ANALYSIS OF CLINDAMYCIN AND CLOTRIMAZOLE BY SIMULTANEOUS ESTIMATION 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

INTRODUCTION

Chromatography is the most powerful and versatile technique available to the modern analyst. In a single step process it can separate a mixture into its individual components and simultaneously provide a quantitative estimate of each constituent [1]. Samples may be gaseous, liquid or solid in nature and can range in complexity from a simple blend of two enantiomers to a multi component mixture containing widely differing chemical species. The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist, M.S.Tswett invented chromatography in 1902. The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). The acronym HPLC, coined by the late Prof. Csaba Horvath for his 1970 Pittcon paper originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns [2]. In the beginning, pumps only had a pressure capability of 500 psi. This was called high pressure

liquid chromatography, or HPLC. New HPLC instruments could develop up to 6,000 psi of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time (smaller particles, even higher pressure), the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography. HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low [3].

Materials and Methods

Water (HPLC grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), Ortho phosphoric acid (G.R), KH_2PO_4 (G.R), K_2HPO_4 (G.R), 0. 22µ Nylon filter (HPLC grade), 0.45µ filter paper (HPLC grade), Clindamycin and Clotrimazole (In- House).

Method development for the simultaneous estimation of Clindamycin and Clotrimazole by using RP-HPLC.

1. Selection of mobile phase
2. Selection of detection wavelength
3. Selection of column
4. Selection of solvent delivery system
5. Selection of flow rate
6. Selection of column temperature
7. Selection of diluent
8. Selection of test concentration and injection volume

1. Selection of mobile phase

- Sodium acetate buffer : Methanol (30 : 70% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
  Above 8: dissolution of silica [4].
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution.

2. Selection of wavelength: 10 mg of Clindamycin and Clotrimazole was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Clindamycin and Clotrimazole The isobestic point was taken as detection wavelength [5].

3. Selection of column

Heart of HPLC made of 316 grade stainless steel packed with stationary phase.

Silica based columns with different cross linking's in the increasing order of polarity are as follows:

←------- Non-polar----------moderately polar--------Polar----------→

C_{18}< C_8< C_6< Phenyl < Amino <Cyano< Silica

In reverse phase chromatography, hydrophobic interaction between drug molecule and the alkyl chains on the column packing material [6]. Column is selected based on solubility, polarity and chemical differences among analysts and Column selected: i.e. Thermosil C18 column (4.6 x125 mm) 5 µ.

Reasons : Better separation,

Good tailing factor.

4. Selection of solvent delivery system [7]

Always preferable solvent delivery system.

More chance of getting reproducible result on retention time of analytes.

More economic than gradient technique.

5. Selection of flow rate

Acceptable limit: - Not more than 2.5 ml/min

- Flow rate selected was 1ml/min
- Flow rate is selected based on
  1. Retention time
  2. Column back pressure
  3. Peak symmetry.
  4. Separation of impurities.

Reasons:

- For earlier elution of analyte and elution of all impurities within 6.0 min.
- Information from the reference method in literature.

6. Selection of diluent [8]

Selection of diluent is based on the solubility of the analyte

Diluent selected: Sodium acetate buffer : Methanol (30 : 70% v/v)

7 Selection of column temperature:

- Preferable temperature is ambient or room temperature.

  Reasons:
  - To elute all impurities along with analyte with in 10.0 min of run time.
  - Less retention time
  - Good peak shape

- ❖ Higher theoretical plates.
- ❖ Good resolution.

8. Selection of test concentration and injection volume [9]

Test concentration is finalized after it is proved that API is completely extractable at the selected test concentration.

- ➢ Test concentration is fixed based upon the response of API peak at selected detector wavelength.
- ➢ And the test concentration selected is 10 ppm.
- ➢ Injection volume selected was 10µL.

Reason: good peak area, retention time, peak symmetry.

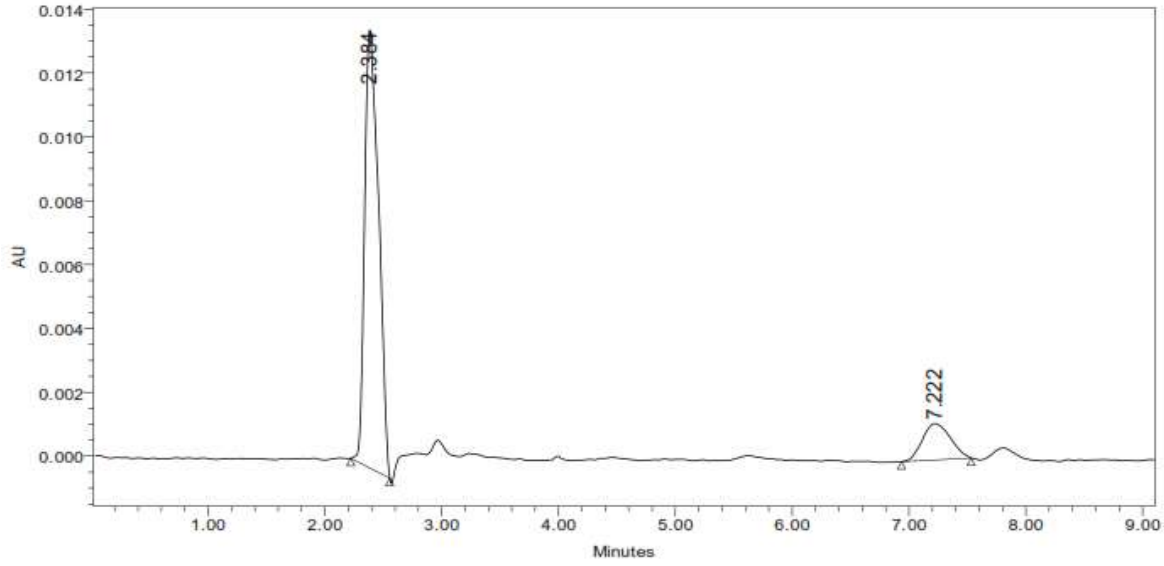
9. Chromatographic trials for simultaneous estimation of Clindamycin and Clotrimazole by RP- HPLC [10].

