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Research Article

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Method development and validation of nicardipine hydrochloride in bulk and formulation using UV spectrophotometric method

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ABSTRACT

The present study describes a simple, accurate, precise, specific and highly sensitive method for the determination of Nicardipine Hydrochloride present in pharmaceutical dosage forms. The method is validated for the determination of Nicardipine Hydrochloride in bulk and tablet dosage form. The solvent used was acetonitrile: water (50:50) and the λmax or the absorption maxima of the drug was found to be 235nm. A linear response was observed in the range of 5-25µg/ml with a regression coefficient of 0.999. The linear regression equation obtained by least square regression method were y=0.249X+0.008, where y is the absorbance and x is the concentration of the pure drug solution. The. The method was validated for several parameters like accuracy, precision as per ICH guidelines. The values of relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of Nicardipine Hydrochloride in bulk and pharmaceutical formulation.

Keywords: Nicardipine hydrochloride, λmax, ICH, UV-VIS spectroscopy

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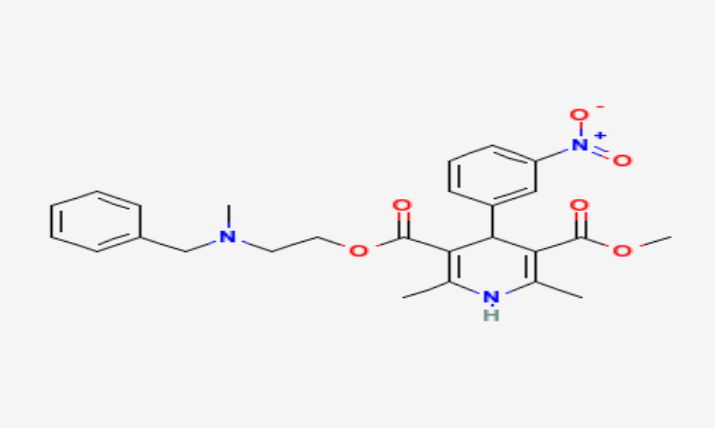
INTRODUCTION

Nicardipine hydrochloride, 2-(N-benzyl-N-methylamino)ethyl methyl 1,4-dihydro- 2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylate monohydrochloride, is a calcium antagonist with highly potent vasodilating activity and has been widely used for the treatment of hypertension and cerebrovascular disease[1]. Although nicardipine is rapidly and completely absorbed from the gastro-intestinal tract after oral administration to humans and laboratory animals, its plasma concentrations are relatively low due to extensive first-pass metabolism in the liver [1,2].

Several analytical methods for nicardipine were reported including spectrophotometry[3- 6], voltammetry[7-9], high performance liquid chromatography[10-14], liquid chromatography-mass spectrometry[15-18], and capillary electrophoresis[19]. A study on forced degradation, degradation kinetics and photo stability of nicardipine were also reported in literature[20-22]. Most of these methods were used for quantitative determination of nicardipine hydrochloride in biological fluids.Hence the objective of proposed study was to develop simple, accurate, precise and rapid this UV spectrophotometric method for the estimation of nicardipine hydrochloride in acetonitrile with water system. Hence, it can be employed for routine analysis in Quality Control Laboratories

Fig.1-Nicardipine structure:



EXPERIMENTAL SECTION

Instrument

UV-Visible SpectrophotometerT60 (model), Analytical technologies Limited, connected to the digital system loaded with UV-Win software ver.5.1.1 have an wavelength accuracy of ±5.0nm with quartz cells of 1cm path length.

Reagents and materials

Working standard of pharmaceutical grade Nicardipine Hydrochloride was procured locally and other chemicals used were of AR grade and purchased from SD fine chemicals, Mumbai.

Preparation of standard stock solution

10 mg of pure Nicardipine Hydrochloride was dissolved separately in acetonitrile solvent and final volume was made up with water to produce a concentration of 100 µg ml^{-1} which is the standard stock solution.

