Volume 3, Issue 1, 2024, Page 621-630

International Journal of Pharma Research and Technology

Quality by Design Approach to Develop and Validate Stability-Indicating RP-HPLC Method for Nicorandil

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Abstract

Nicorandil, a particular medication with a dual mode of action, is prescribed to treat angina. A productive experimental design is suggested on the framework of a Central composite design using QbD. As such there is no HPLC method development of Nicorandil using QbD. So, an attempt was made to determine the presence of Nicorandil in bulk drugs and formulations, a novel, sensitive, and stability-indicating reverse-phase high-performance liquid chromatography technique was developed. A chromatographic separation was achieved on Cosmosil C18 (250mm x 4.6ID, Particle size: 5 microns) mobile phase as Methanol: Water (40:60) at a 0.9 min/ml flow rate. The method was found to be linear with R2 = 0.9998 for the range of 10-50 µg/ml at 225nm. The assay of drug was found to be 100.39 ± 0.61%. The detection and quantitation limits were found to be 0.0332 µg/ml and 0.1005 µg/ml as LOD and LOQ respectively. Forced degradation studies were carried out using HCL, NaOH, H2O2, thermal and photolytic. The validated method was found to be specific, selective, and robust to the Nicorandil API.

Keywords: Quality by design (QbD), RP-HPLC Method Development, Validation, Nicorandil

Introduction

For the treatment of angina, Nicorandil (NIC, N-[2-(nitrox) ethyl]-3-pyridine-carboxamide) with a molecular weight of 211.175 g/mol is a unique drug with dual mechanism of action: as a potassium channel opener, it dilates large coronary arteries and arterioles, and as a nitrate compound, it dilates veins by stimulating guanylate cyclase, which guards the heart against hypoxia-induced apoptosis.[1-2] Nicorandil possesses both nitro vasodilator (NO donor) and potassium channel opening properties, making it a venous and arterial dilator Fig. No. 1.[3-4] It enhances coronary blood flow by causing prolonged dilatation of both artery resistance and conductive channels; although its action on coronary arteries is independent of the coronary steal phenomenon.[5] Nicorandil increases pooling in capacitance vessels with a decrease in preload, resulting in improved blood flow and reduced infarct size. This is achieved through the reduction of end-diastolic pressure and decreased extravascular resistance.

Angina, a typical sign of ischemic heart disease, is a leading global cause of morbidity and death. A comprehensive history and

physical examination are essential for identifying individuals with acute coronary syndrome since it might have cardiac or non-cardiac origins. Angina can be categorized as stable or unstable, with stable angina necessitating quick assessment and treatment. For patients to have better results, it is crucial to identify and treat this symptoms.[6] According to open research, numerous kinds of angina pectoris patients responded well to nicorandil therapy.[7] Nicorandil does not involve the coronary steal phenomenon. Nicorandil's therapeutic effectiveness is mediated by two different processes. Nicorandil is an ATP-sensitive (ATP-dependent) potassium channel (KATP channel) activator and an opening made up of sulfonylurea receptor (SUR) subunits and Kir6.x-type subunits.[8]

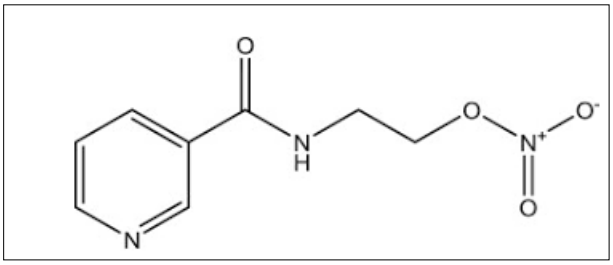


Figure No. 1: Structure of Nicorandil

The term "Quality by Design" (QbD) refers to an approach that focuses on improving scientific understanding of key process and product characteristics, developing controls and tests based on the limits of scientific knowledge during the development phase, and utilizing knowledge gained throughout the product's lifecycle to work on a continuous improvement environment. The pharmaceutical development QbD strategy involves formulation design, development, and manufacturing procedures to uphold the required product quality.[9] RSM (response surface methodology) is a multivariate approach to robustness testing that allows for a complete study of interaction effects and an accurate description of an experimental region around a centre of interest, but its large number of experiments discourages its use in validation.[10]

