CHAPTER – 9

DETERMINATION OF DRUG RELEASE DURING DISSOLUTION OF NICORANDIL IN TABLET DOSAGE FORM BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Determination of drug release during dissolution of Nicorandil in tablet dosage form by using reverse phase high performance liquid chromatography

9.0 INTRODUCTION

The objective was to develop a method for determination of percentage drug release of Nicorandil in tablet dosage form. The method, after development, was validated as per ICH guidelines Q2 (R1).

The target for this research work was to present comprehensive methods of critical tests for each drug product. Thus this work can be treated as a monograph for the drug products.

The main aim for this current study was to develop the analytical determination/ parameters of drug release rather than the dissolution parameters.

A brief introduction of the molecule has already been included in chapter - 7.

9.1 LITERATURE SURVEY

The literature revealed that the assay of the drug in pure and dosage forms is not official in any pharmacopeia and, therefore, requires much more investigation. The estimation of Nicorandil from biological fluids and/or pharmaceutical formulations has been conducted using several analytical methods include high-performance thin layer chromatography[28,29,30] high-performance liquid chromatography [31,32,33,34,35,36,37] and gas chromatography coupled with mass spectrometry[38]. A review of literature revealed no HPLC method for the drug release of Nicorandil in pharmaceutical formulations.

9.2 PRESENT WORK AND DISCUSSION

9.2.1 Selection of Chromatographic Method

Reverse Phase chromatography is the natural choice for method development because of its ease of handling and robust nature. All development was conducted using reverse phase methods. The analytical methods published in literature for Nicorandil are based on reverse phase chromatographic (RPC) separations.

9.2.2 Selection of Stationary Phase

Typical target for developing an analytical method for dissolution is a short run time. Since the assay method developed already met that criterion, it was decided to check if those HPLC parameters can be utilized in drug release determination also.

9.2.3 Selection of Wavelength for Analysis

The optimum wavelength selected was 262 nm which represents the wavelength where Nicorandil has an absorption maxima. Higher wavelength also helps in reducing interferences from common excipients used in the formulated drug product.

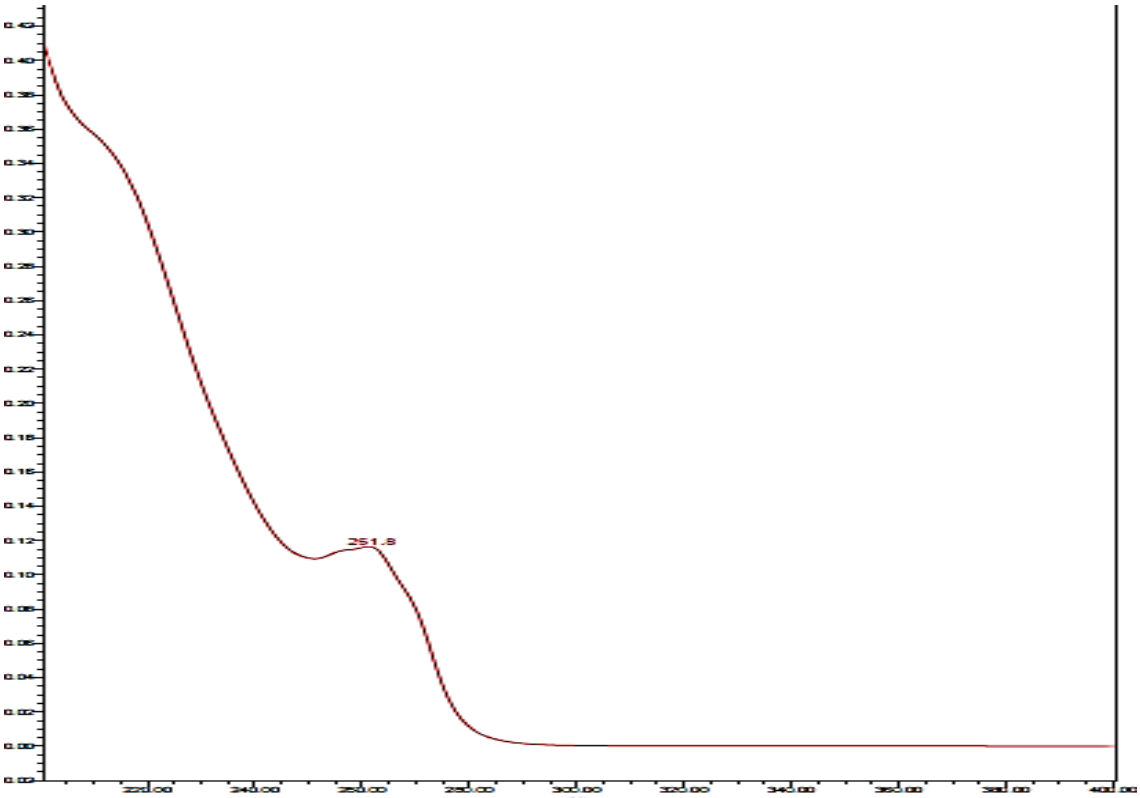


Figure: 9.2.3.1 UV spectrum of Nicorandil

9.2.4 Selection and Optimization of Mobile Phase

From the experience of assay method development and its forced degradation studies, it was decided not to change the parameters any further since there were some closely eluting degradants with respect to main peak. Since these degradations were quite prominent, they were expected to be detected during HPLC run of dissolution samples too. Thus the exact same HPLC method for assay was utilized for dissolution determination. However due to a lower concentration of sample in dissolution, the injection volume was increased to 100µL from the 20µL used in assay.