Selection of wavelength

In order to ascertain the wavelength of maximum absorption (λmax) of the drug, different solutions of the drugs (5 µg/ml and 25 µg/ml) in 50% acetonitrile were scanned using spectrophotometer within the wavelength region of 200 – 400 nm against 50% acetonitrile as blank. The resulting spectra shown in Figure-2 and the absorption curve showed characteristic absorption maxima at 235nm for Nicardipine Hydrochloride . overlay absorption spectrum showed in Figure-3

Figure2:: UV Spectrum of Nicardipine Hydrochloride in acetonitrile

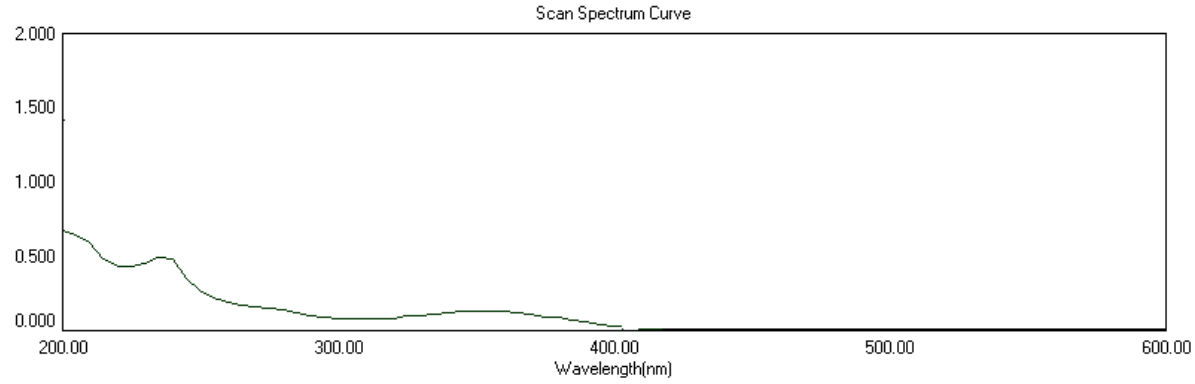
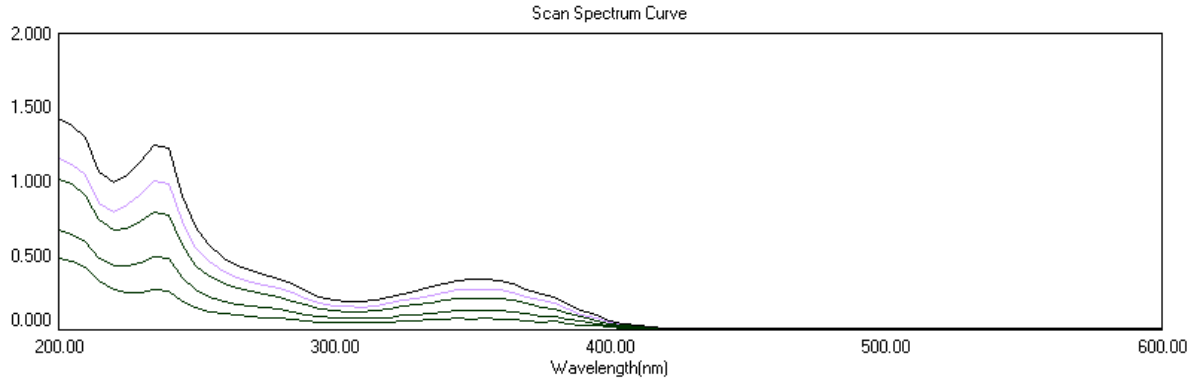


