

**SIMULTANEOUS DETERMINATION OF NICORANDIL IN  
PHARMACEUTICAL DOSAGE FORM BY HPLC METHOD**

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**ABSTRACT**

A rapid and accurate HPLC method was developed for determination of Nicorandil in tablet dosage form. The chromatographic separation was conducted on Agilent 1200 with DA detector; using BDS column; ACE, (150 x 4.6 mm, 5  $\mu$ m). The mobile phase was isocratic consisted of Methanol: Phosphate buffer in ratio of (30: 70 v/v) (buffer is composed of 6.8 gm potassium dihydrogen phosphate per liter and was delivered to the system at a flow rate of 1 ml/min. An injection

volume of 20  $\mu$ l . The detection wavelength ( $\lambda_{max}$ ) was 262 nm. All assays were performed at ambient conditions. The calibration curve in mobile phase was linear with correlation coefficient ( $r^2$ ) = 0.99999; over a concentration range of 5 – 20 mcg/ ml with a retention time of 3.19 minutes. . The percentage recoveries of Nicorandil were 99.84% to 101.71%; respectively. The relative standard deviation (RSD) was found to be 1.06% The proposed method was validated and successfully applied for simultaneous determination of Nicorandil in tablets. The method described is quite suitable for routine analysis of tablets and for their dissolution quantitation.

**KEYWORDS:** Nicorandil; HPLC; BDS column; Method Validation.

## INTRODUCTION

Nicorandil (NIC, N-[2-(nitroxy)ethyl]-3-pyridinecarboxamide) is a novel kind of compound in the treatment of angina. It possesses dual mechanism of action: as a potassium channel opener that causes vasodilation of arterioles and large coronary arteries and as nitrate compound that produces venous vasodilation through stimulation of guanylate cyclase, which protects heart against hypoxia-induced apoptosis.<sup>[1-2]</sup>

However, NIC is not chemically stable and its amide group and nitrate (ester) are easily dissociated, oxidized, reduced and displaced.

Being kept at 60°C for 420 min, NIC produces 2-(3-pyridyl)-2-oxazoline, 2-aminoethyl nicotinate, HEN and NA at pH 4.0, 7.0 and 9.0.<sup>[3]</sup> Upon storage at room temperature NIC also produces NI, HEN and NA. In vivo, metabolites of NIC are HEN, N-methylnicotinamide, nicotinamide and NA.<sup>[4]</sup>

The determination of NIC has been reported by many efficacious analytical methods, such as HPLC, single-sweep oscillography, spectrophotometry, UV spectrophotometry etc.<sup>[5]</sup> At the same time, there are several techniques used simultaneously to determine NIC and one of its degradation products, such as NI by HPLC and HEN by HPLC, HPCE and HPTLC.<sup>[6-7]</sup>

## MATERIALS AND METHODS

**Materials:** All chemicals and reagents used were HPLC grade. Pure standards of Artemether were obtained from Hetero Drugs, Hyderabad. Acetonitrile was HPLC grade from Romil chemicals. Water for chromatography was from Merck.

Nicorandil was obtained from Sinochem, China. Methanol and Potassium dihydrogen phosphate was HPLC grade from Fisher chemicals.

### Methods

#### Analytical procedure for simultaneous determination of Nicorandil in tablet dissolution

**Chromatographic condition:** Agilent 1200 with DA detector was used. BDS column; ACE, (150 x 4.6 mm, 5 µm) was used as a stationary phase. Mobile phase was isocratic consisted of Methanol : Phosphate buffer in ratio of (30 : 70 v/v) (buffer is composed of : 6.8 gm potassium dihydrogen phosphate per liter, delivered to the system at a flow rate of 1 ml/min, An injection volume of 20 µl was used. The detection wavelength ( $\lambda_{max}$ ) was 262 nm, run time was 5 minute. The column was maintained at ambient temperature.

***Preparation of stock and working standard solution***

Standard stock solution (10 µg/ml) of Nicorandil, were prepared separately in mobile phase. Working standard solutions were prepared and further diluted in mobile phase to contain a nicorandil in over the linearity range from 5 - 20 µg/ml respectively.

