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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPIC METHOD FOR ESTIMATION OF NICORANDIL IN BULK AND ITS PHARMACEUTICAL FORMULATAION

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ABSTRACT

Objective: The aim of the present work was to develop and validate a simple UV spectroscopic method for the determination of nicorandil, which is a nicotinamide nitric oxide ester derivative with vasodilatory and cardioprotective effect and it also act as a nitric oxide donor, increasing intercellular CAMP protection leading to venodilation and pain suppression. **Method:** The UV spectrophotometric analysis was performed using Systronics UV-spectrophotometer 2704 X visible double beam by using solvent system ammonium acetate buffer and acetonitrile in the ratio 8:2. Detection was performed at a wavelength of 260nm. Method validation was carried out according to ICH Q2R1 guidelines by taking the parameters linearity, accuracy, precision, ruggedness and robustness, LOD and LOQ. **Results:** The UV spectrophotometric was found linear in the range 10-50µg/ml. The method was rugged and robust with % relative standard deviation less than 2. The extraction recoveries was found to be higher than 99%in all experimental conditions. **Conclusion:** Based upon the performance characteristics, the proposed method was found accurate, precise and rapid and suitable for the determination of nicorandil for routine analysis.

KEYWORDS: Nicorandil, UV- Spectrophotometry, Method development, Validation.

INTRODUCTION

Nicorandil[1,2] 2- [3-pyridine carboxamide] ethyl nitrate is a nicotinamide nitric oxide ester with vasodilatory and cardioprotective effects. It is best known as a potassium channel activator that targets vascular nucleosides, diphosphate-dependent K^+ channel and cardiac ATP-sensitive K^+ channel (EC 50-10µM).[3] In addition to driving dilation of peripheral and coronary arterioles, K^+ channels activation by nicorandil delays the induction of mitochondrial permeability transition in response to oxidative stress.[4] Nicorandil can also act as a nitric oxide donor, increasing intracellular CAMP production leading to venodilation and pain suppression.[5] It is a white to off white amorphous powder, sparingly soluble in aqueous solutions but soluble in organic solvent DMSO. As per investigation of literature, the UV spectrophotometric, HPLC analytical method were developed on determination of nicorandil in Human plasma, biological fluids and pharmaceutical tablet dosage form or bulk drug samples. The rational of this work to develop a simple, accurate, rapid, precise, reproducible and cost-effective spectrophotometric method for the direct quantitative determination of nicorandil. In this method, we developed a method for determination of nicorandil in bulk drug sample and tablet dosage form and validation as per International Conference on Harmonization [ICH] Guidelines.[6]