Trial-1

Chromatographic conditions

Column	:	Thermosil C18 4.6x150mm, 5µm
Mobile phase ratio	:	MeOH: H_2O (60:40%v/v)
Detection wavelength	:	252 nm
Flow rate	:	1ml/min
Injection volume	:	10µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	10min
Retention time	:	2.384 min&7.222 min

Figure 1: Chromatogram showing trial-1 injection



Observation:

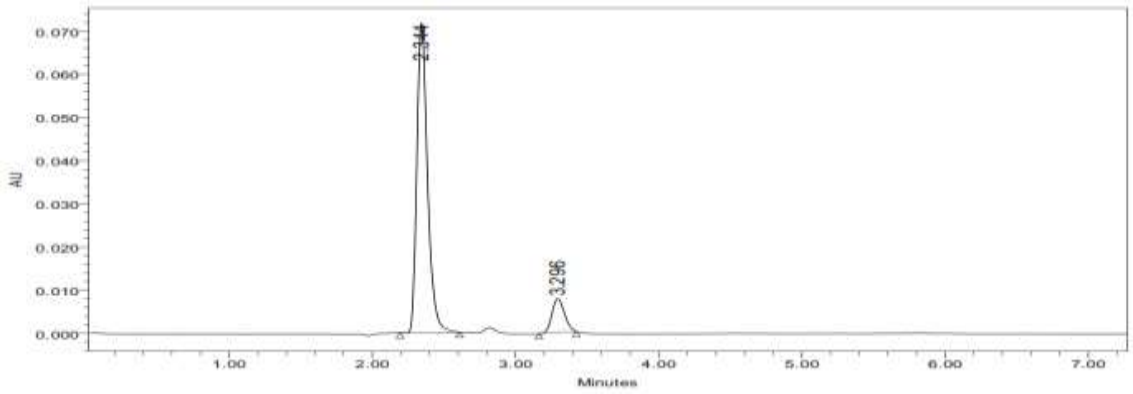
The trial shows no proper separation peaks in the chromatogram, so more trials were required for obtaining peaks.

Trial-2

Chromatographic conditions

Column	:	Zodiac sil RPC18 4.6×250mm 5µm
Mobile phase ratio	:	ACN: pH 3 buffer (65:35% v/v)
Detection wavelength	:	252 nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	7 min
Retention time	:	2.344 mins &3.296 mins

Figure 2: Chromatogram showing trial-4 injections



Observation:

The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks.

Procedure

Preparation of phosphate buffer

6.8 grams of sodium acetete was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid [11]. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 30 ml (30%) and 70 ml of Methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration [12].

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual Clindamycin standard preparation

10 mg of Clindamycin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it

completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluents [13].

Preparation of the individual Clotrimazole standard preparation

10 mg of Clotrimazole working standard was accurately weighed and transferred into a 10 ml 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 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Assay

Assay preparation of the standard and sample solution

Sample solution preparation:

1mg of Clindamycin and 10 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 diluents [14].

Standard solution preparation

1mg Clindamycin and 10 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.

Procedure

10μL of the blank, standard and sample were injected into the chromatographic system and areas for the Clindamycin and Clotrimazole the peaks were used for calculating the % assay by using the formulae.

System suitability

- Tailing factor for the peaks due to Clindamycin and Clotrimazole in standard solution should not be more than 1.5.
- Theoretical plates for the Clindamycin and Clotrimazole peaks in standard solution should not be less than 2000.

Assay calculation

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Clindamycin mg/ml.

ANALYTICAL METHOD VALIDATION [15-18]

Validation parameters

- Specificity
- Linearity
- Range
- Accuracy

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

Preparation of stock solution

1 mg of Clindamycin and 10 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.

Procedure

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

Acceptance criteria

- Correlation coefficient should be not less than 0.999.

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 5µg/ml-25µg/ml and 50µg/ml-250µg/ml of Clindamycin and Clotrimazole respectively.

4. Accuracy

Preparation of standard stock solution

1mg of Clindamycin and 10 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 (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

0.5mg of Clindamycin and 5 mg of Clotrimazole working standard 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 make volume up to the mark with the same solvent (Stock Solution).Further pipette out 10 ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 100% solution (with respect to target assay concentration)

1 mg of Clindamycin and 10 mg of Clotrimazole working standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml 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 above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for Clindamycin and Clotrimazole and calculate the individual % recovery and mean % recovery values.

Acceptance criteria

The % recovery for each level should be between 98.0 to 102.0%

RESULTS AND DISCUSSIONS

Method Development

Figure 3: Spectrum showing overlapping spectrum of Clindamycin and Clotrimazole

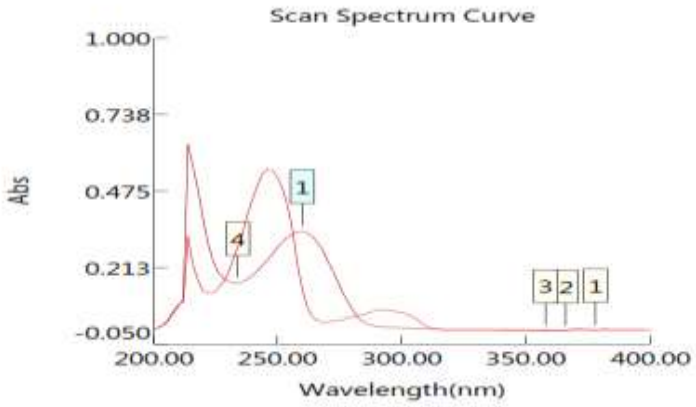


Figure 4: Spectrum showing wavelength of Clindamycin

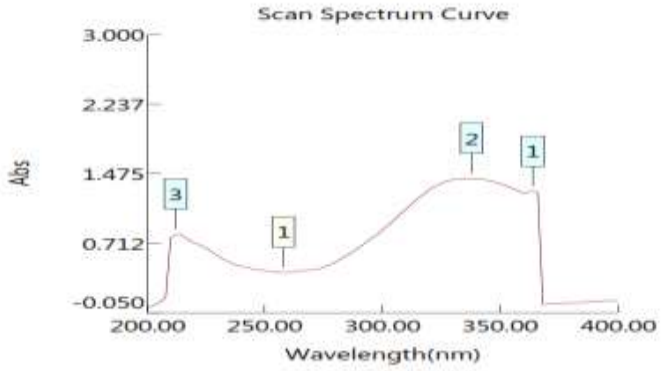
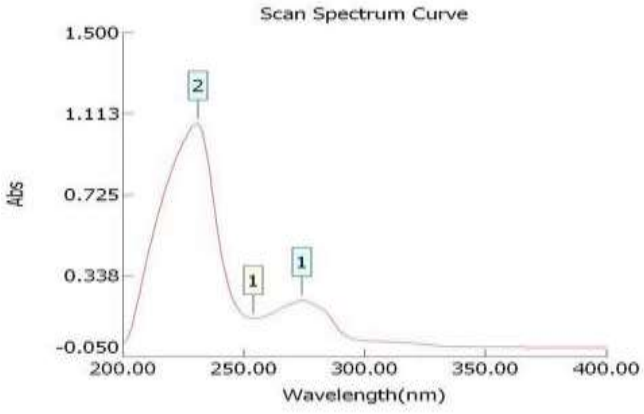


