Original Article

Analytical Method Development And Validation For The Simultaneous Estimation Of Neomycin And Clotrimazole By Rp- Hplc Method

J.Harika[1] , P. Sowjanya[2] , Dr.Gampa Vijaya Kumar[3]

[1,2,3] Department of Pharmaceutical Analysis, KGR Institute of Technology and Mangement, Rampally, Keesara, Rangareddy, Telangana, India
*Corresponding Author
Dr. Gampa Vijaya Kumar ,
Ph.D, Professor,&Principal,
KGR Instiute Of Management And Technology
Email id :Vijaytanu71@gmail.com
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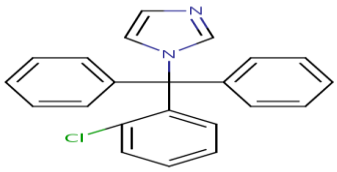
Abstract

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Neomycin and Clotrimazole was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Neomycin and Clotrimazole were found to be from 100-500 µg/ml of Neomycin and 1-5µg/ml of Clotrimazole. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Neomycin and Clotrimazole. LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEY WORDS : Methanol: Phosphate buffer, Inertsil C_{18} column, Neomycin and Clotrimazole.

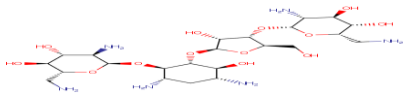
INTRODUCTION

Clotrimazole IUPAC name 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole It is soluble in DMSO (25 mM), chloroform (50 mg/mL), DMF, ethyl acetate, ethanol, It is an anti-fungal agent, 14-alpha demethylase inhibitor and anti-infective agent.



Clotrimazole

Neomycin sulphate IUPAC name (2R, 3S, 4R, 5R, 6R)-5-amino-2-(aminomethyl)-6-{[(1R, 2R, 3S, 4R, 6S)-4, 6-diamino-2-{[(2S, 3R, 4S, 5R)-4-{[(2R, 3R, 4R, 5S, 6S)-3 amino-6-(aminomethyl)-4, 5- dihydroxyoxan-2-yl]oxy}-3-hydroxy 5 (hydroxymethyl)oxolan-2-yl]oxy}-3-hydroxycyclohexyl]oxy}oxane-3, 4-diol.



Neomycin

MATERIALS AND METHOD

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine

Chemicals

Neomycin and Clotrimazole Potassium dihydrogen orthophosphate, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

METHOD DEVELOPMENT

Trial-5 (Optimised)

Chromatographic conditions

Column	:	Inertsil C18 (4.6 x 250mm, 5µm)
Buffer pH	:	3.0.
Mobile phase	:	30% buffer 70% Methanol
Flow rate	:	1.0ml per min
Wavelength	:	260 nm
Temperature	:	ambient.
Run time	:	10min.

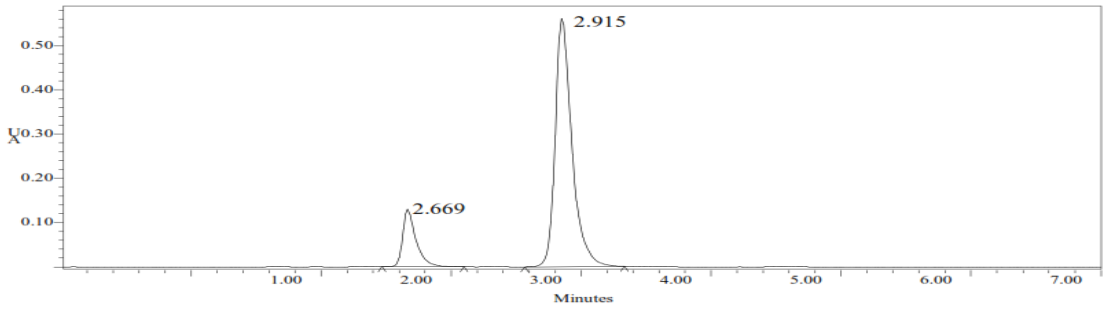


Fig.No.1. Chromatogram showing trial-5 injection

Sample solution preparation

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Neomycin and Clotrimazole (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 3 ml of Neomycin e and Clotrimazole of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD VALIDATION