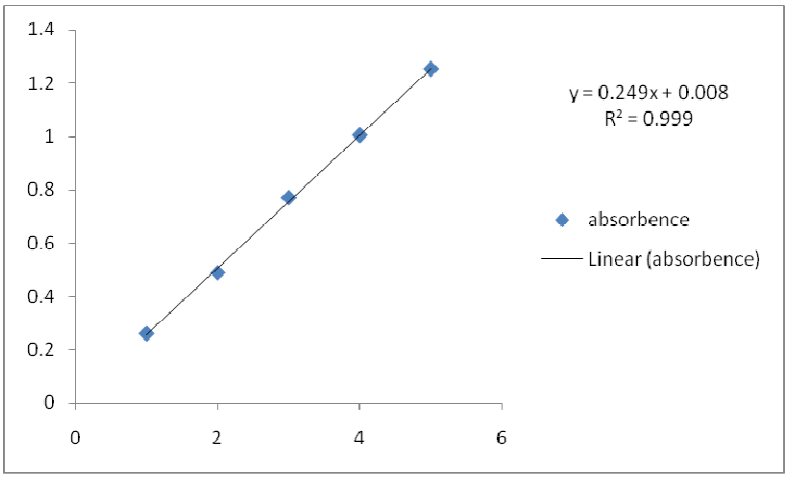
Figure.3:: Overlay Absorption Spectrum of Nicardipine Hydrochloride



Calibration standards

From the standard stock solution of nicardipine hydrochloride , different concentrations were prepared respectively in the range of 5-25µg/ml and measured absorbance at 235nm.The calibration curves were plotted **(Figure-4**) and data presented in Table 1.

Figure 4: Calibration curve of Nicardipine Hydrochloride



VALIDATION

Validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics[10]. The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection(LOD), Limit of quantification(LOQ) according to ICH guidelines**.**

TABLE 1:Linearity table of Nicardipine Hydrochloride

Concentration (µg /ml)	Absorbence
5	**0.263**
10	**0.49**
15	**0.771**
20	**1.004**
25	**1.252**

Linearity

The linearity of the analytical method was its ablity to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of

the standard solution of the drug were prepared from stock solution and analysed. The drug showed linearity in the range of 5-25µg/ml with correlation coefficient 0.999. Linearity data are shown in **Table1.**

Precision

Precision studies were carried out to ascertain the reproduciblity of the proposed method. Repeatablity was determined by preparing six replicates of same concentration of the sample and the absorbance was measured. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision.

The results was reported as %RSD. The precision result showed a good reproducibility **(Table 2)** with percent relative standard deviation less than 2. The results of intraday and interday precision studies are shown in (**Table 3 and Table 4).**

TABLE 2: Precision results showing repeatability

Concentration (µg /ml)	Absorbence	Statistical Analysis
15	0.771	
15	0.779	Mean=0.7731
15	0.768	SD=0.00376
15	0.774	%RSD=0.486
15	0.775	
15	0.772	

TABLE 3: Intraday precision

Concentration (µg/ml)	Absorbence.1 (Morning)	Absorbence.2 (Afternoon)	Absorbence.3 (Evening)	Avg%RSD
15	0.778	0.781	0.774	
15	0.776	0.776	0.769	
15	0.772	0.77	0.771	
15	0.779	0.788	0.774	
15	0.775	0.777	0.775	
15	0.778	0.779	0.781	
%RSD	0.332	0.765	0.529	0.542

TABLE 4: Interday precision

Concentration (µg/ml)	%RSD			Average %RSD
	Day1	Day2	Day3	
15	0.775	0.774	0.778	0.7756

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (80%,100%,120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % Recovery was calculated. The results are shown in (**Table 5).**

TABLE 5: Accuracy readings of Nicardipine Hydrochloride

Labelled claim (mg)	Level of Addition(%)	Amount of pure drug added(mg)	%Recovery	Statistical Analysis		
				MEAN	SD	%RSD
30	80	24	100.05			
30	80	24	98.89	99.085	0.275772	0.278318
30	80	24	99.28			
30	100	30	101.5			
30	100	30	100.8	100.6	1.014869	1.008836
30	100	30	99.5			
30	120	36	99.54			
30	120	36	100.94	100.1267	0.727003	0.726083
30	120	36	99.9			

Ruggedness

Ruggedness was determined by carrying out analysis by two different analyst and the respective absorbance was noted and the results was indicated as % RSD **(Table6)**