Figure 5: Spectrum showing wavelength of Clotrimazole

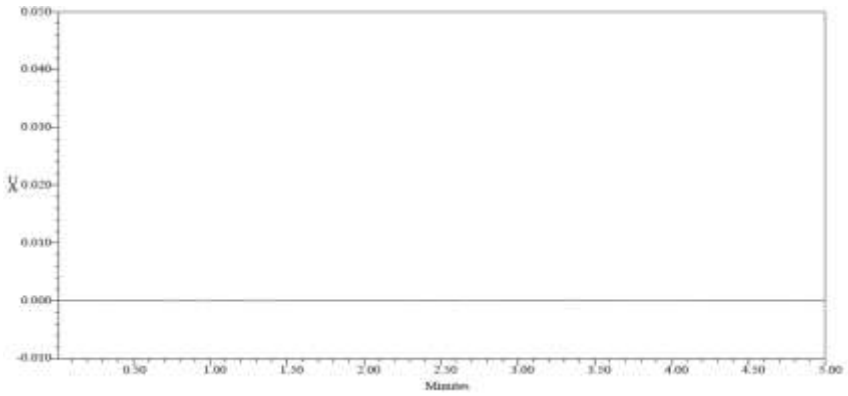


Optimized chromatographic conditions for simultaneous estimations of Clindamycin and Clotrimazole by RP-HPLC method

Column	:	Thermosil C18 (4.0×125 mm) 5.0 µm
Column temperature	:	Ambient

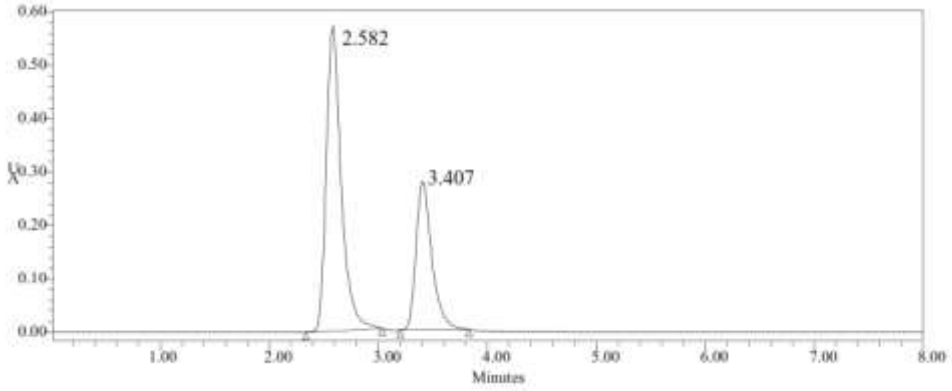
Wavelength	:	252 nm
Mobile phase ratio	:	70:30 Methanol: Sodium acetate buffer
Flow rate	:	0.7 ml/min
Auto sampler temperature	:	Ambient
Injection volume	:	10µl
Run time	:	10.0 minutes

Figure 6: Chromatogram showing blank preparation (mobile phase)



Assay calculation for Clindamycin and Clotrimazole: The assay study was performed for the Felodipine and Simvastatin . Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Fig. No. and results are tabulated in Table.No .

Figure 7: Chromatogram showing assay of sample injection-1,2.



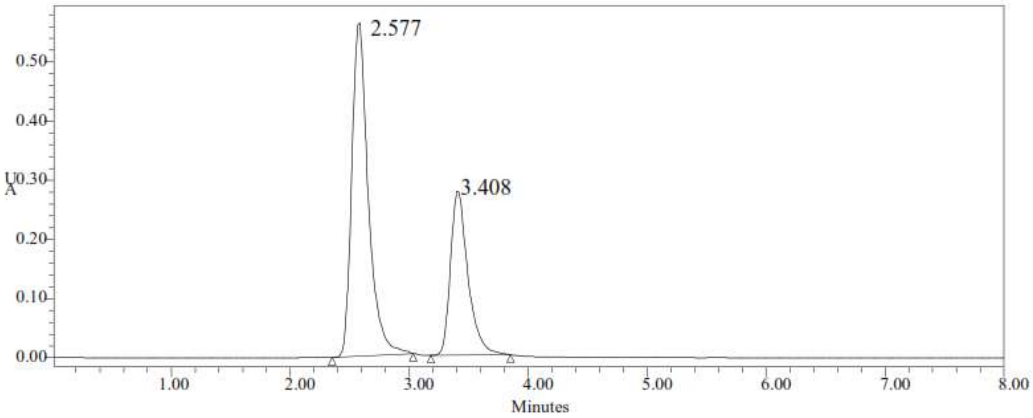


Figure 8: Chromatogram showing assay of Standared injection -1,2.