The literature survey for Nicorandil identified some HPLC methods and their validation. Mukhopadhyay et.al put forward an HPLC method in a tablet dosage form, which had a lengthy retention time and the mobile phase includes a buffer which may reduce the life of the column.[11] Emad Mahmoud Abd El-Halim et al. employed a method for simultaneous determination but it had a very short retention time and buffer was used also the validation results are not in detail.[12] There are some bioanalytical methods for the estimation of Nicorandil in human plasma [13,14,15] etc also technique like Quality by design have not been utilized for method development. Analytical QbD produces a well-known, reliable, and resilient approach, like a process that gives the desired output over the course of its lifecycle.[16]

Based on the literature findings the current study has been aimed to establish regular analytical technique development and validation utilizing a quality-by-design methodology for Nicorandil which has been

prescribed frequently for the lifestyle disease angina. In QbD, the central composite design was employed. The RP-HPLC parameters including linearity, accuracy, precision, and robustness were used to validate the approach. Further, an assay of the marketed formulation was performed utilizing the developed method.

Materials and Methods

Chemicals and Reagents

Nicorandil API (procured as a gift sample from Vital Labs Pvt. Ltd.) and Nicorandil tablet (Korandil Sun Pharma lab ltd) were employed for the assay. All the reagents and chemicals were of analytical grade and all the solvents used were of HPLC grade.

Instruments

HPLC 3000 series of Analytical Technologies Ltd. with UV-3000-M as detector and P-3000-M reciprocating pump was employed for the analysis of the drug. The separation was achieved with Cosmosil C18 (250mm x 4.6ID, particle size: 5 micron).

The samples were weighed and then sonicated utilizing Wenser High Precision Balance and Ultra Sonicator.

With the use of chromatographic conditions, an acceptable separation and peak symmetry for the medication were obtained. With the help of central composite design, the HPLC technique for Nicorandil was optimized for the mobile phase, flow rate, and wavelength.

Preparation of Standard Stock Solution

The preparation of a standard stock solution involved dissolving 10 mg of the pure drug in 10 ml of solvent, giving 1000µg/ml. By serial dilutions a working solution of 10µg/ml was prepared and sonicated for a few min.

Assay

Twenty tablets were weighed and powdered. Accurate powder equivalent to 10mg of Nicorandil was weighed and transferred to 100 ml of a volumetric flask.25 ml of solvent was added and sonicated until the powder was dissolved. The mobile phase was then used to make up the volume to specification. The resulting solution was filtered. The solution was analyzed by HPLC with the same chromatographic condition as linearity.

λ_{max} detection

The standard solution of 10µg/ml was employed to scan in the UV range of 200-400nm, and 255nm was selected as the λ_{max} depicted in Figure No. 2.

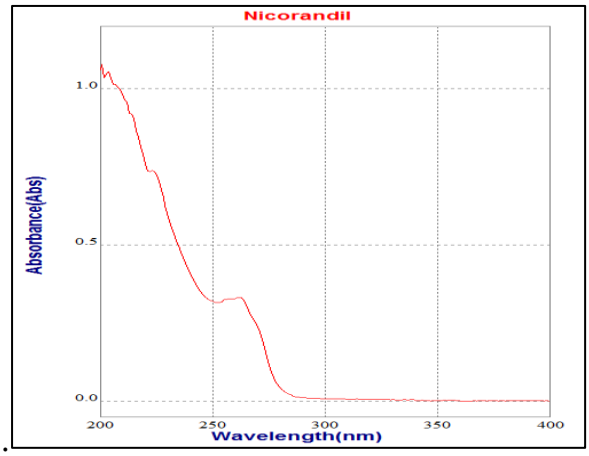


Figure No. 2: UV Scanning of Nicorandil

Optimization of the design of the experiment for method development of nicorandil

