CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.8283605

Available online at: http://www.iajps.com *Research Article*

ESTIMATION OF NETILMICIN IN INJECTION FORMULATION BY RP-HPLC METHOD

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Abstract:

In the present investigation, a simple, sensitive, precise and accurate Reverse phase high performance liquid Chromatography method developed and validated for estimation of Netilmicin in injection formulation. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Netilmicin was freely soluble in ethanol, methanol and sparingly soluble in water. ACN: Methanol: water (10:30:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. 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. This method can be used for the routine determination of Netilmicin in injection formulation and in other pharmaceutical dosage forms.

Key words: *RP-HPLC, Netlimicin, injection.*

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Please cite this article in press H. Padmalatha et al, ***Estimation Of Netilmicin In Injection Formulation By RP-HPLC Method****, Indo Am. J. P. Sci, 2023; 10 (08).*

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. The bactericidal effect of netilmiicin is not fully understood. It is for the treatment of bacteremia, septicaemia, respiratory tract infections, skin and soft-tissue infection, burns, wounds, and peri-operative infections caused by susceptible strains. 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.[1-4] IUPAC name is 4-amino-3-{[(2S,3R)-3-amino-6-(aminomethyl)-3,4-dihydro-2H-pyran-2-yl]oxy}-6-(ethylamino)-2-hydroxycyclohexyl]oxy}-5-methyl-4 (methylamino)oxane-3,5-diol. Molecular formula $C_{21}H_{41}N_5O_7$. Molecular Weight is 475.5.

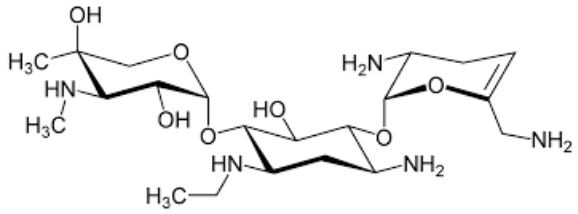

Figure 1: Structure of Netilmicin

The literature survey revealed that There are very few methods reported in the literature for analysis of Netilmicin alone or in combination with other drugs in the pure form and pharmaceuticals formulations.[5-15] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Netilmicin estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Netilmicin. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Netilmicin in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Netilmicin Gift samples obtained from Hetero Drugs Ltd, Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 260 nm with column XBridge C18 (4.6×250mm) 5µ, dimensions at Ambient temperature. The optimized mobile phase consists of ACN: Methanol: Water (10:30:60% v/v). Flow rate was maintained at 0.8 ml/min.

Preparation of solutions:

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 0.7ml 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 ACN: Methanol: Water in proportion 10:30:60 v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like Gemini,ODS column, and X Bridge C18 column. XBridge C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of mobile phase:

Accurately measured 100 ml (10%) of HPLC grade ACN, 300ml Methanol (30%) and 600 ml of Water (60%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.8 ml/min for 6 minutes to equilibrate the column at ambient temperature. The overlay spectrum of Netilmicin was obtained and the Netilmicin showed absorbance's maxima at 260 nm. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into XBridge C18 (4.6×250mm) 5µ column, the mobile phase of composition ACN: Methanol: Water (10:30:60% v/v) was allowed to flow through the column at a flow rate of 0.8 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.
Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Netilmicin in injection dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.
Validation of Analytical method:
Linearity: The linearity study was performed for the concentration of 20 µg/ml to 100 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%,150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Netilmicin and calculate the individual recovery and mean recovery values. The results are shown in table 4.
Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 5.
Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

$LOD = 3.3\sigma/S$ and
$LOQ = 10\ \sigma/S$, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