The important factor that needed to be considered was the impact on the peak shape due to change in diluent when compared to assay method. Since the dissolution medium does not have a scope of organic solvents, it was essential to ascertain that the peak shape is proper when fully aqueous sample was injected. This was of further importance since the injection volume was now a considerable amount.

BCS solubility study was performed on the molecule. 20mg of Nicorandil was shaken in an orbital shaker in 250ml buffers with pH ranging from 1 to 7.4 at 37ºC. The results are given in Table 9.2.1

Table 9.2.1

Medium	% dissolved (Nicorandil)
Water	101.7
0.1 N HCl	101.1
0.01 N HCl	102.7
0.001 N HCl	102.2
Citrate pH 3.0 buffer	99.1
Acetate pH 4.5 buffer	100.9
Acetate pH 5.0 buffer	101.5
Phosphate pH 6.8 buffer	100.4
Phosphate pH 7.4 buffer	99.9

All the samples were analysed by the HPLC method and peak shapes were acceptable proving that this method is capable to accept fully aqueous injections and thus suitable for dissolution study.

Optimized Chromatographic Conditions:

Chromatographic condition for dissolution:

Dissolution Conditions;

Instruments/Equipment	:	Dissolution Apparatus, Make-Electrolab, Model-TDT-08L with fraction collector, or equivalent
Apparatus	:	USP Apparatus II (Paddle)
Dissolution medium	:	0.1 M HCL
Volume	:	900 ml
Temperature	:	37°C ± 0.5°C
Speed	:	50 rpm
Time	:	45 minutes

Preparation of 0.1 M HCl:
Transfer 8.87 ml of concentrated Hydrochloric acid in 1000 ml water and mix.

The method employed for Nicorandil tablets is separation using isocratic HPLC with detection by UV.
Chromatographic condition for dissolution:

Instruments/Equipment	:	HPLC, Make – Waters, Alliance, 2695 Separation Module, (UV/PDA), or equivalent. Analytical Balance, Make –Mettler Toledo, Model-XS205DU, or equivalent.
Column	:	Inertsil ODS-3V, 250 x 4.6 mm, 5µm or equivalent
Flow rate	:	1.5 ml/minute
Column temperature	:	30°C
Wavelength	:	262 nm
Sample temperature	:	15°C
Injection volume	:	100 µl
Run time	:	8 minutes
Retention time	:	about 6.0 minute
Diluent	:	Mixture of Water: Methanol in the ratio 50:50 v/v, mix.

Buffer:

Weigh accurately 1.42 gm of Disodium hydrogen phosphate anhydrous and transfer into 1000 ml of water. Adjust the pH 6.4 with ortho phosphoric acid.

Preparation of Mobile Phase:

Prepare a mixture of Buffer: Acetonitrile: Methanol in the ratio 70:15:15 v/v/v. Mix and degas.

9.3 EXPERIMENTAL WORK

9.3.1 Instrumentation

Equipment	Make	Model
HPLC	Waters	2695Alliance Separation Module, (PDA/UV Detector) 2996/2487
Dissolution Apparatus	Electrolab	Model-TDT-08L with fraction collector, or equivalent
Column	AKZO NOBEL	Inertsil ODS-3V, 250 x 4.6 mm, 5µm
pH meter	Thermo Electron Corp.	Orion-4star 1117000
Analytical Balance	Mettler Toledo	XS205DU
Ultrasonnicator	Spectralab	-
Photostability Chamber	Thermolab	400litr
Water Bath	Spectralab	

9.3.2 Chemicals and Reagents

Name	Grade	Manufacturer
Disodium Hydrogen phosphate anhydrous	HPLC grade	Merck
Acetonitrile	HPLC, Gradient grade	Rankem
Methanol	HPLC, Gradient grade	Merck
Ortho-Phosphoric acid	GR	Merck
Hydrochloric acid	GR	Merck
Water	HPLC milli-Q	In-house

9.3.3 Working Standard

Working Standard:

Standard	Lot .No.	Potency (as is) %
Nicorandil	6712008002	99.4

Test Sample:

Batch. No.	Label claim
NCT/20/23	20mg

Placebo:

Batch. No.
NCT/23P

9.3.4 Solution Preparation

Preparation of Standard solution:

Accurately weigh and transfer about 40.0 mg of Nicorandil standard and transfer to a 100 ml amber colour volumetric flask, add 70ml of diluent, sonnicate to dissolve and make up the volume with diluent. Dilute 5ml of this solution to 100ml with dissolution medium.

