of harm when used by mouth during pregnancy but this has been less well studied. When used by mouth, greater care should be taken in those with liver problems. It is in the azole class of medications and works by disrupting the cell membrane Benzyl N-[3-(acetylthio)-2-benzylpropanoyl]glycinate

CLOTRIMAZOLE



MATERIALS AND METHODS:

Clindamycin and Clotrimazole, Ortho phosphoric acid, Acetonitrile, Methanol, KH_2PO_4, K_2HPO_4, Water.

INSTRUMENTS

HPLC-auto sampler –UV detector Separation module2695, UV.detector2487 Empower-software version-2 Waters U.V double beam spectrometer Digital weighing balance (sensitivity 5mg) pH meter Sonicator

Chromatographic conditions (optimised method)

Column	:ACE C18 (4.6×150 mm) 5.0 µm
Column temperature	:Ambient
Wavelength	:240 nm
Mobile phase ratio	:70:30 Methanol: Phosphate buffer
Flow rate	: 1.2 ml/min
Auto sampler temperature	: Ambient
Injection volume	: 10µl
Run time	:10.0 minutes

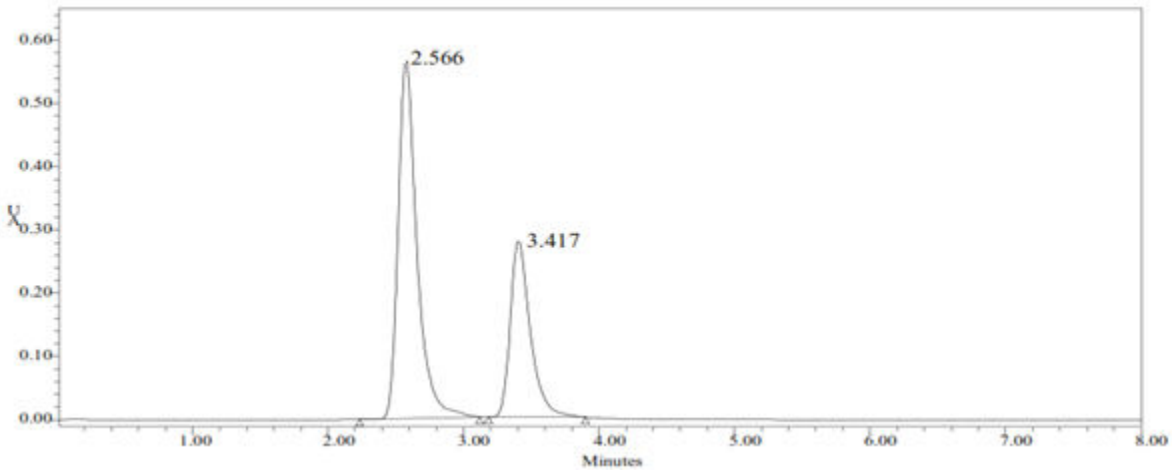


Fig. no.1 Chromatogram showing trial-5injection

Observation

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Preparation of the Clindamycin and Clotrimazole standard and sample solution

Sample solution preparation:

10 mg of Clindamycin and 1 mg Clotrimazole tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Clindamycin and 1 mg Clotrimazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

METHOD VALIDATION

1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

2. Linearity

10 mg of Clindamycin and 1 mg of Clotrimazole working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

3. Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50µg/ml-250µg/ml and 5µg/ml-25µg/ml of Clindamycin and Clotrimazole respectively.

4. Accuracy

10mg of Clindamycin and 1mg of Clotrimazole working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

5. Precision

10 mg of Clindamycin and 1 mg of Clotrimazole working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

6. Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

7. Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

8. Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines

9. Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

10. System suitability

10 mg of Clindamycin and 1 mg of Clotrimazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

RESULTS AND DISCUSSION:

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Clindamycin and Clotrimazole was obtained and the isobestic point of Clindamycin and Clotrimazole showed absorbance's maxima at 240 nm. The spectrums are shown in Fig2.

