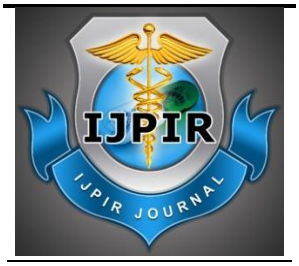
Research Article



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Reverse phase high performance liquid chromatography method development and validation and validation for estimation of Netlimicin in injection formulation

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Netilmicin, in its pure form as well as in injection dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and water (45:55% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 260nm. The retention time of the Netilmicin was 2.379 ±0.02min respectively. The method produce linear responses in the concentration range of 24-120mg/ml of Netilmicin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Netilmicin, Reverse phase, HPLC, Validation, Injection formulation, Symmetry column, C18, Methanol.

INTRODUCTION

Netilmicin is an Anti-Bacterial Agent with a pKa value 12.49. Literature review reveals that several analytical mehods were performed to estimate Netlimicin in pure and different dosage form. To develop new simple, sensitive, accurate and economical analytical method for the estimation of Netilmicin. To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Netilmicin in dosage form.

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Figures

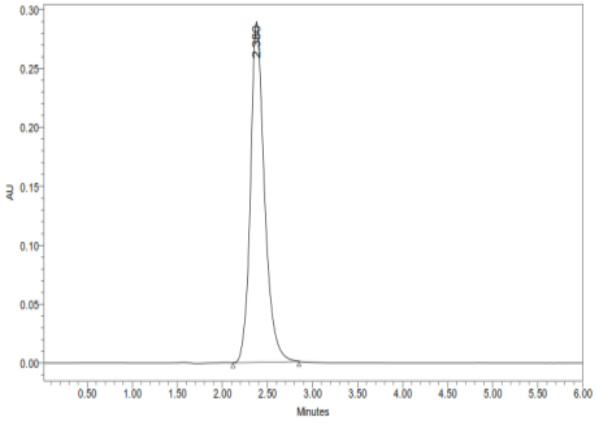

Figure: 1. Optimized Chromatogram

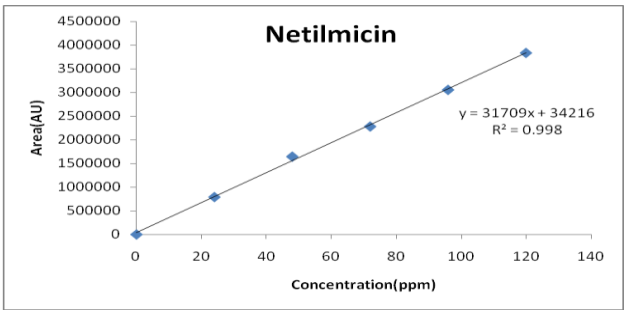

Figure: 2. Linearity Plot

Tables

Table:1. Precision results for Netilmicin.

Repeatability	Mean Area	Standard deviation	%RSD
	2275724	9476.485	0.416416
Intermediate Precision	2241395	4333.851	0.193355

Table: 2. Accuracy results for Netilmicin.

%Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1172485	36	35.8	99.4	99.5%
100%	2314753	72	71.6	99.4	
150%	3480210	108	107.9	99.9	

Table: 3. Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.8mL/min	3119086	2.379	5837	1.2
Less Flow rate of 0.7mL/min	2640811	2.763	5361	1.2
More Flow rate of 0.9mL/min	2640354	2.234	5231	1.2
Less organic phase	2640758	2.765	4503	1.5
More organic phase	2640125	2.236	4491	1.5

MATERIALS AND METHOD

Literature review reveals that estimation was performed with mobile phase methanol: trifluoroacetic acid (20 : 80)[1], mobile phase consisting of (A) pentafluoropropionic acid–water–acetonitrile (0.1:96:4, v/v/v) and (B) trifluoroacetic acid–water–acetonitrile (1:96:4, v/v/v)[2].The present The method was performed on WATERS Alliance 2695 separation module for estimation of Netlimicin with methanol water combination with less retention times.

EXPERIMENTAL

Method development

The method was developed with mobile phase methanol: water (45:55) ratio with a Column (Symmetry C18 (4.6 x 150mm, 5µm)), flow rate 0.8ml/min, Wavelength (260nm), Injection volume (10µl)and the Run time (6minutes).the standard and sample stocks were prepared by using methanol and further dilution was done by further pipetting 0.72ml of the above Netilmicin stock solution into a 10ml volumetric flask with methanol.

The system suitability and specificity was performed by injecting the standard solution and the results were tabulated.

The Linearity was performed with a concentration range of 24ppm to 120 ppm and correlation coefficient is calculated and values are tabulated.

Method Validation

Precision

Repeatability was performed by injecting the standard solution five times and measured the area for all five injections in HPLC. The %RSD was tabulated.

Intermediate precision was evaluated on different days by maintaining same conditions. The %RSD was tabulated.

Accuracy

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Calculated the Amount found and Amount added for Netilmicin and calculated the individual recovery and mean recovery values.

Robustness

The analysis was performed in different conditions in terms of altered mobile phase composition (±5%) and with a flow rate (±0.2ml/min) to find the variability of test results and the results was tabulated.

RESULTS AND DISCUSSIONS

It was found from above data that all the system suitability parameters for developed method were within the limit. (Theoretical plates>2000and Tailing factor was within the range of 0.9 to 2, % RSD Values are <2).

Analytical method was tested for specificity to measure accurately Netilmicin quantity in drug product and the impurities, degradation products, and matrix components are not interfered with test results.

Linearity was assessed and Correlation Coefficient (r) was 0.99, and the intercept was 34216. These values meet the validation criteria. Precision was also assessed and the %RSD values were found within 2.

The Accuracy results obtained in terms of percentage recovery at 50%, 100%, 150% and the results were within the limits (98-102%). Hence method is accurate.

The robustness was performed and there was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The LOD and LOQ values are acceptable.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Netilmicin in bulk drug and pharmaceutical dosage forms without any preliminary chemical derivatisation or purification steps.

Netilmicin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: water was chosen as the mobile phase.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

LIST OF ABBREVIATIONS

RP-HPLC-Reverse Phase High Performance Liquid Chromatography.
LOD- Limit of Detection.
LOQ-Limit of Quantitation.
RSD-Relative Standard Deviation.
PPM-Parts Per Million.

Conflict of interest

Financial support has been provided by the management of Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar, and Telangana, India.

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