World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

QUANTITATIVE ESTIMATION OF NETLIMICIN IN PURE AND PHARMACEUTICAL FORMULATION BY RP- HPLC

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Article Received on 08/05/2025 **Article Revised on 28/05/2025** **Article Accepted on 18/06/2025**

ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Netilmicin. **Methods:** A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Netilmicin. The chromatographic strategy utilized Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Methanol and Water were consists of 45:55 % v/v. A flow rate of 0.8 ml/min and a detector wavelength of 260 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. **Results:** LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range. **Conclusion:** The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

KEYWORDS: Netilmicin, RP-HPLC, Method Development, Validation, Accuracy, Robustness.

INTRODUCTION

Netilmicin is a semisynthetic 1-N-ethyl derivative of sisomycin, an aminoglycoside antibiotic with action similar to gentamicin, but less ear and kidney toxicity. Netilmicin inhibits protein synthesis in susceptible organisms by binding to the bacterial 30S ribosomal subunit and interfering with mRNA binding and the acceptor tRNA site.

Aminoglycosides like netilmicin "irreversibly" bind to specific 30S-subunit proteins and 16S rRNA. Specifically netilmicin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes, leaving the bacterium unable to synthesize proteins vital to its growth.

EXPERIMENTAL WORK

INSTRUMENTS USED

Table: Instruments used.

S.No.	Instruments and Glasswares	Model
1	HPLC	Shimadzu LC-10 AT VP
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil

6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table: Chemicals Used.

S.No	Chemical	Brand names
1	Netilmicin (Pure)	AR labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for LC	Merck

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Netilmicin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.72ml of the above Netilmicin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 45:55 v/v respectively.

Optimization of Column

The method was performed with various C18 columns like ODS column, Xterra, and X Bridge C18 column. Symmetry C18 (4.6 x 150mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Shimadzu LC-10 AT VP
Temperature : Ambient
Column : Symmetry C18 (4.6 x 150mm, 5□m)
Mobile phase : Methanol: Water (45:55% v/v)
Flow rate : 0.8ml/min
Wavelength : 260nm
Injection volume : 10µl
Run time : 6minutes

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Netilmicin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.72ml of the above Netilmicin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of the Powder and weight 10 mg equivalent weight of Netilmicin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.72ml of Netilmicin above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula.

%ASSAY =

$$\times \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \text{Weight of tablet} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

Accurately weigh and transfer 10 mg of Netilmicin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (24ppm of Netilmicin)

Pipette out 0.24ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – II (48ppm of Netilmicin)

Pipette out 0.48ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – III (72ppm of Netilmicin)

Pipette out 0.72ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – IV (96ppm of Netilmicin)

Pipette out 0.96ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – V (120ppm of Netilmicin)

Pipette out 1.2ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Optimized Chromatogram (Sample)

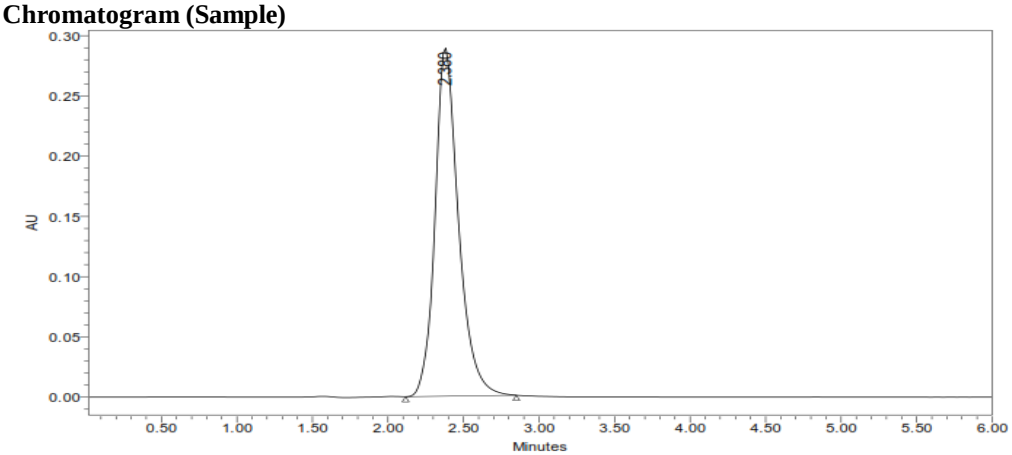

Figure: Optimized Chromatogram (Sample).

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Netilmicin	2.380	3118812	258374	1.2	5264

Table: Optimized Chromatogram (Sample).

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Netilmicin	2.317	2274631	239458	5728	1.2
2	Netilmicin	2.302	2284721	239582	5093	1.2
3	Netilmicin	2.323	2238127	236493	5391	1.2
4	Netilmicin	2.343	2259349	249482	6139	1.2
5	Netilmicin	2.321	2204850	239452	5281	1.2
Mean			2252336			
Std. Dev.			31827.08			
% RSD			1.41307			

Table: Results of system suitability for Netilmicin.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Netilmicin	2.354	2255919	248281	1.2	6582	1
2	Netilmicin	2.350	2255538	249382	1.2	5928	2
3	Netilmicin	2.354	2253363	241533	1.2	5291	3

Table: Peak results for assay standard.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Netilmicin	2.354	2258820	243782	1.2	5639	1
2	Netilmicin	2.350	2258600	248236	1.2	6198	2
3	Netilmicin	2.354	2257284	247382	1.2	5928	3

Peak results for Assay sample

LINEARITY

LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Netilmicin is a straight line.