The goal of this project was to provide flexibility to enable ongoing product innovation without the requirement of expensive adjustments to be made after a market has been authorized. In order to explore the effects of three factors on the four important response variables, three randomized response surface designs with a Central Composite Design were employed with 17 trial runs as given in Table 1. In this design, the mobile phase composition (A1), flow rate (A2), and Wavelength (A3) were selected as independent variables, and retention time (RT), Area Under Curve (AUC), Theoretical plates (TP) and Asymmetry factor (AF) were selected as dependent variables. The resultant data was analyzed statistically using analysis of variance (ANOVA) and F-Test after being fitted into Design Expert 13 software.

Results and Discussion

Design screening for appropriate chromatographic conditions using Central composite design (CCD)

The screening studies were carried out utilizing several columns, such as C18 and C8, and mobile phases such as acetic acid, acetonitrile, methanol, and water, to choose the most appropriate column and mobile phase. The C18 column and methanol: water (40:60) ratio were chosen from six trials that were completed to analyze Nicorandil. The active pharmaceutical ingredient (API) was successfully separated under the chromatographic conditions, with a 6.38-minute retention time Figure No.3. Thus, Nicorandil was successfully separated under the best chromatographic conditions.

Previous chromatographic separation experiments served as the foundation for the parameters and range selection. In the Nicorandil method development the independent variables chosen were mobile phase composition (A1), flow rate (A2), and wavelength (A3). The limits for the higher and lower levels for the three respective selected factors were as follows: for A1- 80% and 40%; for A2 - 1.0 and 0.8 mL/min and, for A3 - 225 and 221 nm respectively. The retention time, area, theoretical plates, and asymmetry factor were the dependent variables (or responses) as depicted in Table 1.

The 17 runs recommended by the Central Composite Design had been carried out to determine the best possible chromatographic condition. Three of the 17 runs with Rt values of 6.38, 6.38, and 5.7 were taken into consideration. To get the desired response, one run out of three with methanol: water (40:60) as mobile phase composition, a retention time of 6.38, a flow rate of 0.9ml/min, and a wavelength of 225 was selected. The other two runs lacked the necessary flow rate or wavelength.

Table 1: Optimized conditions by central composite design and observed response values

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4

Run	(A1) Mobile Phase Composition	(A2) Flow rate	(A3) Wavelength	Retention Time	Area	Theoretical Plates	Asymmetry Factor
	%(Methanol)	ml/min	Nm	Min	Area Units	Units	Units
1	80	0.8	223	3.96	1.06E+06	4270	1.32
2	60	0.8	221	4.749	921266	7036	1.28
3	80	0.9	221	3.538	1.04E+06	3170	1.36
4	40	0.9	225	6.384	1.19E+06	7924	1.27
5	60	0.9	223	4.248	920253	7388	1.33
6	60	0.9	223	4.248	920253	7388	1.33
7	80	1	223	3.237	934318	4218	1.31
8	40	0.8	223	7.184	871679	6875	1.34
9	60	0.9	223	4.248	920253	7388	1.33
10	60	0.8	225	4.722	917370	7565	1.3
11	40	0.9	221	6.383	778281	7603	1.28
12	40	1	223	5.798	716345	7468	1.27
13	60	1	225	3.901	862263	7370	1.31
14	60	1	221	3.908	784208	6764	1.34
15	60	0.9	223	4.248	920253	7388	1.33
16	60	0.9	223	4.248	920253	7388	1.33
17	80	0.9	225	3.568	1.03E+06	3260	1.35