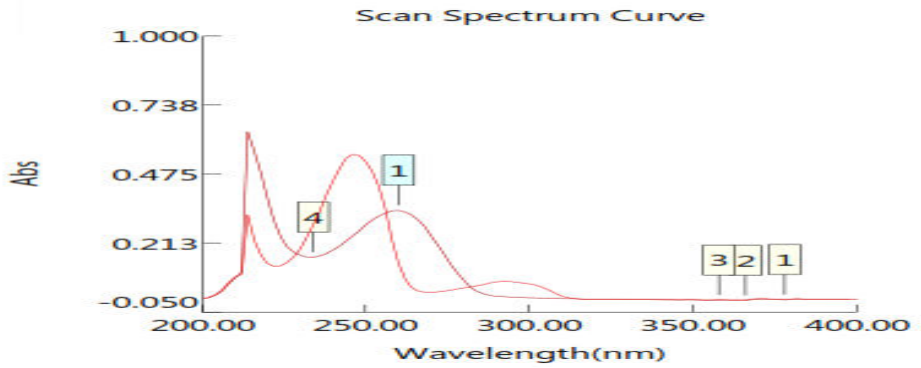
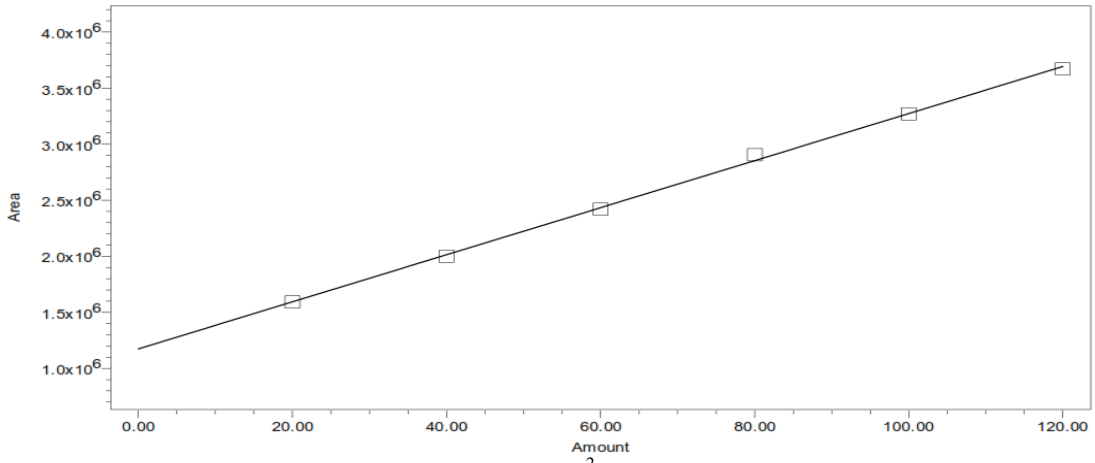


Fig.No.2. Spectrum showing overlapping spectrum of CLIN and CLOT

Table.No.1 Linearity Results for Clindamycin:

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	471543
2	II	100 ppm	656277
3	III	150 ppm	794999
4	IV	200 ppm	946124
5	V	250 ppm	1002139
Correlation Coefficient			0.999



Clindamycinr^2 = 0.999

Fig.No.3. showing Calibration graph Clindamycin

Table.No.2 Linearity Results for clotrimazole:

S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999

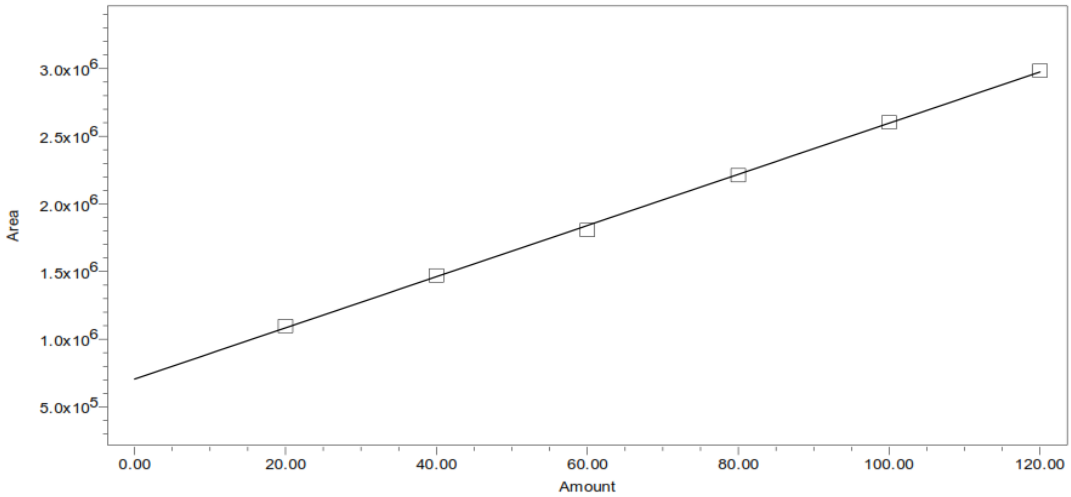


Fig.No 4. Showing calibration graph for clotrimazole

clotrimazole = 0.999

Accuracy

Table.No.3. Showing accuracy results for Clindamycin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	656659	5	4.96	99.91%	99.56%
100%	1304258	10	9.98	99.18%	
150%	1854608	15	15.02	99.60%	

Table.No.4. Showing accuracy results for clotrimazole

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	65312	0.5	0.99	99.53%	99.47%
100%	124509	1.0	1.05	99.38%	
150%	178517	1.5	1.495	99.52%	

Precision

Table.No.5. Showing% RSD results for Clindamycin

Peak Name : Clindamycin

	Peak name	RT	AREA	HEIGHT
1	Clindamycin	2.343	1302729	248455
2	Clindamycin	2.344	1309759	248699
3	Clindamycin	2.344	1302947	249526
4	Clindamycin	2.345	1303977	246695
5	Clindamycin	2.345	1303236	250012
MEAN			1304529.8	
STD.DEV.			2961.1	
%RSD			0.2	

Table.No.6. Showing %RSD results for Clotrimazole

Peak Name : Clotrimazole

	Peak name	Rt	area	height
1	clotrimazole	3.285	124263	19458
2	clotrimazole	3.287	124487	19634
3	clotrimazole	3.287	124175	19600
4	clotrimazole	3.288	124894	19327
5	clotrimazole	3.288	124495	19540
Mean			124462.7	
Std.dev.			278.6	
%RSD			0.2	

Intermediate precision/Ruggedness

Table.No.7 Showing results for intermediate precision of Clindamycin

Peak Name : Clindamycin

	Peak name	RT	AREA	HEIGHT
1	Clindamycin	2.342	1305937	247870
2	Clindamycin	2.343	1306476	246764
3	Clindamycin	2.344	1304520	247140
4	Clindamycin	2.344	1300148	247280
5	Clindamycin	2.345	1308271	250012
MEAN			1305070.2	
STD.DEV.			3061.8	
%RSD			0.2	

Table.No.8. Showing results for intermediate precision of Clotrimazole

Peak Name : Clotrimazole

	Peak name	Rt	area	height
1	**clotrimazole**	**3.278**	**122962**	**19165**
2	**clotrimazole**	**3.281**	**122487**	**19115**
3	**clotrimazole**	**3.281**	**122632**	**19073**
4	**clotrimazole**	**3.281**	**122626**	**19003**
5	**clotrimazole**	**3.283**	**122702**	**19123**
Mean			**122681.8**	
Std.dev.			**174.8**	
%RSD			**0.1**	

Detection limit

Table .No.9 Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Clindamycin	382625.50	572175863	3.17
clotrimazole	5862.40	467579210	0.0172

Quantitation limit

Table.No.10. Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Clindamycin	381727.80	583265980	5.80
clotrimazole	5681.30	469828490	0.212

