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SPECTROSCOPIC ANALYSIS OF NETILIMICIN BY DERIVATIZATION USING QUALITY BY DESIGN FOR BULK DOSAGE FORMS

Anita. A[a,*], Bincy Raj[b].

Dayananda Sagar University, Bangalore -560078, Karnataka, India

*Corresponding author E-mail**: anita19965@gmail.com**

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ABSTRACT

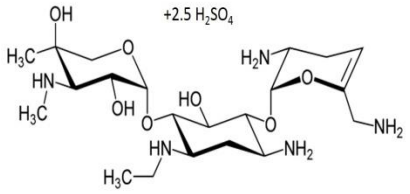
ICH guidelines specifies the pharmaceuticals intended for human use in the category of Q 8,10,11 for the efficacy quality and safety of the intended product are in sync with the Quality by Design (QbD) principles, as quality by design is a risk based approach with pure scientific logics to establish a quality target product profile[1] .Spectroscopic method approach for analysis of netilimicin was performed as per ICH Q8 (R2) guidelines. Quality by Design (QbD) was also incorporated by derivatization for which absorption spectra and absorption maxima were used, making it very simple and inexpensive methods. Spectroscopic analysis of netilimicin was performed using UV spectroscopy keeping in view the QbD principles as well as the limitation of beer's law ,the accuracy and precision of the method used were compared with the standard UV reference method ,for this three simple and sensitive methods were utilized, where pure sample of netilimicin was used in bulk dosage forms . (kumar et al, 2018)Ishikawa diagram were used to depict the systemic approach to the study. Various critical parameter were studied for the proposed method, implemanding QbD principlies through spectrophotomerty utilized various method input variables like study of intensity of absorbance, absorbance maxima,spectral shape ,which were the validated as per ICH guidelines .

INTRODUCTION

An antibiotic with a bacterilogical activity focussing especially on gram negative bacreria which inhibits protien synthesis and containining a molecule as amino modified glycoside is usually termed as an Aminoglycoside [2].They are basically classified as 1st generation aminoglycosides which include streptomycin. Neomycin, monomycin and kanamycin, 2nd generation gentamycin, tobromycin, syzomycin, 3rd generation as netilimicin and amicacin. Netilimicin mechanism of action is its irreversible binding to 30s ribosomal subunit of bacterial cell SR and that too specifically the 16RNA and S12 protein subunits ,therefore it Inhibits the initiation of protein synthesis as it interferes with the conjoining of mRNA and bacterial ribosome which otherwise would have formed a complex. Netilimicin is obtained from sisomicin a naturally occurring aminoglycoside antibiotic which is produced by the fermentation of Micromonospora inyoensis,netilimicin basically occurs in a salt form in sulfate form hence available as netilimicin asulphate it is semisynthetic and wter soluble in nature which requires to be used in its formulation netlimicin additionally acts by change in translational frame shift of

mRNA,by misreading the template leading to premature termination of protein synthesis eventually leading cell death. (National Center for Biotechnology Information. PubChem Database. Netilmicin sulfate)