Y = mx + c

Slope (m) = 31709 Intercept (c) = 34216

Correlation Coefficient (r) = 0.998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 34216. These values meet the validation criteria.

PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Table: Results of Repeatability for Netilmicin.

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Netilmicin	2.356	2259464	245362	5938	1.2
2	Netilmicin	2.356	2275915	248293	5827	1.2
3	Netilmicin	2.357	2282117	240795	5032	1.2
4	Netilmicin	2.358	2278675	230139	5978	1.2
5	Netilmicin	2.359	2282448	249605	6183	1.2
6	Netilmicin	2.385	2296854	249605	6183	1.2
Mean			2279246			
Std. Dev			12093.69			
%RSD			0.5306			

Table: Results of Intermediate precision for Netilmicin.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Netilmicin	2.380	2236184	202188	5472	1.2
2	Netilmicin	2.383	2238020	201837	6193	1.2
3	Netilmicin	2.385	2239352	201273	5980	1.2
4	Netilmicin	2.385	2242466	203923	7163	1.2
5	Netilmicin	2.389	2244692	202938	6182	1.2
6	Netilmicin	2.389	2247654	201982	7684	1.2
Mean			2241395			
Std. Dev.			4333.851			
% RSD			0.193355			

Table: Results of Intermediate precision Analyst 2 for Netilmicin.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Netilmicin	2.380	2236184	217363	5928	1.2
2	Netilmicin	2.383	2238020	218467	6183	1.2
3	Netilmicin	2.385	2239352	218346	5927	1.2
4	Netilmicin	2.385	2242466	221736	5163	1.2
5	Netilmicin	2.389	2244692	228361	4827	1.2
6	Netilmicin	2.346	2263431	217553	5019	1.2
Mean			2244024			
Std. Dev.			9988.458			
% RSD			0.445114			

ACCURACY

Table: Results of Accuracy for concentration-50%.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Netilmicin	2.375	1175619	331631	1.2	5838	1
2	Netilmicin	2.379	1173851	319383	1.2	5029	2
3	Netilmicin	2.380	1167986	318371	1.2	5726	3

Table: Results of Accuracy for concentration-100%.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Netilmicin	2.379	2319086	272611	1.2	5833	1
2	Netilmicin	2.380	2318812	274521	1.2	5029	2
3	Netilmicin	2.379	2306361	274826	1.2	5827	3

Table: Results of Accuracy for concentration-150%.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Netilmicin	2.383	3477226	517362	1.3	5462	1
2	Netilmicin	2.384	3477511	518371	1.2	5928	2
3	Netilmicin	2.384	3485894	529182	1.1	5391	3

Table: The accuracy results for Netilmicin.

%Concentration (at specification Level)	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	

LIMIT OF DETECTION FOR NETILMICIN

LOD= 3.3 × σ / s

where

σ = Standard deviation of the response S = Slope of the calibration curve **Result:**

=5.5µg/ml

QUANTITATION LIMIT

LOQ=10×σ/S

Where

σ = Standard deviation of the response S = Slope of the calibration curve Result:

=16.7µg/ml

ROBUSTNESS

Table: 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

CONCLUSION

Netilmicin was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), slightly soluble in methanol, Soluble in water.

Methanol: water (45:55 v/v)

SUMMARY

Maximum absorbance was found to be at 260nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area.

The column used for study was Symmetry C18 (4.6×150mm) 5µm particle size because it was giving good peak.

40 ºC temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 0.8ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol and water was taken in the ratio of 45:55 % v/v was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study.

Methanol was selected because of maximum extraction sonication time was fixed to be 6min at which all the drug particles were completely soluble and showed good recovery.

Run time was selected to be 8.0 min because analyze gave peak around 2.379min and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.

The analytical method was found linearity over the range of 24-120ppm of the Netilmicin target

concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

ACKNOWLEDGMENT

In forwarding this thesis entitled "**Quantitative Estimation of Netlimicin in Pure and Pharmaceutical Formulation by RP- HPLC**'' I would like to express my sincere thanks to my guide A.Rajamani, M.Pharm, Associate Professor, Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar, Telangana. For his remarkable guidance, constant encouragement and philosophical suggestions made us possible to submit my dissertation.My deep sincere of gratitude to our Chairman Sri.P. Yadaiah Guptha and Secretary Sri.P.Narendra Guptha for their constant encouragement throughout this work at Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar, Telangana.I extended my sincere thanks to our Professor cum Principal, Dr.A.Yasodha, Department of Pharmaceutical Analysis, Dhanvanthri College of Pharmaceutical Sciences, Mahabubnagar, Telangana, for providing facilities and permitting me to get carry out all my work successfully.

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