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

RESULTS AND DISCUSSION

System suitability

Table 1: Results of system suitability parameters for Neomycin and Clotrimazole

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Neomycin**	2.5	124505	213642		1.2	4673.4
2	**Clotrimazole**	3.9	1308495	154566	6 0	1.3	6090.3

Precision

Table 2: Results of method precession for Neomycin

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Results of method precession for Clotrimazole

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Intermediate Precession (Ruggedness)

Table 4: Results of Intermediate precision for Neomycin

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5: Results of Intermediate precision for Clotrimazole

Injection	Area
Injection-1	122487

Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Accuracy

Table-6 Accuracy (recovery) data for Neomycin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table-7 Accuracy (recovery) data for Clotrimazole

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Linearity

Table-8 Area of different concentration of Neomycin

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934

2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table-9 Area of different concentration of Clotrimazole

S.No	**Linearity Level**	**Concentration**	**Area**
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

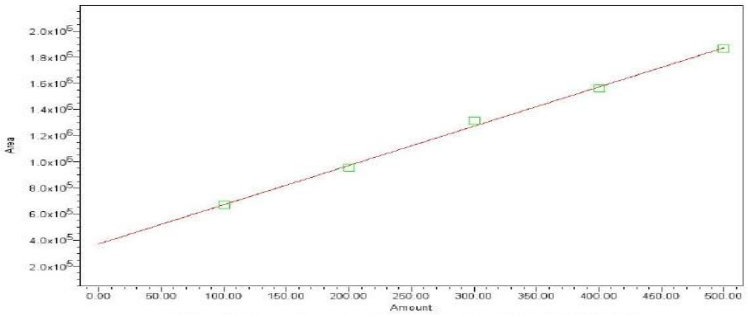


Figure 2 Calibration graph for Neomycin

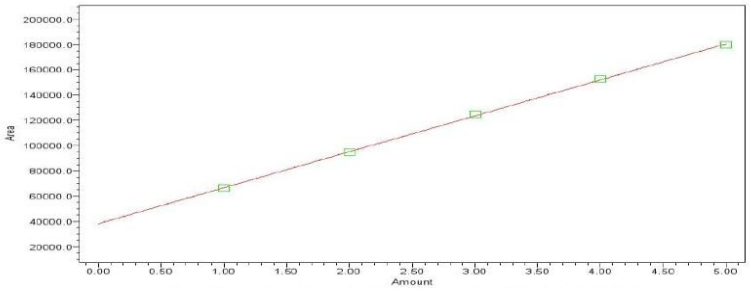


Figure 3 Calibration graph for Clotrimazole

Table-10 Analytical performance parameters of Neomycin and Clotrimazole

Parameters	Neomycin	Naloxone
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

LIMIT OF DETECTION

Table-11 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Neomycin	52	152	2.9
Clotrimazole	52	156	3

LIMIT OF QUANTIFICATION

Table no-12 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Neomycin	52	522	10.03
Clotrimazole	52	524	10.1

Robustness

Table-13 Flow Rate (ml/min) data for Neomycin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table-14 flow rate (ml/min) data for Clotrimazole

		System Suitability Results

S. No	Flow Rate (ml/min)	USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table -15 Change in Organic Composition in the Mobile Phase for Neomycin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table -16 Change in Organic Composition in the Mobile Phase for Clotrimazole

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

SUMMARY AND CONCLUSION

The proposed analytical technique of RP-HPLC is simple, accurate and precise method for the simultaneous estimation of Neomycin and Clotrimazole in pharmaceutical dosage forms has been developed. The method was validated as per ICH guidelines. Statistical analysis proves that method is repeatable, sensitive for the analysis of Neomycin and Clotrimazole in pharmceutical dosage forms.

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