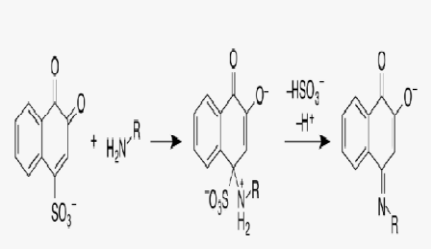
Netilmicin Sulfate



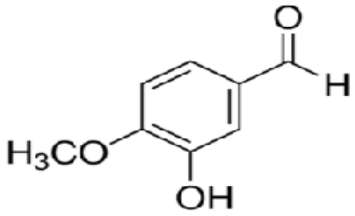
To ensure a predefine quality product (QbD) concepts are used in development of pharmaceutical products and processes, the guidelines are explained in (ICH) guidelines Q8 (R1)(Pharmaceutical development, Q9 (Quality risk management)and Q10 (Pharmaceutical quality system) . As per Q8(R1) QbD is a systemic approach with predefined objectives based on pure scienticfic approach ,it takes care of various aspects like product parameters materials and final products , (QbD) approach is which takes care of the process from beginning to the end.(QbD) approach for analytical techniques contribute to the full understanding of the technical procedure and operating conditions affecting the analytical performance ,it involves study of reagemnts used, technique used and instrumental paramerters ,it involve guidelines ICH Q2(R1) AQbD approach can be used in the development of a robust and cost-effective analytical method Thus, a QbD based UV spectrometric method can be developed by considering the ICH guidelines Q2 (R1). Revalidation techniques are also not mandated if AQbD is implemented if any change in analytical method is required. Netilmicin is an aminoglycoside basically used for treatment against Gram-negative bacteria; it is used in the treatment of urinary tract infections skin infections and skin lesions, and lower respiratory tract infections, as well as in intra-abdominal infections, septicemia and many other infections including pediatric dosage forms. Once a dosage form has proved the superiority of netilimicin over multiple dosage regimens without any compromise on safety and efficacy, the drug has comparable efficacy with the contemporary antibiotics like gentamicin, amikacin or tobramycin.Comparative studies have generally revealed similar clinical and bacteriological efficacies between netilmicin and gentamicin, amikacin or tobramycin. Netilimicin is a effective antibacterial agent useful as a parentral drug therapy in severe systemic infections clinical trials have proved excellent response in bacterial eradication in case of urinary tract infection up to 87%, 90% in cases of septicaemia and 89% for lower respiratory tract infections .it is found effective against species like P. aeruginosa and Serratia ,similarly it showed synergestictic action with tinidazole and clindamycin ,recomended route of administration is IM OR IV . The draw back with netilimicin therapy as with other aminoglycoside was obviously ototoxicity and nephrotoxicity. Method of administration of netilimicin is intramuscular and intravenous injection dosage usually varies from 1.5 to 3.5 mg/kg/day as adult dose depending on the type of infection. The goal of the present investigation was to developed a simple, rapid, robust, flexible and economical UV spectrometric method for netilimicin sulphate.Implemending the qbd approach for uv spectophotometric analysis the shape of the spectra, intensity of the absorbance and absorption maxima with the method of input variables were analysed and various critical parameters were observed[4,5]. Further studied were performed, after which the methods were validated as per ICH guidelines Q2 (R1).

2. MATERIALS AND METHODS:

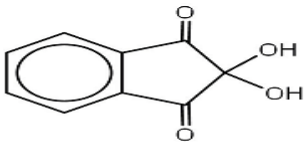
2.1- Method A :Reaction between netilimicin with Folin –ciocalteu regent.In presence of sodium bicarbonate (NA_2 CO_3) was oxidized followed by complexation, the absorbance for A was 760nm .The Folin-Ciocalteu reaction depends on presence of aromatic rings of polyphenol which has no of hydroxyl group ,the presence of these hydroxyl groups in the phenolic compounds is responsible for the oxidation reaction and formation of the product having colour directly proportional to the concentration of the metabolite present [6,7,8]



2.2 **Method B;** was based on condensation reaction where the product was colored in acidic conditions namely (3-methoxy-4-hydroxy benzaldehyde).the absorbance was found out to 555nm.



2.3 **Method C** was based on reaction of netilimicin with ninhydrin in presence of ascorbic acid to give a blue violet colored product, for which the absorbance was 560nm.The results obtained were statistically validated as well as were reproducible and were found suitable for various pharmaceutical formulation



Ninhydrin

The results of the study proved the factors affecting the derivatization reaction were very significant and thus were identified, the method developed by the spectrophotometery and HPTLC were totally comparable and sensitive too.The purpose of QbD for spectroscopy[9] , robustness and ruggedness should be verified early in the method development stage to ensure method performance over the lifetime of the product. Quality by design principles are applied to build in a more scientific and risk based multi-factorial approach to the development and validation of analytical methods using spectroscopy. (Bhusnure O.G.*, 2017)

3. Instrumentation: Spectrophotometer (UV1601 PC) was used, the made was SHIMATDZU UV-VIS DOUBLE BEAM, The spectral band width used was 2nm and wavelength was 2800.0 nm The bundled software is UV PC personal Spectroscopy version,Model used was TCC-240 .The aim of the work conducted was to establish a systematic yet simple sensitive and rapid method of analysis of netilimicin by QbD approach

4. Reagents: All the chemicals and reagents used were of analytical grade and the solutions were prepared in doubly distilled water. Aqueous solutions of FC Solution and 9.43 x 10 'M Na2CO3 were prepared for method A. (Loba Chemicals) Methanolic solution of vanillin (BDH. 2.63x10 ° M) and H_2SO_4 were prepared for Method B(Merck, 18 M) Ninhydrin (bdh), 1%, s.678xl0'w net (05 - solution in acetone, aqueous solution of ascorbic(bdh, 0.1%, 5.678x10'w) and ph 5.0 buffer were prepared for method C.