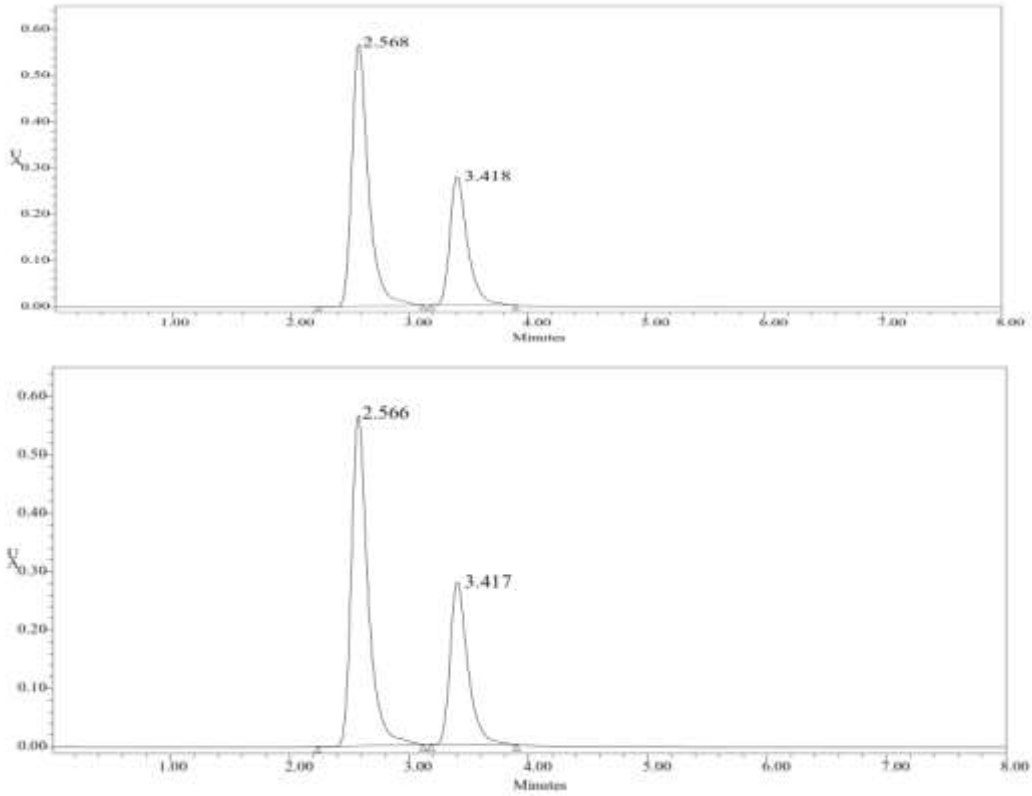


Table 1: Showing assay results

S.No	Name of compound	Label claim	Amount taken	%purity
1	Clindamycin	500	754.7	99.24
2	Clotrimazole	2.5	735.6	101.04

VALIDATION REPORT

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 study was performed by injecting blank. The chromatograms are shown in Figure 9, 10, 11.

Figure 9: Chromatogram showing blank (mobile phase preparation)

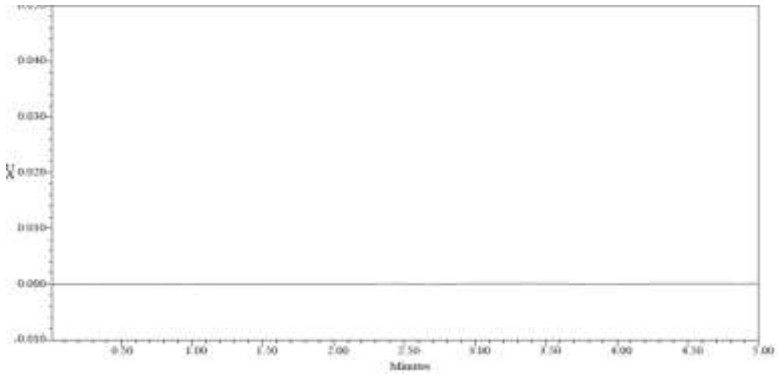


Figure 10: Chromatogram showing standard injection

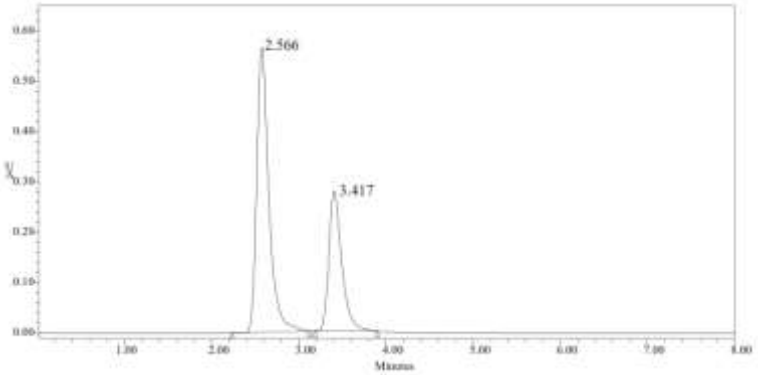
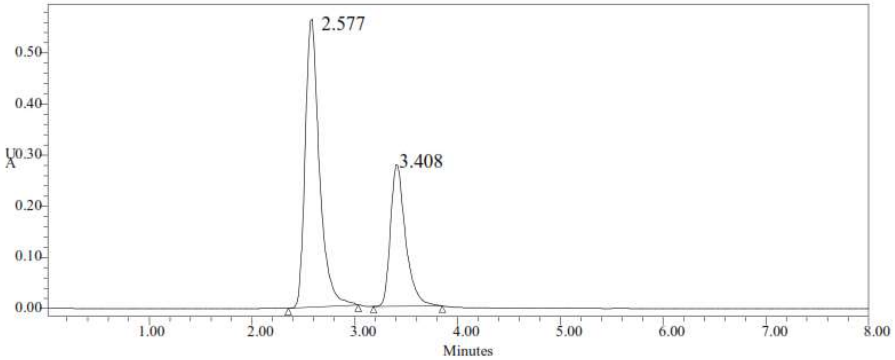