TABLE 6: Results showing Ruggedness

Analyst.1		
Concentration(µg/ml)	**Absorbance**	**Statistical analysis**
15	**0.785**	
15	**0.776**	**MEAN=0.778**
15	**0.782**	**SD=0.0041**
15	**0.779**	**%RSD=0.530**
15	**0.775**	
15	**0.775**	
Analyst.2		
15	**0.778**	
15	**0.776**	**MEAN=0.778**
15	**0.773**	**SD=0.0039**
15	**0.779**	**%RSD=0.510**
15	**0.785**	
15	**0.778**	

Robustness

Analysis was carried out at two different temperatures, room temperature and at 180c to determine the robustness ofthe method and the respective absorbance was measured. The results was indicated as %RSD **(Table7)**

TABLE7: Results showing robustness

Room temperature		
Concentration(µg/ml)	**Absorbance**	**Statistical analysis**
15	**0.771**	
15	**0.776**	**MEAN=0.773**
15	**0.768**	**SD=0.0049**
15	**0.769**	**%RSD=0.636**
15	**0.775**	
15	**0.781**	
Temperature 18 degree cenrigrade		
15	**0.788**	
15	**0.778**	**MEAN=0.780**
15	**0.785**	**SD=0.0053**
15	**0.773**	**%RSD=0.691**
15	**0.781**	
15	**0.778**	

TABLE 6: Summary of the method developed

PARAMETER	RESULT
Absorption maximum	**235nm**
Beers law range	**5-25 µg/ml**
Correlation coefficient	**0.999**
Regression equation	**0.249X+0.008**
Slope	**0.249**
Intercept	**0.008**
Accuracy	**98.8-101.5%**
Precision(%RSD)	**Intraday(0.542), interday(0.775)**
LOD	**0.1032 µg/ml**
LOQ	**0.3130 µg/ml**

LOQ and LOD

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ)is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOQ nd LOD was determined using the following equation LOQ-10s/m , LOD-3.3s/m where s is the standard deviation of the response and m is the slope of the related calibration curve.

The values of LOQ and LOD were found to be be 0.1032and 0.3130 µg/ml respectively.

The results of various parameters of the developed method are shown in **TABLE 6.**

Quantification in dosage form

To analyse the concentration of drug in the pharmaceutical formulation, Twenty tablets were accurately weighed and powdered. Tablet powder equivalent to 100mg was accurately weighed and transferred to a 100ml volumetric flask, dissolved in acetonitrile, sonicated, and finally made up the volume with 50% acetonitrile. The solution was centrifuged for the excipients to settle down and the resulting solution was filtered using whatsmann filter paper no1.The solution was suitably diluted so as to obtain a concentration in the linearity range and the absorbance was measured at 235nm against 50% acetonitrile as blank. The result of analysis are shown in (**Table 7)**.

TABLE 7: Quantification in dosage form

Formulation	Label claim(mg)	Estimed amount of drug(mg)	%Purity
Nicardipine Hydrochloride(cardene)	45mg	44.86	99.68

RESULTS AND DISCUSSION

The proposed method provides a simple , accurate, economical and convinient method for the analysis of Asenapine maleate using UV spectrophotometry. The wavelength corresponding to maximum absorbance in methanol was found at 235nm. Beers law was obeyed in the concentration range of 5-25µg/ml with correlation coefficient 0.999. Acurracy of the proposed method was determined by the recovery studies, and good %Recovery (98.8- 101.5%) of the drug obtained indicate that the method is accurate. The method was found to be precise as %RSD values for interday and intraday was found to be less than 2. The method was also found to be rugged and robust as the % RSD values were found to be less than 2. The limit of detection and limit of quantification of the proposed method was found to be 0.1032and 0.3130 µg/ml indicating that the method developed is sensitive. The results of assay obtained was found to be in good agreement with the labeled claim, indicating the absence of interference of the excipients**.**

CONCLUSION

The developed method can be concluded to be simple, accurate, reliable and economical.The proposed method is specific without and interference of excepients and hence can be used for the routine analysis of Nicardipine Hydrochloride in bulk and in pharmaceutical formulation.

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