4.1 **PREPARATION OF THE STANDARD SOLUTION**: For method A and B the stock solution (mg/ml/) was prepared by dissolving 100mg of netilimicin in 100ml of distilled water. To prepare a working solution a portion of this stock solution was diluted stepwide with the same solvent and produced 20 µ/ml for method A and 100 µ/ml for method B. For method C stock solution of 1mg/ml was prepared in methanol and was diluted for working solution with same solvent to 100 µ/ml.For pharmaceutical preparation: The injection powder equivalent to 100mg unit of netilimicin was taken and diluted as per the preparation of standard solution[10].

4.2 Method A: Aliquots of standards netilimicin solutions (1.0 to 3.0) 20mg/ml were transferred into the series of 25ml calibrate d tubes and the volumes were adjusted to 3ml with distilled water ,to each 5ml of NA_2CO_3 and 1.5 ml of Folin-Ciocalteu reagent was added and kept aside for about 15min, the volume was made upto the mark and absorbance was measured at 760nm against blank solution prepared as per same conditions .Amount of drug in the sample was deduced from beer's lamberts plot.

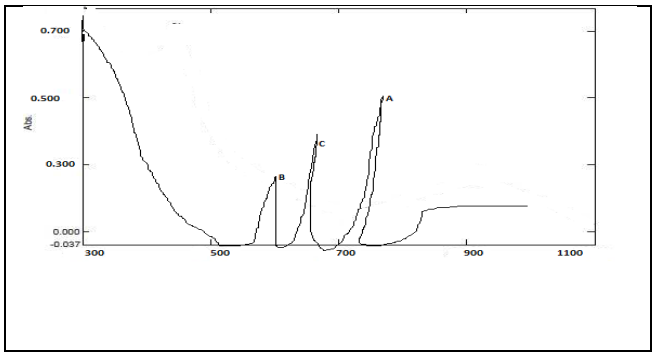


Fig. 1: UV spectrum of NETILIMICIN showing maximum absorbance ((λ _max)

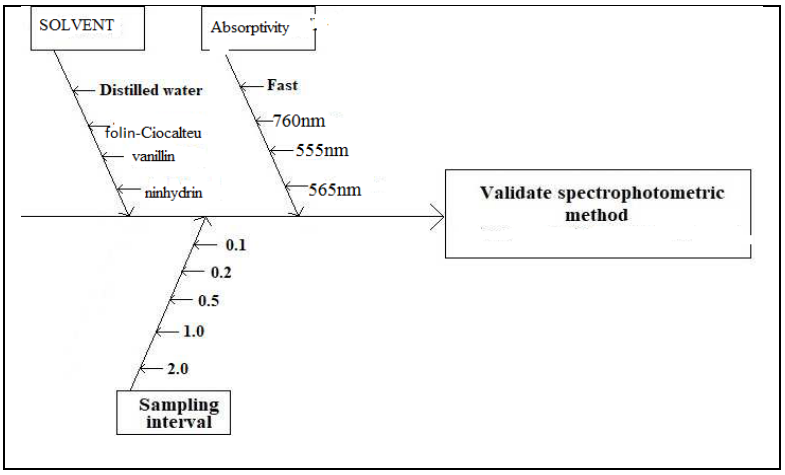


Fig 2: Ishikawa diagram showing the relationship between variable input parameters and the method performance characteristics of the spectrophotometric analytical methods

Table 1: Optical characteristics precision and accuracy of the proposed method for netilimicin

S.NO	PARAMETERS	METHOD 1	METHO 2	METHOD 3
1.	λ_{MAX}	760nm	555nm	560nm
2.	Beer's law limit (µg/ml)	2-12	5-30	5-30
3.	Molar absorbitivity ($1mole^{-1}\,cm^{-1}$)	$2.8631*10^4$	$1.013*10^4$	$1.1818*10^4$
4.	Sendell's sensitivity ($\mu g/cm^2/0.001$)AU	0.017	0.047	0.041
6.	Co-relation coefficient (r)	0.9998	0.9999	0.9999
7.	Relative standard deviation	0.4993	0.3421	0.2509
8.	Range of error	0.417	0.286	0.210
Y=a+bc,where c is the concentration in µ/ml;**from total of six determinations				