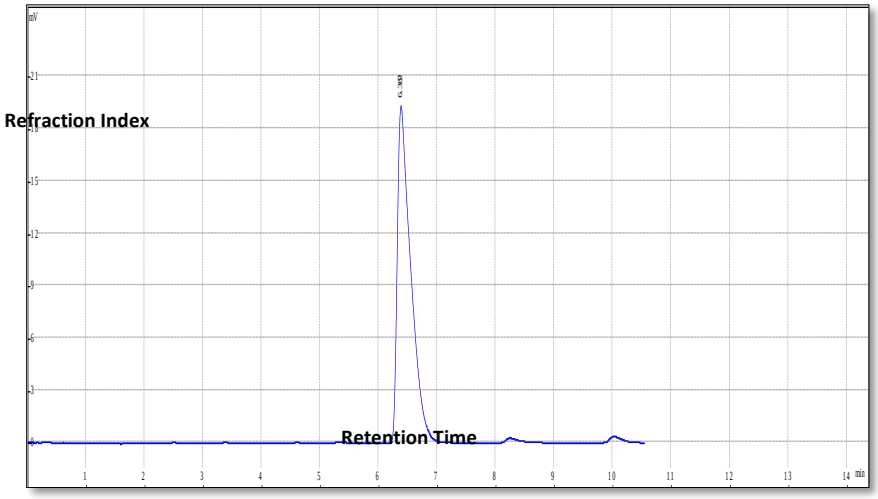


Figure No. 3: Chromatogram of Nicorandil

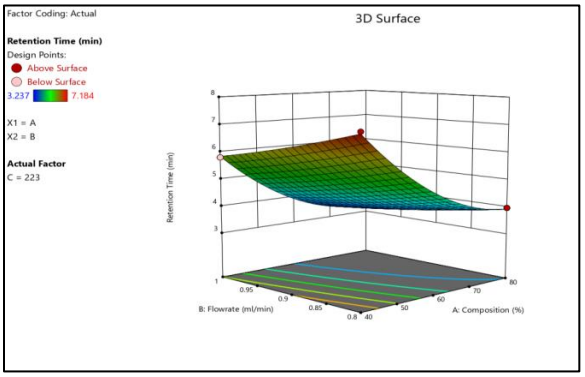
Design Space

To ascertain the effects of flow rate, wavelength, and mobile phase composition on dependent variables, the data were further submitted to the 3-D response surface approach. The study generates three-dimensional graphs by plotting the response model against two of the components, while

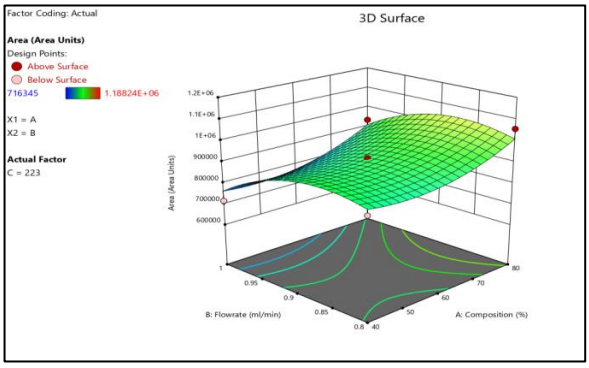
the third is kept constant at a predetermined level, as illustrated in Figure No. 4 a–d.

The responses remained unaffected by the factor wavelength. Thus, the remaining two factors i.e., mobile phase composition and flow rate are taken into consideration keeping the wavelength constant for the 3-D response surface.

The retention time response surface for alterations in flow rate and mobile phase composition, with the wavelength held constant at its optimal value of 255 nm, is depicted graphically in Fig 4a. Retention time increases in direct proportion to the % mobile phase composition since they are directly correlated. As the mobile phase composition increased in Fig. 4b, the area was significantly affected. Since the wavelength is kept constant in Figs. 4c and 4d, the approach may be regarded as being reliable for the experimental outcome.