Chemical Structure of Nicorandil

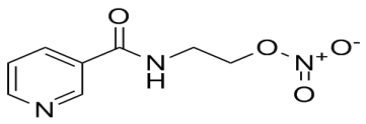


Fig. 1

EXPERIMENTAL

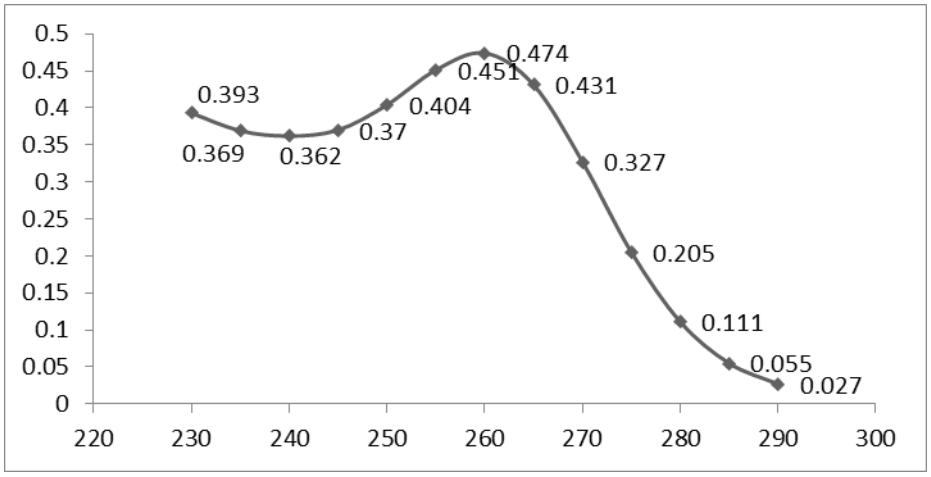
MATERIALS AND METHODS

INSTRUMENTS

Spectral runs were made on a Systronics UV-spectrophotometer 2704 X Visible double beam was employed with spectral band width of 1nm and wave length accuracy of ± 0.3 nm with automatic wave length corrections with a pair of 10mm quartz cell. Wenser Analytical single pan balance was used. Glasswares used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

LOCATION OF λ max

The working standard solution was scanned in UV range (200-400) in 0.1 cm quartz cell against solvent blank. The UV spectra of the drug show the spectrum wavelength selected for the estimation of drug was 260 nm as λ max. At 260 nm Nicorandil show maximum absorbance (Fig.2).



(Fig.2) UV Spectrum of Nicorandil.

MATERIAL

Nicorandil was kindly gifted from Sahana Pharmaceuticals, Nagercoil, TamilNadu, India. The commercially available tablets Sarandil, Korandil, AV-COR, K-COR, SS Zynicor were obtained from the market. Acetonitrile (HPLC grade), ammonium acetate (analytical reagent) and glacial acetic acid (analytical reagent) were used as solvent obtained from Shiv Scientific Industries and distilled water was used obtained from water purification unit.

PREPARATION OF STANDARD SOLUTION

A standard stock solution was prepared by accurately weighed 25 mg of nicorandil in 25 ml of volumetric flask and dissolved in diluent to obtain a concentration of 1mg/ml or 1000µg/ml (standard stock solution-I). Further diluting 5 ml of stock solution to 50 ml with diluent to get desired concentration of 100 µg/ml (standard stock solution – II).[6-8]

SELECTION OF WAVELENGTH FOR ANALYSIS OF NICORANDIL

Accurately measured 1 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted 10 ml of give concentration of 10µg/ml and it was used for initial spectral scan in the UV range of 290-230 nm to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution by allegation method.

PREPARATION OF SERIES DILUTIONS

The serial dilutions were prepared from the standard stock solution to get a respective concentration of 10, 20, 30, 40 and 50 µg/ml.

METHOD VALIDATION

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, sensitivity and specificity according to ICH Q2 (R_1) guideline and USP guidelines.[9-10]

LINEARITY AND RANGE

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure to the interval between the upper end lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n=3) at a concentration range of 10-50 µg/ml. The absorbance obtained at respective concentration was recorded and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software.

ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is termed as trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation). The final concentration of nicorandil was determined at each level of the amount, three determinations were performed. The percentage recovery was calculated as mean ± standard deviation.

PRECISION
The precision of an analytical method is the degree of reproducibility among individual test results when the procedure was applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as standard deviation.

LIMIT OF DETECTION (LOD)
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

The limit of detection (LOD) was determined by preparing solution of different concentration from 10-20 µg/ml

$$LOD = 3.3 \times SD/S$$

Where SD = Standard deviation
S = Slope

LIMIT OF QUANTIFICATION (LOQ)
The detection limit is the lowest amount of analyte in a sample which can be detected but not quantitates. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve

$$LOD = 10\ SD/S$$

Where SD = standard deviation
S = slope

SENSITIVTY
The sensitivity of the method was determined by calculating the different parameter like molar absorptivity and Sandell's sensitivity.

ROBUSTNESS
The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method the solutions of 10 µg/ml of standard nicorandil solution was prepared and analysed by a change in wavelength. The wavelength was selected λmax ± 1 (i.e.) 259-261nm respectively for the standard nicorandil solution.

RUGGEDNESS
The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days.

To establish ruggedness of the proposed method, the solution of 10 µg/ml of standard nicorandil solution was prepared and analysed with the change in the different analyst.