Sample	Labelled amount (mg)	Amount found by proposed Method			Ref method	% Recovered by proposed method		
		Method A	B	C		A	B	C
Inj 1	10	10.00 ⟨±) 0.054 F=1.70 T=0.92	10.04⟨±) 0.037 F=3.6 T=1.4	9.97⟨±) 0.040 F=2.8 T=0.27	9.99⟨±) 0.067	100.01⟨±) 0.532	100.40⟨±) 0.38	99.77⟨±) 0.40
Inj 2	10	9.97⟨±) 0.044 F=1.94 T=0.39	9.98⟨±) 0.02 F=1.1 T=1.86	9.96⟨±) 0.021 F=1.12 T=1.35	9.98⟨±) 0.019	99.74 ⟨±) 0.443	99..97⟨±)0.20	99.64⟨±) 0.21
Inj 3	25	24.96⟨±) 0.05 F=1.06 T=0.99	24.9/0.04⟨±) F=1.73 T=0.99	24.94+0. ⟨±)05 F=1.07 T=1.82	25.03⟨±) 0.05	99.86 0.22⟨±)	99.90 ⟨±) 0.16	99.76⟨±) 0.22
Inj 4	50	49.89⟨±) 0.306 F=2.30 T=0.68	49.89⟨±) 0.184 F=1.2 T=0.35	24.94+0.05⟨±) F=1.07 T=1.82	49.75⟨±) 0.202	99.61⟨±) 0.61	99.66 ⟨±) 0.37	100.32⟨±) 0.42

Table 2: *average (±) standard deviation of the triplets :t- and f- values refers to the comparision of the proposed method with the reference method .Theoritical values at 95% confidence limit ,t+2.57,f+5.05:**After adding 3 different amounts of the pure labeled drug of the various pharmaceutical formulation ,each value is an average of 3 determines**.**

4.3 Method B :To each of the calibrated tube aliquots (0.5-2.5) 100mg/ml of standard netilimicin solution 1ml of vanillin and 3ml of con H_2SO_4 were added successively and volume in each flask was brought about 9ml by addition of methanol then placed it on water bath for about 15 minutes, flask were cooled and made upto the mark with the solvent and absorbance was measured at 555nm against blank solution prepared as per same conditions, amount of netilimicin present in the sample was deduced with beer's lamberts' plot.

4.4Method C: Aliquots of the standard netilimicin (05-2.5ml)100mg/ml solution in were transfered into series of calibrated tubes containing 4.0 ml buffer (pH 5.0) 1mL of ninhydrin solution and 6 mL of ascorbic acid solution.The volume in each tube was adjusted upto 9ml with distilled water and was kept in water bath ,after 10 min the tubes were removed and chilled in ice water for some time ,then the volumes in the test tubes were made up to 10ml with distilled water and absorbance was measured at 560 nm against blank solution prepared as per same conditions, amount of netilimicin present in the sample was deduced with beer's lamberts' plot**[11,12,13].**

5. Implementation of AQbD approach in the development of the analytical method

For AQbD approach, Ishikawa diagram was used to study the relationship between variable input parameters and the method performance characteristics of the spectrophotometric analytical methods [14] (fig. 3).

6. Analysis: Weight of 10 tablet of netilimicin tablet was taken by crushing it in mortar and pestle, and 10mg of drug was taken in 100ml volumetric flas and methanol was added to adjust he volume upto 100ml, working solution for various strength were prepared usin g mobile phase concentration and was analyzed by UVspectroscopy and % purity of the drug was analyzed.

7. Validation: To achieve analytical target profile the selected critical parameters should comply with the method performance characteristics of analytical method as per the guidelines laid by ICH Q2(R1) specifically for analytical methods, Appropriately for the spectroscopic method to be validated the parameters were used to implement AQbD were according of the ICH guideline

development of various other critical parameters were also validated by same guidelines .limit of quantification ,limit of detection ,precision, linearity ,accuracy system suitability were specific characteristic studied.[15,16]

8. System suitability: The suitability of the UV spectrophotometer used in the study is done by system suitability studies, three replicates of various strength of netilimicin sulphate were prepared from the stock solution from suitable compatible solution aand the absorption was determined using the UV spectrophotometer,absorbance measurement also helped in calculation of percentage relative standard deviation(%RSD).[17]

9. Linearity: Test results which are directly propotional to the concentration of the analytes in the sample in an analytical procedure are expressed by linearity as per ICH guidelines. For linearity studies minimum of three solution of each method A,B,C were prepared in methanol from stock solution of netilimicin and absorbance was noted .The calibration curve was plotted for absorbance versus concentration then by regression analysis method the correlation coefficient , %RSD was calculated. (Ahmeda*, 2018)

10. Precision: The closeness of the result obtained by various measurements of the same sample **,**In an analytical procedure is called as precision ,it is usually performed as repeatability (intra-day) and intermediate precision(inter-day) as per ICH guidelines here the triplicates of the sample were analysed on the same day %RSD were calculate ,and inter day parameters the triplicate were analysed on three consecutive days and %RSD was analysed.[18]

11. Accuracy: The result Which is closest to the true conventional values in analytical procedure is called as accurate and shows the accuracy, in this study accuracy was studied by the recovery of the netilimicin ,to the known amount of the standard solution and to the known amount of stock solution were added to get the final concentration and these solution were then again subjected to analyze the drug content ,they were performed in triplicates and %RSD and recovery of sample were calculated.