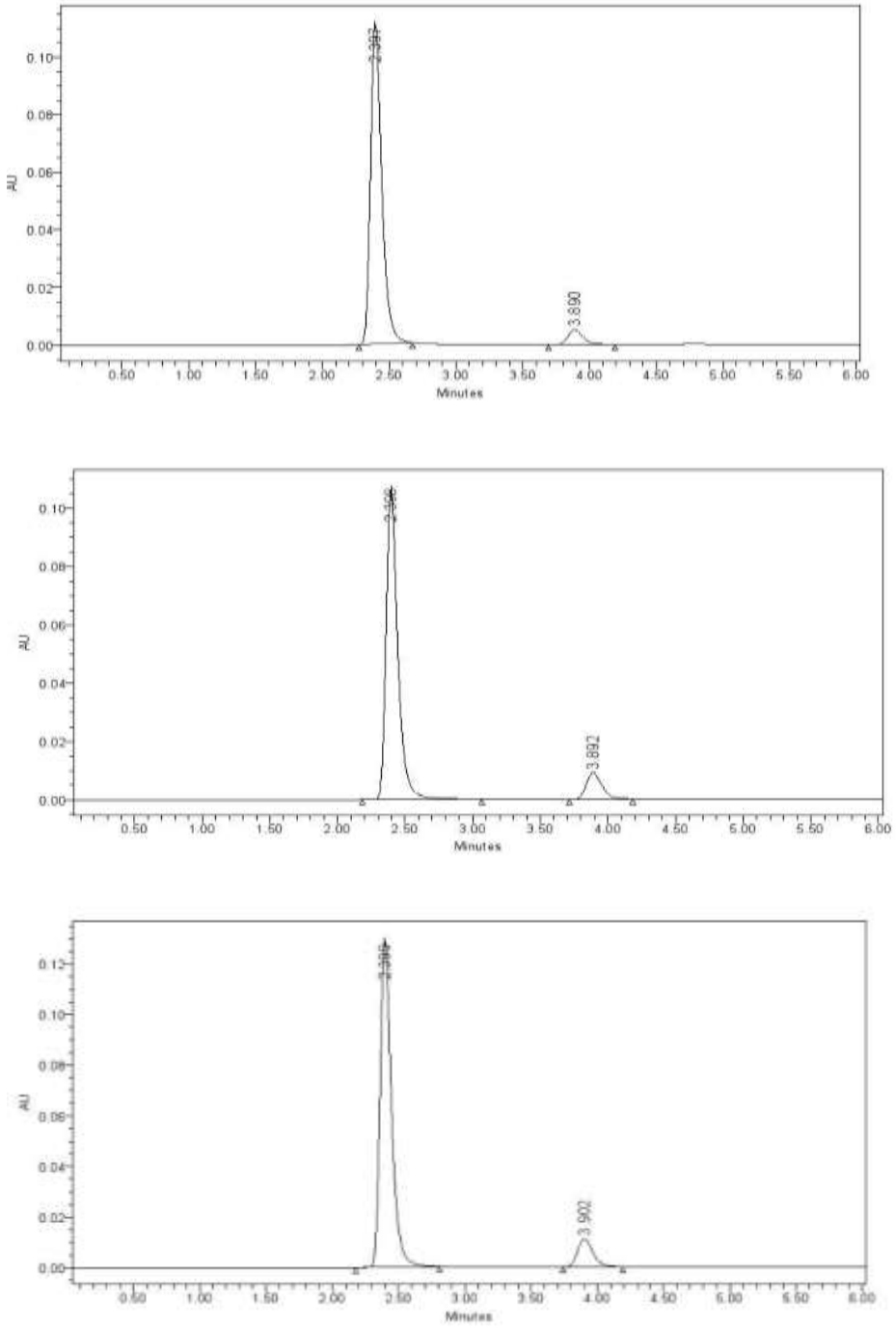
Figure 11: Chromatogram showing sample injection



The specificity test was performed for Clindamycin and Clotrimazole. It was found that there was no interference of impurities in retention time of analytical peak.

Linearity

Figure 12: Chromatograms showing linearity level-1 to level 5 (5ppm-25 ppm of Clindamycin and 50ppm -250ppm of Clotrimazole) injections.



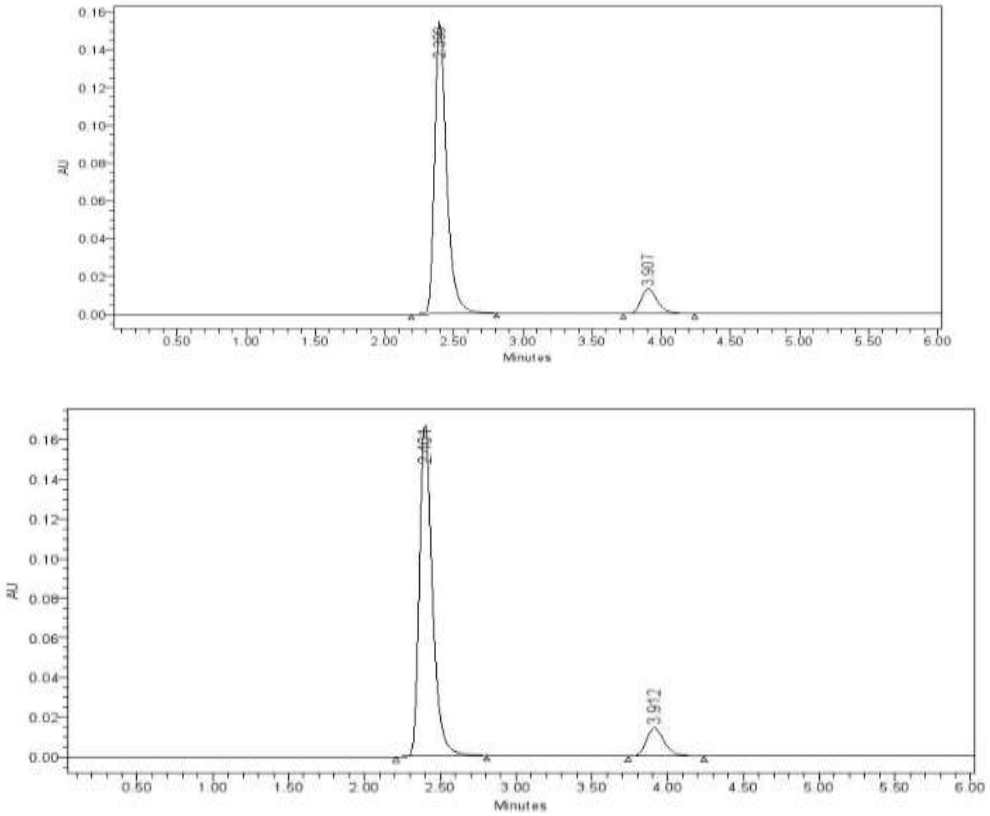


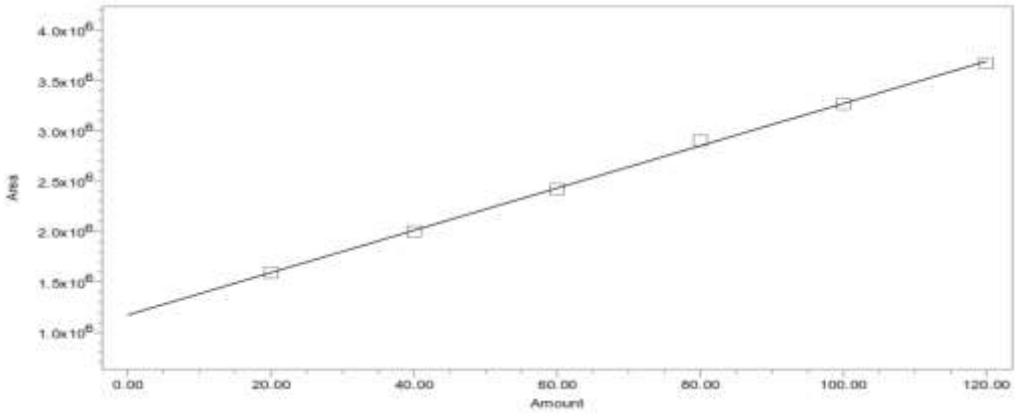
Table 2:Linearity Results for Clindamycin