(4 a)



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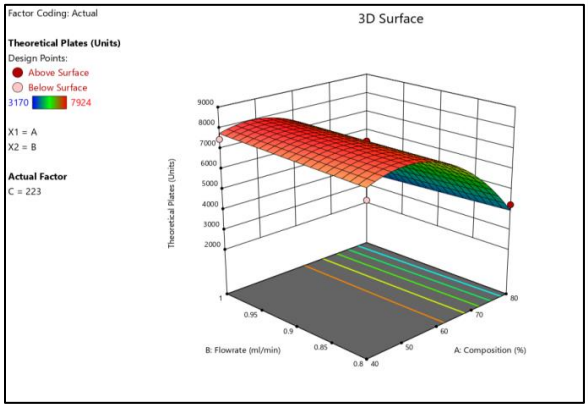

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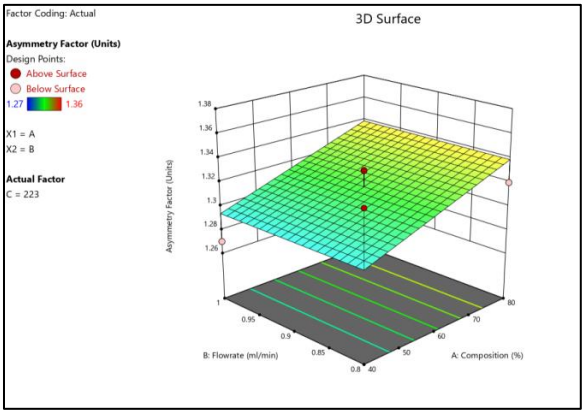

(4 d)

Figure No. 4: 3-D response surface for Retention time, Area, Theoretical plate, and Asymmetry factor.

Validation parameters for the RP-HPLC method

The QbD CCD outcomes were further utilized for validation of the devised RP-HPLC method as per the ICH guidelines. The parameters specified below were employed to evaluate the liquid chromatographic (LC) technique.

Calibration Curve: After a repeat investigation of five standards with Nicorandil concentrations of 10, 20, 30, 40, and 50 g/ml, a calibration curve was created. After calculating the peak height ratio of the drug and plotting the AUC versus concentration, data was analyzed using least-squares linear regression to obtain the equation for the best-fit line and the correlation coefficient (R2) to validate linearity. Samples were injected, peaks at 255 nm were recorded, and a graph of drug concentration vs. peak area was created. The calibration curve is depicted in Figure No. 5. The value of R2 was found to be 0.9998 which falls under the acceptable limit of 0.999.

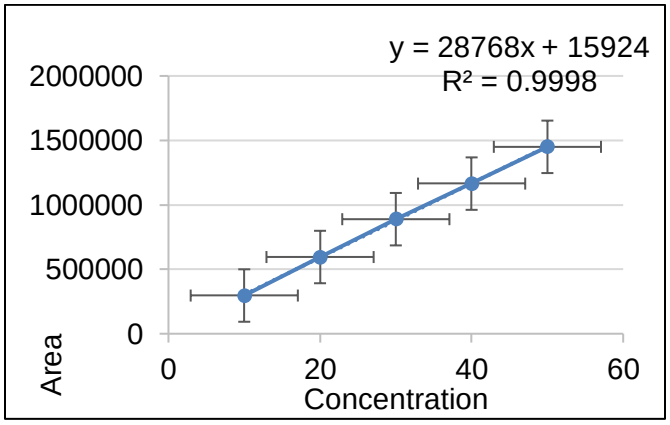

Figure No. 5: Calibration Curve

Precision: The examination of three replicates of one concentration i.e. 30 µg/ml) resulted in the establishment of intra-day (repeatability) precision. On the same day, analysis was conducted for a brief period of time. The concentration's inter-day accuracy or repeatability was evaluated for two consecutive days utilizing the same concentration, and both intra- and inter-day precision were assessed using the percent relative standard deviation (% RSD). The %RSD for inter-day and intra-day was found to be 0.0332 and 0.5005 respectively. The results are very well within the given limit of 2% as specified by International Council for Harmonisation (ICH) guidelines.