12. Limit of detection (LOD) and limit of quantification (LOQ): The lowest concentration of an analyte present in the sample is determined by limit of quantification(LOQ) and the lowest amount of analyte that can be detected but not necessarily quantified is called as limit of detection(LOD),As per ICH GUIDELINES the parameters for netilimicin for LOD and LOQ were determined by method based on the calibration curve prepared in the linearity studies ,standard deviation of the calibration curve was also recorded. [19]

$$LOD = \frac{3.3\sigma}{S} \quad LOQ = \frac{10\sigma}{S}$$

σ = standard deviation

S = slope of calibration curve

13. Specificity: Presence of impurities, additives, excipients which are present in the sample and tend to modify the results specificity test is also performed as per ICH guidelines to determine them and to show a standard solution(10 μg/ml) of the exciepients diluents and additives were performed and compared .

14. RESULTS AND DISCUSSION: The color development of various methods were established by maintaining optimum conditions in varying parameters keeping other method fixed while checking others one at a time, optical characteristic like beer's law limit molar absorptivity for each method were depicted in Table 1 .The precision of each method was found by measuring the absorbance of six replicated samples containing known amount of drug sample and the obtained are incorporated in the Table 2. Regression analysis using the method of least squares was made to evaluate the slopwe (b) intercepts (a) and the correlation coefficient (R) for each methods (*TABLE 1) .The accuracy of each method was ascertained by comparing the results by proposed and reference method (UV) stastically by the t- AND F- TEST (Table 2) .This comparision shows that there is not much of a significance difference between the

proposed and reference method .The similarity of the results is obvious evidence that during the applications of these method ,the addtives and the exciepients are usually present in the tables do not interfere in the analysis of the proposed method. As an check of accuracy of proposed method and recovery experiment were performed by adding a fixed amount of drug to the preanalyzed formulations, the amount of drug and its %content were analysed by the same procedures. [20, 21] Optimal conditions for the experiments: For method A various parameters on the Folin-Ciocalteu like its strength ,nature, alkaline content ,time for color development ,order of addition of reagents, stability of colored species and reduction of folin- Ciocalteu reagent by netilimicin were studied. the use of 1-2ml of reagent and 4-5 ml of Na_2CO_3 were found necessary to produce absorbance value .15 min of waiting time was also require doe complete color development which wa sstable fro 5min only. For method B Netilimicin was allowed to condense with the vanillin in presence of con H_2SO_4 The effect of reagent concentration ,temperature ,heating time ,and the order of addition of the reagents ,development of maximum color ,sensitivity were studied by means of control experiments .the use of 1.5-3 ml vanillin and 2-4 ml con H_2SO_4 were found to be necessary to produce constant absorbance values .heating time of 10-20 min at temperature 40-50^{0}C temperature was found to be necessary for complete color development .Change in the order of addition resulted in low absorbance values. The colored complex formed was stable for about one hour. In order to establish the optimum conditions in method c ,which involves the reaction between netilimicin and Ninhydrin reagent to produce bluish violet colored product ,1ml of ninhydrin was found to be necessary for color product formation and to cover broad range of Beer's law limit .No added advantage was observed even when excess ninhydrin was used. Among the two reducing agents tried (ascorbic acid and $SnCl_2$) ascorbic acid was found to be more efficient with sensitivity and reproducibility .0.5ml of ascorbic acid was found to be adequate for the development of the color development. A heating time of 10min and at 40 to 50 ^{0}C temperature was found to be necessary for the complete color development .The absorbance of the colored product decreased slowly with after 1 hr.

CONCLUSION:

The proposed methods are applicable for the analysis of the drug netilimicin and have advantage of wider range under Beer's law limit .The decreasing order of sensitivity and max among the proposed method are A>B>C respectively.All the methods are applicable in to determine netilimicin in bulk form and in formulations,using quality by design" (AQbD) approach The proposed spectroscopic methodmethods are simple ,selective and can be used in routine determination of the netilimicin in bulk samples and in formulation with reasonable precision and accuracy as it does not involve any complexity and has an economic advantage too moreover the selected method was also validated as per ICH guidelines also.

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Conflicts of Interests: Declared none

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