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

Table 3: Linearity Results for clotrimazole:

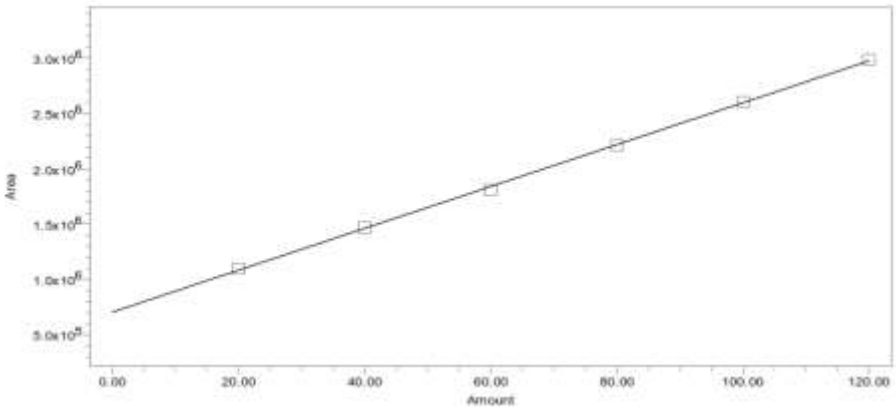
S.No	Linearity Level	Concentration	Area
1	I	5ppm	56472
2	II	10 ppm	73841
3	III	15ppm	92655
4	IV	20ppm	111541
5	V	25ppm	130567
Correlation Coefficient			0.999

Figure 13: showing Calibration graph Clindamycin



Clindamycinr^2 = 0.999

Figure 14: Showing calibration graph for Clotrimazole

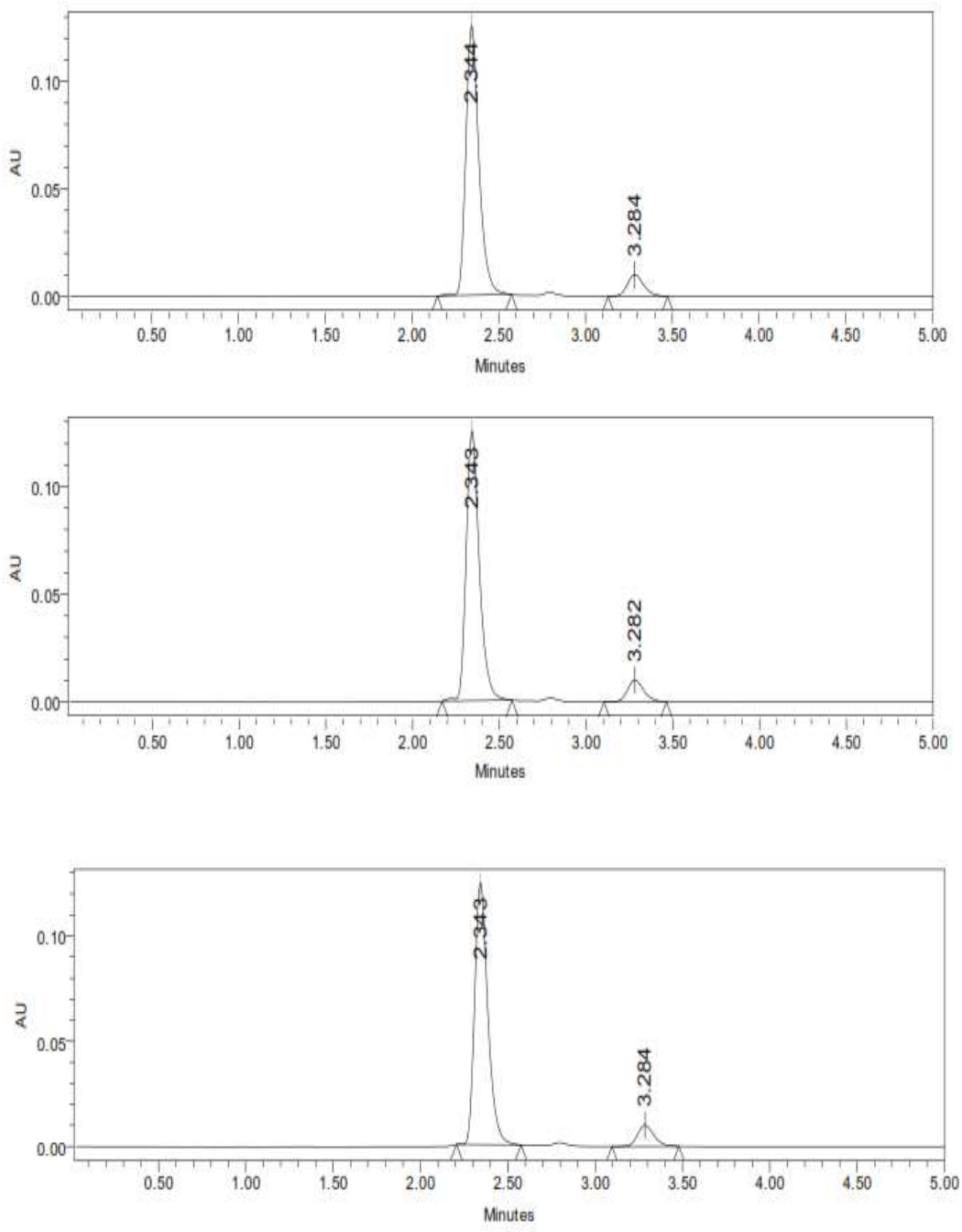


Clotrimazole r^2 = 0.999

The linearity study was performed for concentration range of 5.µg-25µg and 20µg-100µg of Clindamycin and Clotrimazole and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999).