RESULTS AND DISCUSSION
The proposed method for determination of Nicorandil showed molar 2.3×10^3 $mole^{-1}$ cm^{-1}. From the calibration curve it was found that it shows linearity in the range 10-50 µg/ml with regression coefficient 0.0265. Linear regression of absorbance on concentration gave the equation y = 0.0263x + 0.0034 with a correlation coefficient (r) of 1.1402. The detection wavelength showing λmax (maximum wavelength) at 260nm.

ACCURACY
The percentage recovery and % RSD were calculated the mean percentage recovery and % RSD where found to be within limits and its less than 2, which explains the present research paper is accurate in method development of Nicorandil. The mean, standard deviation and percentage relative standard deviation (%RSD) where calculated. The results were shown in table

PRECISION
Repeatability of the method was studied by precision experiment. The %RSD of Nicorandil was found to be 0.2280.

APPLICATION OF THE PROPOSED METHOD
The proposed method was successfully developed and validated for the determination of Nicorandil in pharmaceutical formulations. The proposed method was compared with the reference method.[11]

REFERENCE METHOD
The average weight of tablet was calculated. The tablet powder equivalent to 50mg of nicorandil was accurately weighted and dissolved in distilled water to make 100 ml and filtered through Whatmann filter paper No.41. It was diluted to 500mcg/ml an aliquots of 0.2 to 1.6 ml portion of sample solution were transferred to a series of 10 ml volumetric flasks. To each flask, 0.2 ml of ammonia solution and 2.5 ml of cyanogens bromide solution were added and mixed. Then 1.0ml of sulphanilic acid reagent was added to each flask and kept aside for 10 min for development of colour and volume in each flask was adjusted 10 ml with distilled water. The absorbance of the solution in each flask was measured at 460 nm against the reagent blank and calibration curve was plotted. The amount of nicorandil is obtained 99.1 ± 0.74.

STUDY OF FORMULATIONS
Accurately measured standard stock solution was diluted upto 10ml with diluent to get the concentration range 10 to 50 µg/ml. The absorbance of each of the solution was measured at 260 nm against blank (diluent). a calibration curve was found to be linear.

QUANTIFICATION OF FORMULATIONS
10 tablets of Nicorandil were taken for the analysis. The average weight of tablet was calculated and the tablet was powdered in a glass mortar. Tablet powder

equivalent to 25 mg was accurately weighed was dissolved in diluent. It was filtered and the residue was washed with diluent and then the volume was made up to 25 ml with diluent (solution-I) 5ml of solution-I was pipette into 50ml volumetric flask and the volume was made up to 50ml with diluent(solution-II). From this 2ml was pipetted into 10ml volumetric flask followed by the addition of ammonium acetate buffer pH 9 to produce final drug concentration of 20µg/ml. The absorbance of solution was measured at 260nm against blank. The same procedure was repeated five times. In similar manner standard absorbance was measured with pure drug in same final concentration that of assay method. The readings were recorded in the table no:1

VALIDATION OF PROPOSED UV-SPECTROPHOTOMETRIC METHOD ACCURACY

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

STANDARD SOLUTION

Standard solution was prepared as per described in standard dilution.

SAMPLE SOLUTION

In order to justify the reliability and suitability of the proposed method of the recovery studies were carried out.

The recovery experiment was performed on the tablet of Nicorandil. The powder equivalent to 25mg was weighed accurately and dissolved in diluent. It was filtered and the residue was washed with diluent an aliquot of 5ml of standard solution (1mg/ml) of pure sample of Nicorandil was added to the flask. It was shaken well and the volume was made up to 25 ml with diluent and the procedure for the assay of the tablet was followed. The experiment was repeated 5 times. The results were shown in table no:2. The percentage of recovery was calculated by using the formula,

$$\% \text{ Recovery} = \frac{A}{B + C} \times 100$$

Where,

A ⟶ Total drug estimated (mg)
B ⟶ wt(mg) of drug contributed by tablet powder
C ⟶ Amount of pure drug added (mg)