**Analytical method validation**

**a. Selectivity:** It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resolved from any other peak by resolution of minimum 2.

This could be done injecting placebo and compare it with that of standard and dissolution samples, then peak purity was ascertained by use of DAD

**b. Linearity:** is defined by the correlation coefficient, which should be found NLT 0.99, using peak area responses, Linearity for single point standardization should extend to at least 20% beyond the specification range and include the target Conc. This was performed by preparing 5 different concentrations, and then making 3 replicates of each concentration The linear working range were determined from the constructed standard calibration curve.

**c. Intraday Precision:** This study was conducted by performing multiple analyses on a suitable number of portions of a homogeneous sample.

This was performed by assaying multiple aliquots with the same concentration starting from the first step to the final step of analysis.

The analytical precision of the method was determined by the relative standard deviation.

**d. Inter-day Reproducibility (method ruggedness):** the degree of reproducibility determined by analysis of samples from homogeneous lot of materials, under different but typical test conditions The method is to be rugged, at any item if the pooled %RSD of the total number of replicates that have been made in this item is within the acceptance criteria, 3 replicates of a single sample of powder material are used for each determination. First day: 3 replicates, on a second day: 3 replicates, then on third day: 3 replicates of freshly prepared test from the same sample are analyzed, under the same conditions.

**e. Accuracy and Recovery:** Accuracy was evaluated by spiking standard solution. The measurements are made at a Conc. of Standard Mix which is found to be the target concentration, and at suitable intervals around this point.

The dissolution samples was spiked with known quantities of St. Using three determinations over five Concentrations level covering the specified range (i.e. five concentrations and three replicates).

Relative recoveries of all concentrations of Nicorandil used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation

### **Specificity**

The PDA chromatograms of the Nicorandil in standard and sample were recorded. In the chromatograms of the formulations, some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks, which demonstrate that the assay method is specific. Furthermore, the purity of the peaks was studied by peak purity studies. The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

### **Linearity**

The response for the detector was determined to be linear over the range of 5-20 µg/ml (5, 8, 10, 16 & 20) as shown in figure (4) . Each of the concentrations was injected in triplicate to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. They were represented by the linear regression equation

$$Y_{\text{Nicorandil}} = 660.6626 X - 360, r^2 = 0.9999$$

Slopes and intercepts were obtained by using regression equation ( $Y = mx + c$ ) and least square treatment of the results used to confirm linearity of the method developed.

### **Quantification limit**

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified

with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of Nicorandil found to be  $0.35\mu\text{g/ml}$  respectively. The LOQ to be  $1.07\mu\text{g/ml}$  respectively

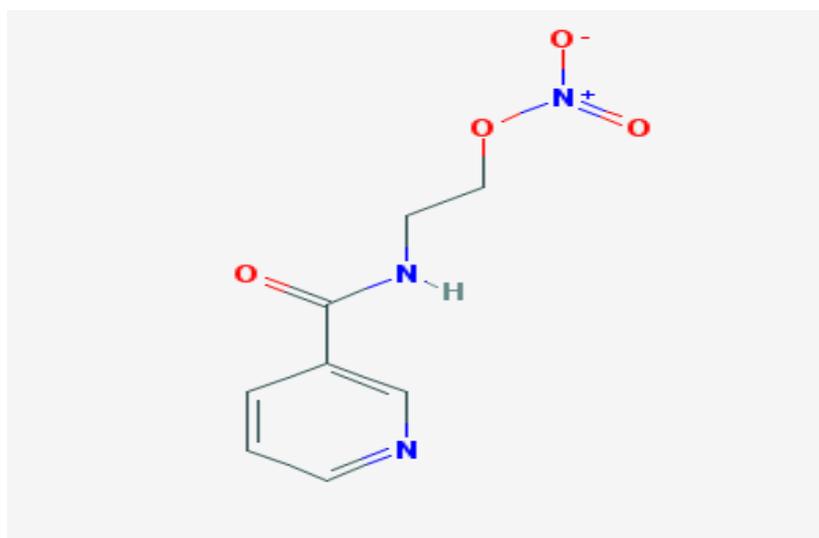
**Solution Stability:** In this study, the mobile phase, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