Accuracy

Figure 15: Chromatograms showing accuracy-50% injection-1,2,3



Accuracy -100%

Figure 16: Chromatogram showing accuracy -100% injection-1,2,3

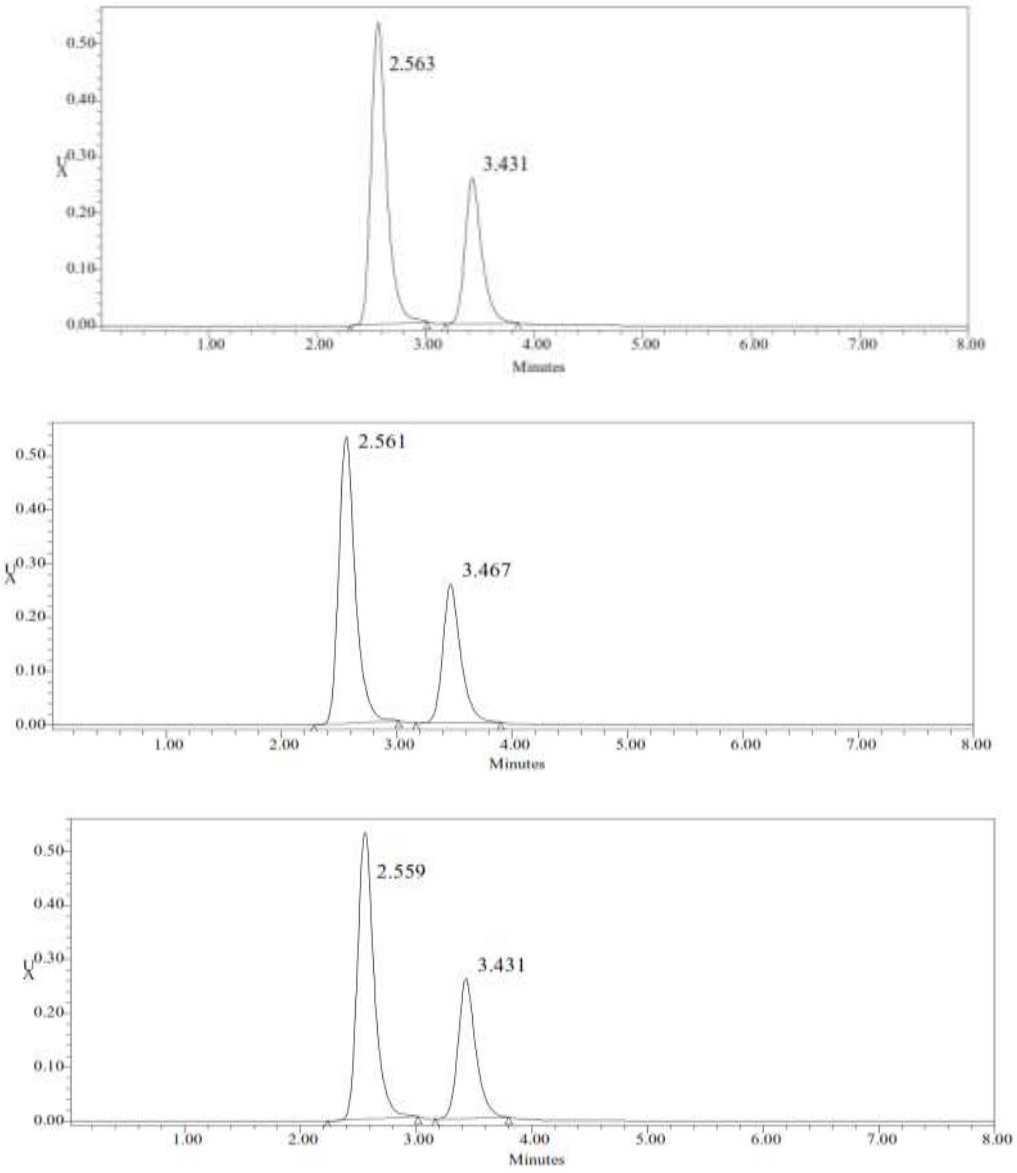


Table 4: Showing accuracy results for Clindamycin

%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%	

Table 5: 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%	

The accuracy study was performed for % recovery of Clindamycin and Clotrimazole. The % recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%)

Detection limit

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.

Formula:

$$\text{LOD} = 3.3 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD)

S - Slope

Figure 17: Showing results for Limit of LOD

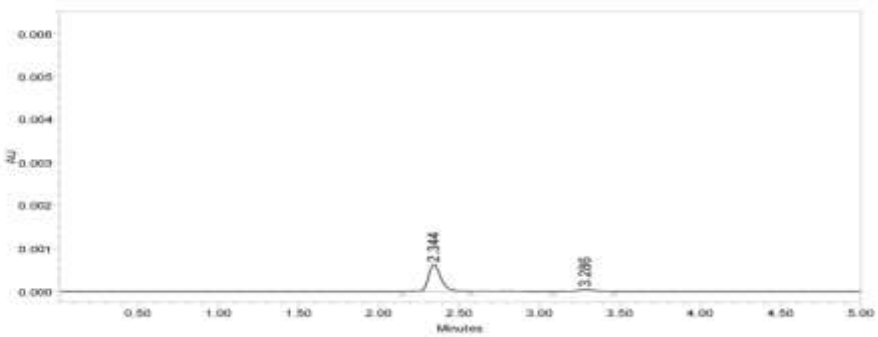


Table 6: Showing results for Limit of Detection

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

The LOD was performed for Clindamycin and Clotrimazole was found to be 3.17and 0.0172 respectively.

Quantitation limit

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.

Formula:

$$\text{LOQ} = 10 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

Figure 18: Showing results for Limit of LOQ

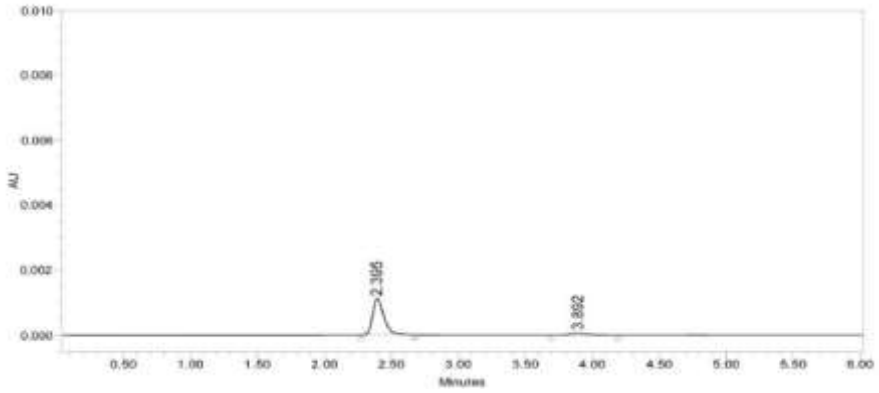


Table 7: Showing results for Limit of Quantitation

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

The LOQ was performed for Clindamycin and Clotrimazole was found to be 5.80 and 0.212 respectively.