Robustness

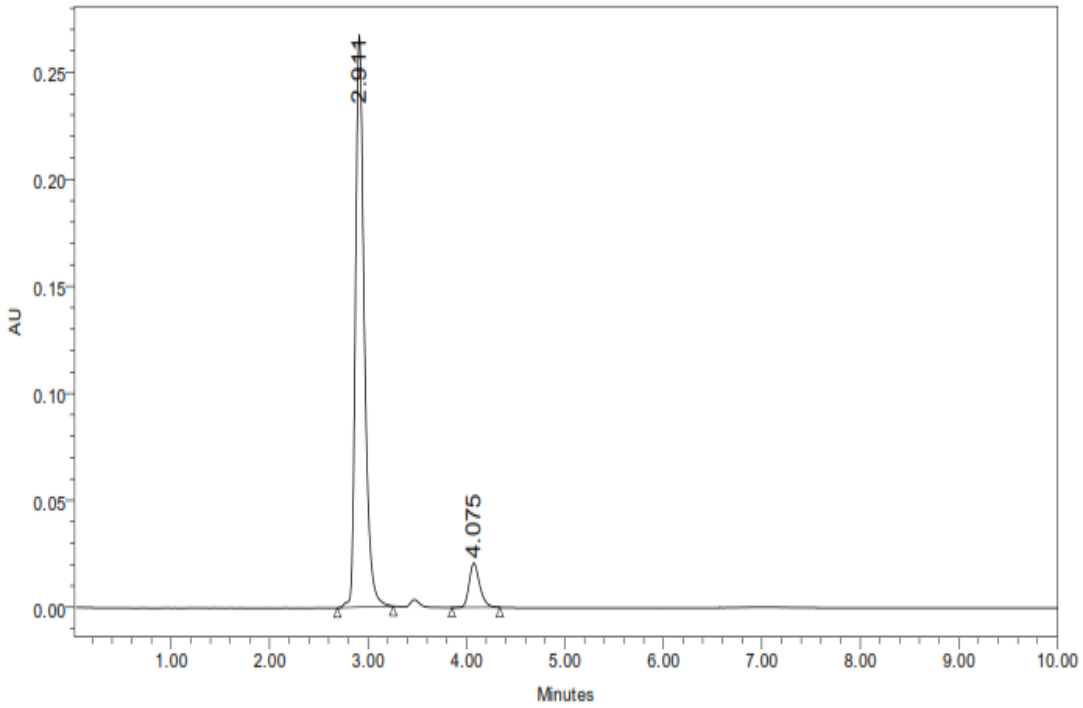


Fig.No.5. Chromatogram showing less flow rate 0.8ml/min

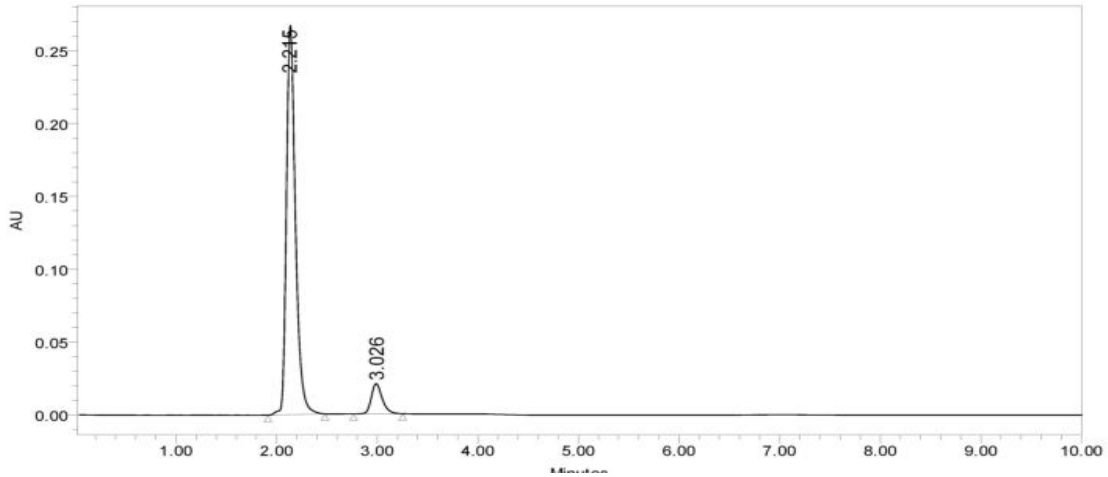


Fig.6. Chromatogram showing less flow rate 1.2 ml/min

The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

Table.No.11 Showing system suitability results for Clindamycin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339	1.4
2	**1**	**4668**	**1.3**
3	1.2	5216	1.4