Accuracy: Percent recovery study was performed with 3 concentration levels, each in triplicate. It was carried out by adding 50%, 100% and 150% of working level concentration to the standard stock of 20 ppm. Results for percent recovery have been summarized in Table 2. According to ICH guidelines, the acceptable range for percentage recovery is 98–102% of the standard addition. Nicorandil's recovery percentage has been found to be well within the ICH recommended range.

Table No. 2: Nicorandil accuracy results

% Composition	% Recovery	Conc. Taken

	(%)	(ppm)
50% Recovery	100.3870089	30
100% Recovery	100.1358282	40
150% Recovery	99.69565202	50

Robustness: To demonstrate the robustness of the method, system suitability parameters were verified by a deliberate change in chromatographic conditions, i.e., change in wavelength at 220 and 230 nm, change in pH 6.2 and 6.6. The calculated % relative standard deviation (RSD of the peak area was found to be 0.208% and 0.0902% respectively. The method's results were reliable within an acceptable operating range of HPLC conditions during operation.

LOD and LOQ: The detection limit (LOD) and the quantification limit (LOQ), respectively, are the lowest drug concentrations that can be detected and distinguished from the background and the lowest concentration that can be quantified. In accordance with the ICH recommendations, LOD and LOQ were calculated using the following equation.

$$LOD = 3.3 \times \sigma / SD$$
$$= 3.3 \times 289.25 / 28768$$
$$= 0.0332$$
$$LOQ = 10 \times \sigma / SD$$
$$= 10 \times 289.25 / 28768$$
$$= 0.10055$$

Where,

S= Slope of the calibration curve

σ = Residual standard deviation of response

Forced Degradation: The forced degradation studies were done for the evaluation API and its degradant product. The 20µg/ml sample solution was exposed to stress conditions such as acid, base, hydrogen peroxide (H_2O_2), temperature, and light. The results indicated that maximum degradation was observed in base with sample purity of 87.23%. About 88.6% purity of drug was obtained by acid degradation. Nicorandil showed about 98% purity with photolytic and thermal and 89.6 % purity with H_2O_2 degradation processes.

Assay: The validated approach was successfully utilized to quantitatively Nicorandil in the presence of every excipient included in the commercial formulation within their limits. The % Assay was found to be 100.3989% which falls under the range mentioned in the ICH guideline. Table No.3

Table No. 3: Assay

Sr. No.	Conc.	Area	Average	%Assay

1	30ppm	891827		
2	30ppm	894695	892155	100.3989
3	30ppm	8 89943		

Conclusion

A successful technique for estimating Nicorandil using reversed-phase high-performance liquid chromatography has been developed and validated in accordance with ICH. The percentage of mobile phase, flow rate, and wavelength were all optimized with the assistance of the QbD i.e., Central Composite design. The devised method successfully resolved and separated all peaks even under the circumstances of degradation. The current study demonstrates the development and approval of a quick, easy, and highly sensitive RP-HPLC technique for the quantification of Nicorandil in both pure form and dose forms. The shortcomings of the previously published approach, such as longer retention times, complex mobile phase, and few validated methods have been addressed by the newly developed method. Additionally, this method is economical with the use of inexpensive instruments, solvents, and reagents. This straightforward technique for quality control is dependable and reproducible because of its great accuracy, precision, and sensitivity. Thus, the developed RP-HPLC method can be employed by all for the quantification of Nicorandil in API as well formulation.

Acknowledgments

The authors of this paper are thankful to Vital Laboratories. Pvt. Ltd, Western Express Highway, near Hanuman Temple, Vile Parle (E) for providing Nicorandil API to perform the research work.

Conflict Of Interest

The authors state no conflict of interest with respect to the research, authorship, and /or publication of this article.

Funding

The authors did not receive support from any organization for the submitted work

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