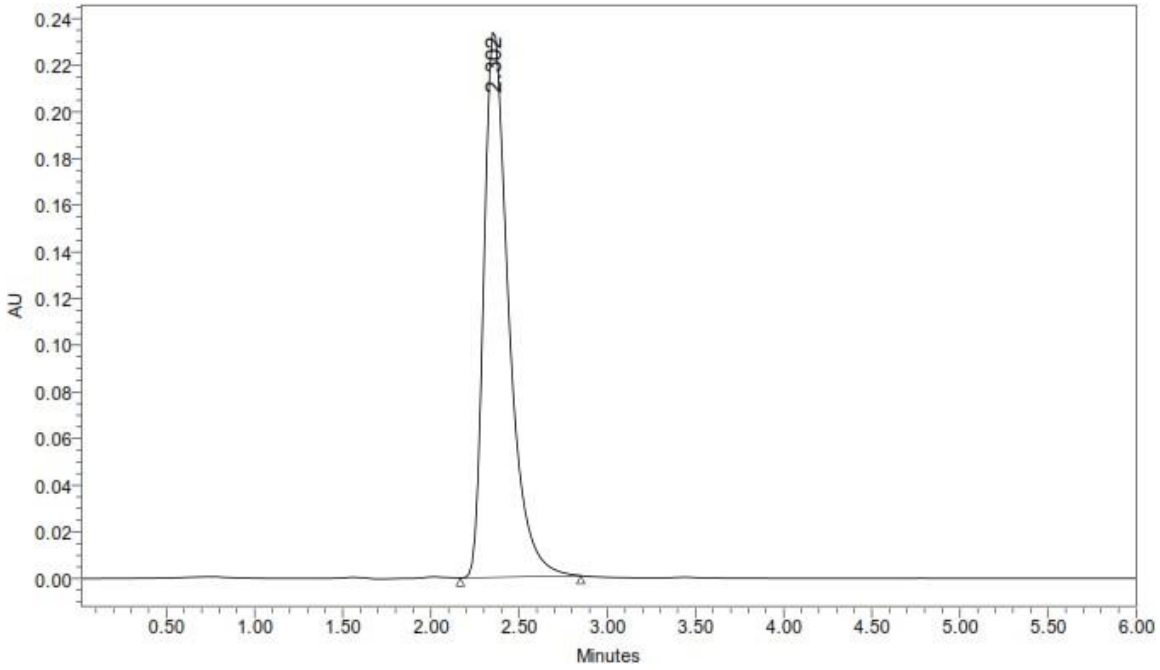

Figure 2: Standard chromatogram

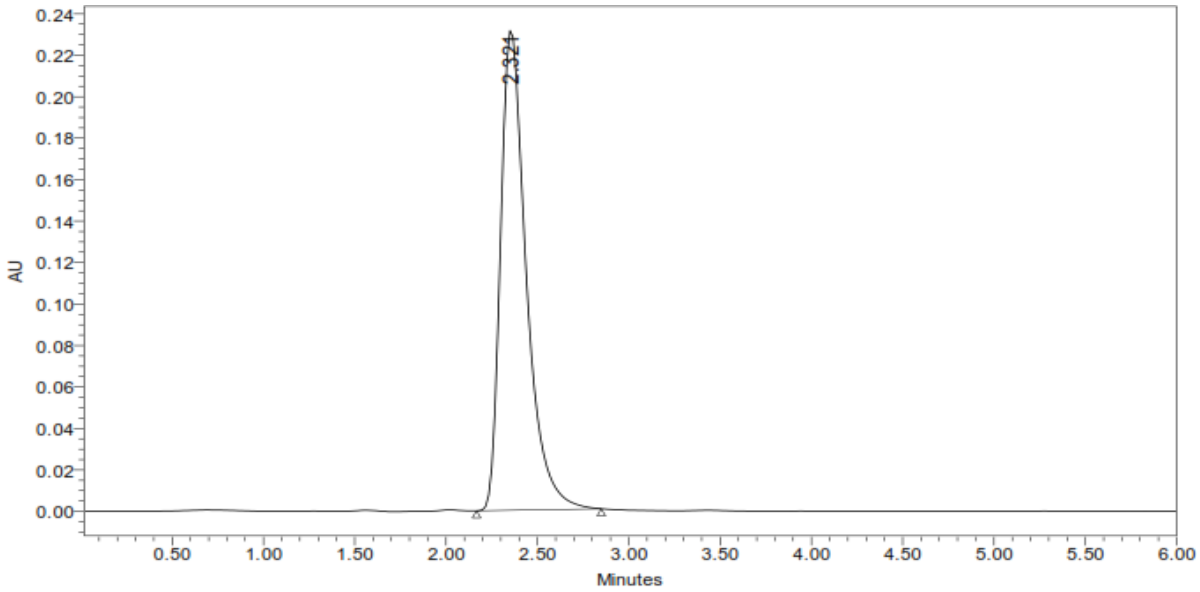

Figure 3: Sample chromatogram

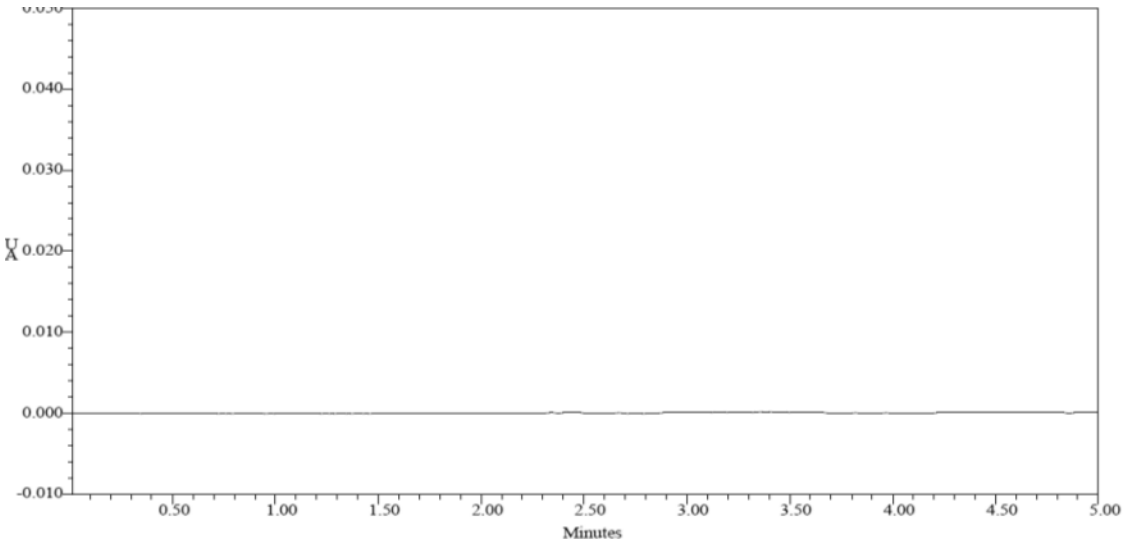

Figure 4: Blank chromatogram

Table 1: System suitability parameters

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Netilmicin	2.321	2204850	239452	5281	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	2.317	2274631	239458	5728	1.2
Mean			2252336			
Std. Dev.			31827.08			
% RSD			1.41307			

Table 2: Assay results for Netilmicin

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

Table 3: Linearity results of Netilmicin

Concentration µg/ml	Average Peak Area
20	791354
40	1657073
60	2293804
80	3158339
100	3939630

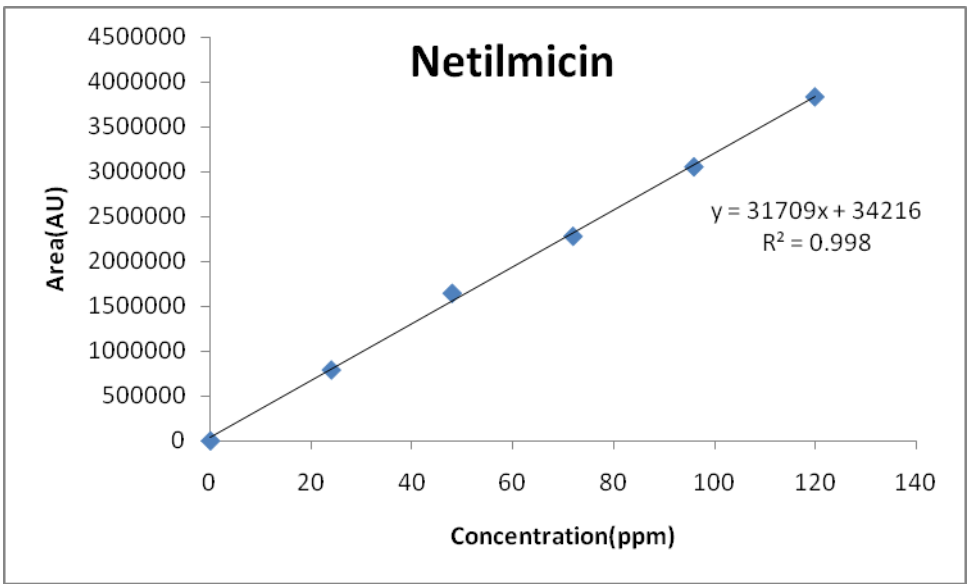

Figure 5: Linearity graph for Netilmicin

Table 4: Showing 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	

Table 5: Precision results for Netilmicin

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Netilmicin	2.356	2279464	245362	5938	1.2
2	Netilmicin	2.356	2285915	248293	5827	1.2
3	Netilmicin	2.357	2282117	240795	5032	1.2
4	Netilmicin	2.358	2288675	230139	5978	1.2
5	Netilmicin	2.359	2272448	249605	6183	1.2
Mean			2275724			
Std.dev			9476.485			
%RSD			0.416416			

Table 6. Ruggedness results of Netilmicin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USPTailing
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			
%			0.193355			

Table 7: Robustness results for Netilmicin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Less Flow rate of 0.7mL/min	2650811	2.765	5551	1.2
More Flow rate of 0.9mL/min	2740254	2.234	5421	1.2
Less organic phase	2740658	2.763	4803	1.5
More organic phase	2740325	2.236	4691	1.5

Table 8: LOD, LOQ of Netilmicin

Drug	LOD	LOQ
Netilmicin	5.5	16.7

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Netilmicin in injection formulation and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Netilmicin in injection formulation and in other pharmaceutical dosage forms.

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