Table.No.12. Showing system suitability results for Clotrimazole

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1. 3

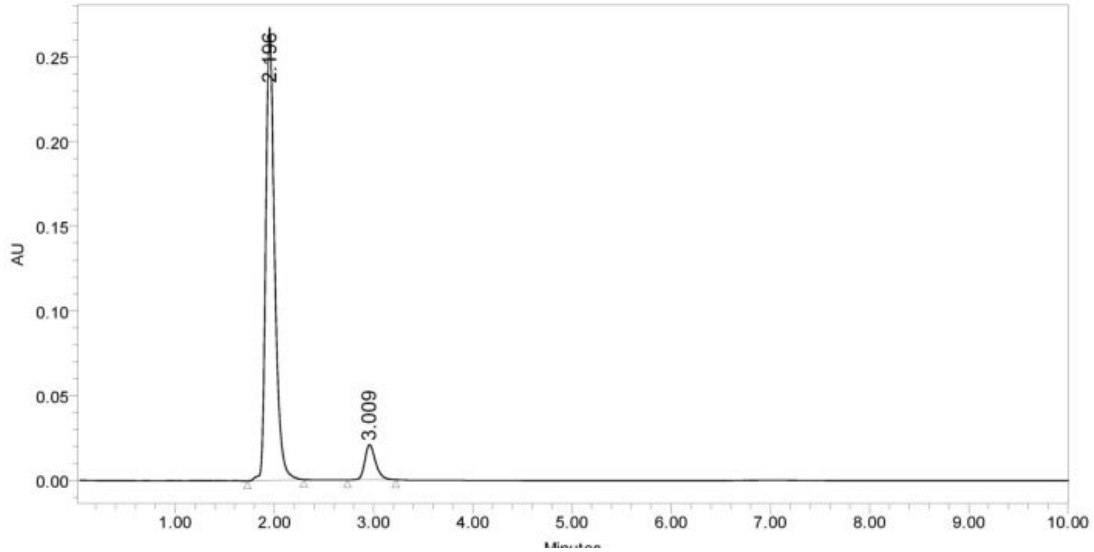


Fig.No.7 Chromatogram showing more organic phase ratio

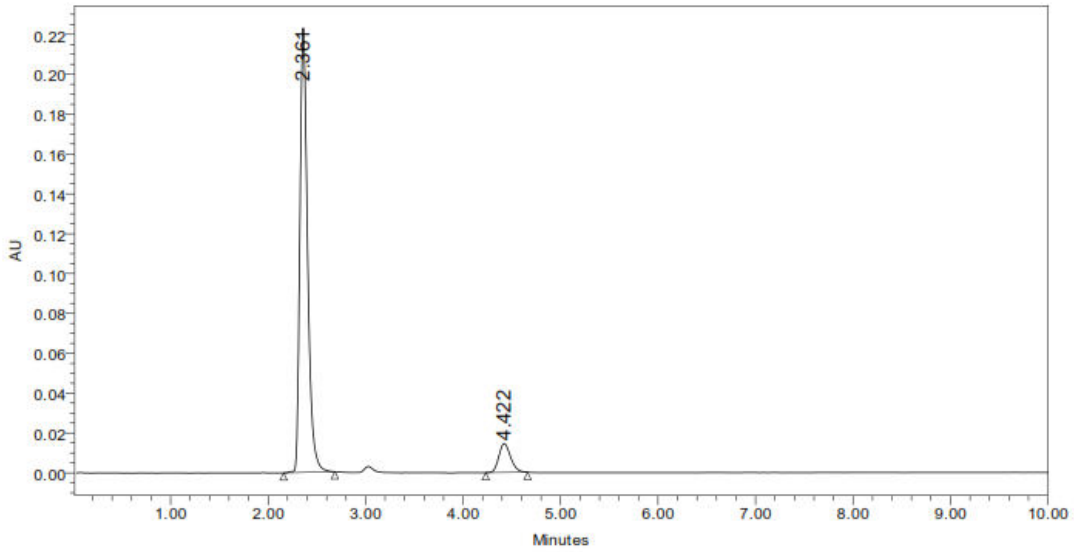


Fig.No 8. Chromatogram showing less organic phase ratio

On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Table.No.13. Showing system suitability results for Clindamycin

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	6232	1.4
2	***Actual**	**4668**	**1.3**
3	5 % more	6387	1.4

Table.No.14. Showing system suitability results for Clotrimazole

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	***Actual**	**6089**	**1.2**
3	5 % more	4817	1.2

SUMMARY AND CONCLUSION:

A new method was established for simultaneous estimation of Clindamycin and Clotrimazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Clindamycin and Clotrimazole by using ACE C18 column (4.6×150mm) 5µ, flow rate was 1.2 ml/min, mobile phase ratio was (70:30 v/v) methanol:Phosphate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.344 mins and 3.284 mins. The % purity of Clindamycin and Clotrimazole was found to be 101.27% and 99.97% respectively. The system suitability parameters for Clindamycin and Clotrimazole such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study in Clindamycin and Clotrimazole was found in concentration range of 50µg-250µg and 5µg-50µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.2 and 0.2, % RSD for

intermediate precision was 0.2 and 0.1respectively. The precision study was precise, robust, and repeatable. LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

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