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Clindamycin and Clotrimazole. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.The chromatograms are shown in Fig and results are tabulated in Table.No.

Figure 19: Chromatogram showing less flow rate 0.8ml/min

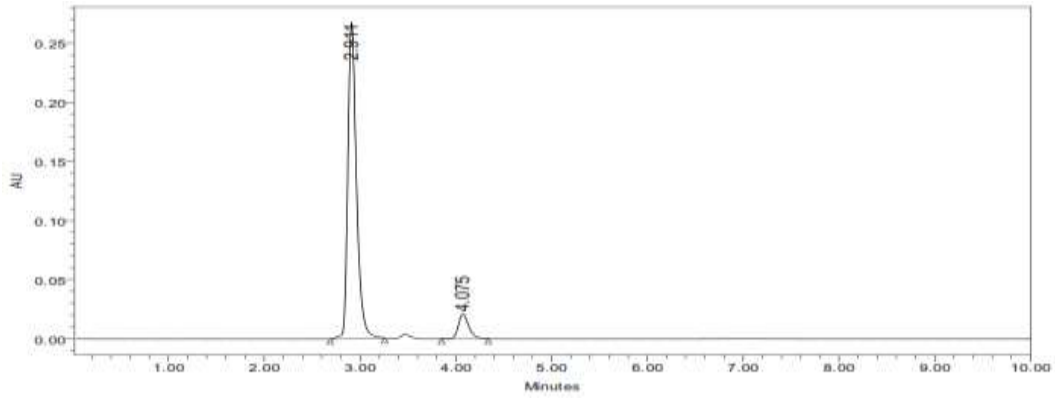
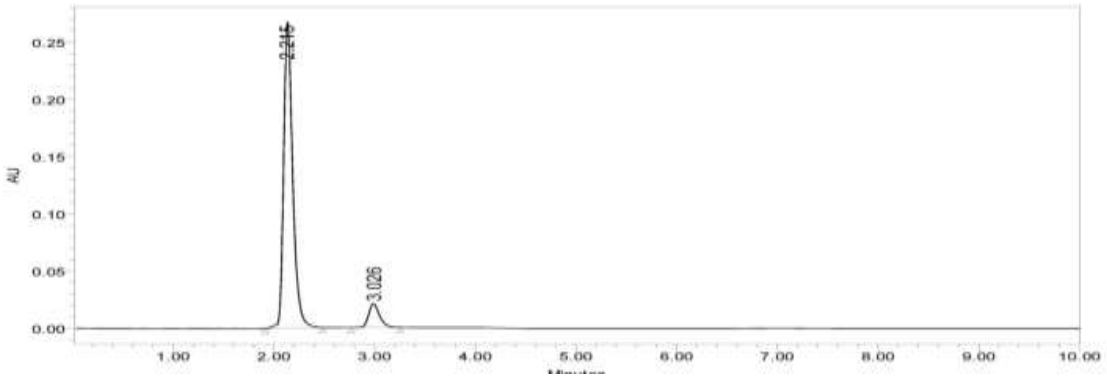


Figure 20: Chromatogram showing less flow rate 1.2 ml/min



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 8: Showing system suitability results for Clindamycin

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

Table 9: 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

Figure 21: Chromatogram showing more organic phase ratio

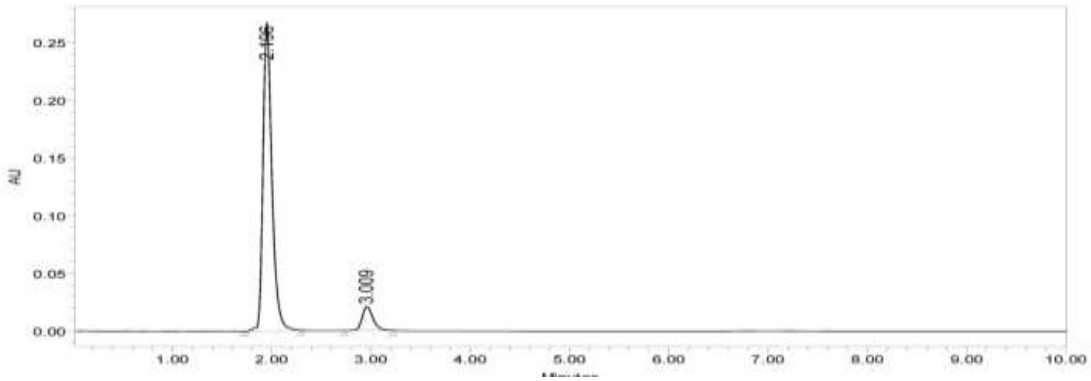
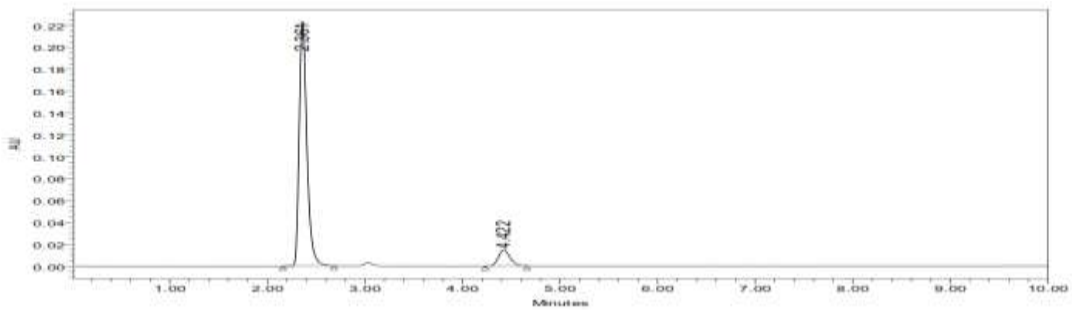


Figure 22: Chromatogram showing less organic phase ratio



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 10: 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 11: Showing system suitability results for Clotrimazole

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

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 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.

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