### System suitability

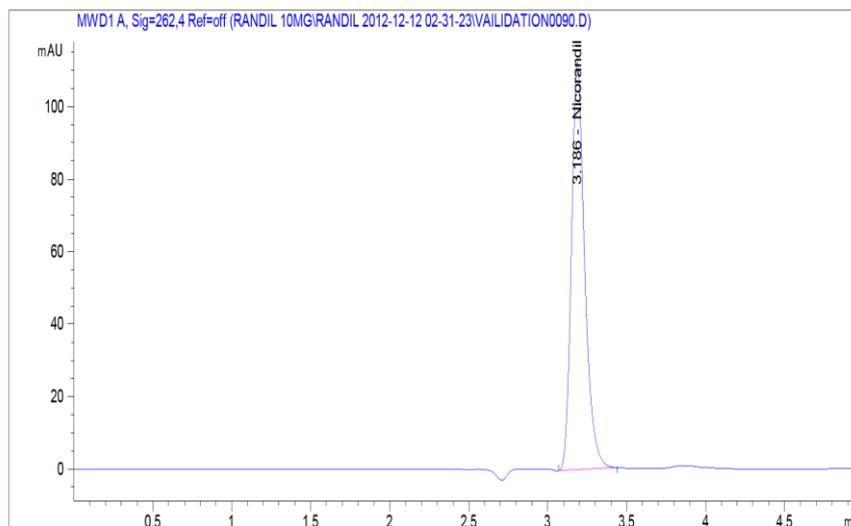
The resolution, capacity factor, theoretical plates/meter,  $R_t$  values and peak symmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within  $\pm 3\%$  standard deviation range during routine performance of the method.

## RESULTS AND DISCUSSION

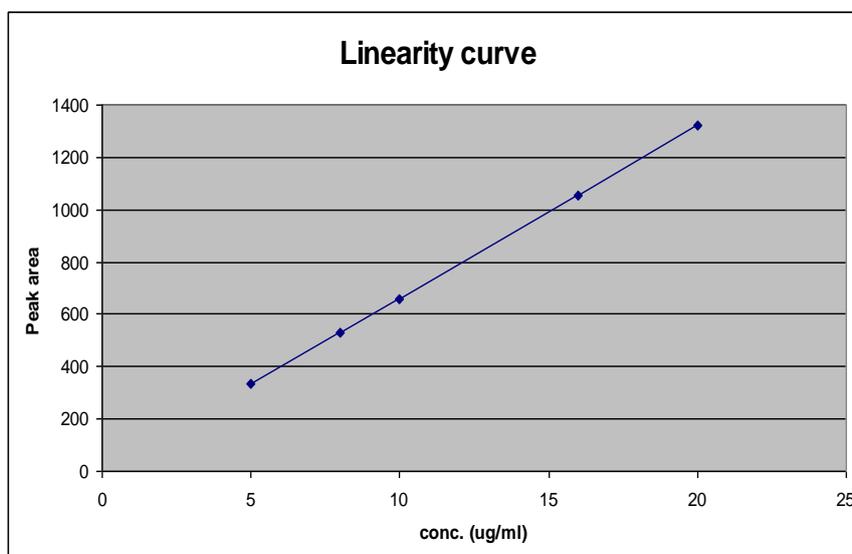
The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries.



**Figure (1): Chemical structure of Nicorandil.**



**Figure (2): HPLC Chromatogram of Nicorandil**



**Figure (3): Calibration curve of Standard Nicorandil**

## CONCLUSION

This method is simple, specific and easy to perform and requires short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control. This method enables simultaneous determination of Nicorandil because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise, linear, and rugged.

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**CONFLICT OF INTEREST**

Authors declare that they have no conflict of interest

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