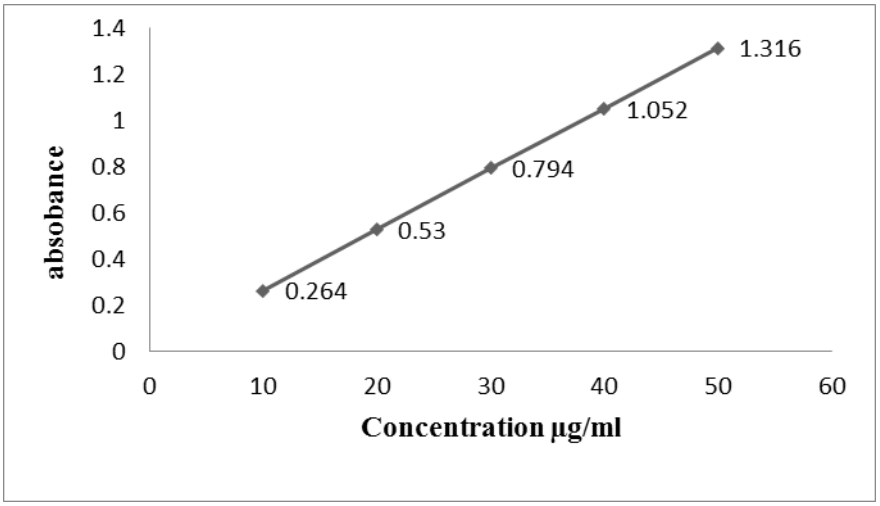


Fig 3 – Linearity of proposed method.

Table 1: Result of Analysis of Tablet Nicorandil.

S. No.	Sample Wt.(mg)	Std (mg)	Absorbance at 260 nm (std)	Sample Absorbance At 260nm	Drug content (mg)	Avg. Cont. (mg)
	49.0	25.0	0.530	0.532	0.098	
	50.0	25.0	0.530	0.338	0.099	
	51.0	25.0	0.530	0.543	0.099	9.9
	52.0	25.0	0.530	0.551	0.100	
	53.0	25.0	0.530	0.559	0.101	

n 9.9 ±SD 1.1402 %RSD 0.2280

Table 2: Result of Recovery of Proposed Method.

S. No.	Sample Wt.(mg)	Amount Std. added (mg)	Absorbance at 260 nm (std)	Abs of recovered sample	% of Recovery
	49.0	5	530	0.540	
	50.0	5	530	0.546	
	51.0	5	530	0.552	100%
	52.0	5	530	0.559	
	53.0	5	530	0.565	

±SD 1.1402 %RSD 0.2280

Table 3: Assay of Reference Method.

Brand name	Average weight (mg)	Wt. of tablet powder (mg)	Std abs	Wt. of tablet powder	Test abs	Content of drug in tablet
SARANDIL (saahana pharmaceuticles)	**19.82**	**25**	**528**	**50**	**0.338**	**9.9**

Table 4: Optical Characteristic, Data, Precision and Accuracy of the Proposed Method for Nicorandil.

Parameter	Meter
λ max	260
Beer's law limits (µg/ml)	10-50
Molar absorptivity (Lit mole^{-1} cm^{-1})	2.37×10^{3} mole^{-1}cm^{-1}
Sandall's sensitivity (µgkm^{2}/0.001 abs unit)	0.0380
Regression equation (y=a+bc)	0.0265
Slope (b)	0.0263
Correlation coefficient	1.1402
% Relative standard deviation*	0.2280%

*Average of five determinations.

CONCLUSION

The proposed UV-Visible Spectroscopy is a simple, low-cost method can be easily be applied to Nicorandil control sample analysis in bulk and pharmaceutical formulations. It has a more comprehensive dynamic range for the study with excellent accuracy and precision value. The proposed method does not require any laborious clean up procedure before analysis and simple methodology for its determination. Therefore, it can easily accommodate in the laboratories of research, and pharmaceutical industries for the quantification of Nicorandil in pure and pharmaceutical dosage forms.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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