Preparation of Sample solution:

Transfer one tablet in each of the six dissolution vessels. Run the Dissolution as per the set parameters. Withdraw about 10 ml of the sample after 45 minutes. Filter through on line Stainless Steel (SS) filter. Discard first 2-3 ml of filtrate and inject the filtrate into the HPLC.

Preparation of Placebo solution:

(To be prepared only for method validation)

Weigh accurately placebo equivalent to one tablet and transfer in the dissolution vessel. Run the Dissolution as per the set parameters. Withdraw about 10 ml of the sample after 45 minutes. Filter through on line SS filter. Discard first 2-3 ml of filtrate and inject the filtrate into the HPLC.

Evaluation of System suitability:

Inject the Nicorandil Standard five times; the relative standard deviation of five replicate injections should not be more than 2.0%. The USP tailing factor for Nicorandil peak should not be more than 2.0. The USP plates should not be less than 2000.

Procedure:

Inject equal volumes of Blank (diluent), Standard (5 replicate) and sample solutions.

Calculation:

Calculate the amount of Nicorandil present in the tablets as per give formula.

$$\% \text{ Release} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{100} \times \frac{900}{1 \text{ Tab}} \times \frac{P}{LC}$$

Where,

AT = Area of peak due to Nicorandil in sample preparation.

AS = Area of peak due to Nicorandil in standard preparation.

WS = Weight of Nicorandil standard in mg.

LC = Label claim of Nicorandil per tablet in mg.

P = Potency of Nicorandil standard on as is basis.

9.4 VALIDATION OF THE DEVELOPED METHOD

9.4.1 Validation parameters and acceptance criteria

The Table 9.4.1.1 summarizes the validation acceptance criteria along with the obtained results.

Table 9.4.1.1: Validation Summary

Sr.No.	Parameters	Acceptance criteria	Result obtained
1.0	System suitability % RSD for Standard solution USP Tailing USP Plate count	 NMT 2.0% NMT 2.0 NLT 2000.	 0.02 1.20 5549
2.0 2.1	Specificity Identification	Results should be comparable with respect to the retention time.	Complies
2.2	Interference	No interference from blank and placebo to main component.	Complies
2.3	Peak purity	Purity angle should be less than purity threshold. Standard peak should be pure for working concentration level.	Complies
3.0	Linearity	Response should be Linear	Response is linear
		Correlation coefficient should not be less than 0.999.	0.9999
		Y- Intercept should be within ± 5.0% of the corresponding Y-co-ordinate of the working level.	Complies

Table 9.4.1.1: Validation Summary (continued)

Sr. No.	Parameters	Acceptance criteria	Result obtained	
4.0	Accuracy (Recovery)	Mean recovery should be in the range of 95.0%- 105.0%.	Level %	% Mean Recovery
			50	100.4
			100	99.2
			150	97.6
5.0	System Precision % RSD for Standard solution USP Tailing USP Plate count	NMT 2.0% NMT 2.0 NLT 2000	0.02 1.20 5549	
5.1	Method Precision % RSD of six determinations	NMT 5.0%.	1.82	
5.2	Intermediate Precision (Ruggedness) % RSD for Standard solution	NMT 2.0%	0.07	
	USP Tailing	NMT 2.0	1.29	
	USP Plate count	NLT 2000.	9067	
	RSD for % release	NMT 5.0%.	2.29	
	Difference for pooled result (Analyst-I and II)	The difference in the mean should not be more than ± 5.	0.0	

Table 9.4.1.1: Validation Summary (continued)

Sr. No.	Parameters	Acceptance criteria	Result obtained
6.0	Stability in analytical solution	The difference should not be more than ± 5.	Sample stable for 24 hours at 15°C
7.0	Filter compatibility	The difference between centrifuged sample and filtered sample should not be more than ± 5.	complies
8.0	Robustness Change in Flow rate (± 0.2 ml/min)	No significant change should be in System suitability parameters. % RSD should be less than 5%.	No significant change. Compiles
	Change in wavelength (± 5nm)	No significant change should be in System suitability parameters. % RSD should be less than 5%.	No significant change. Compiles
	Column oven temperature (± 5°C)	No significant change should be in System suitability parameters. % RSD should be less than 5%.	No significant change. Compiles
	Change in Mobile phase composition (± 2% absolute)	No significant change should be in System suitability parameters. % RSD should be less than 5%.	No significant change. Compiles
	Change in Buffer pH (± 0.2)	No significant change should be in System suitability parameters. % RSD should be less than 5%.	No significant change. Compiles
	Change in Speed of rotation. (± 4% rpm)	No significant change should be in System suitability parameters. % RSD should be less than 5%.	No significant change. Compiles

9.4.2 System suitability:

Single injection of Blank (Diluent) and five replicate Standard solution were made on the system. The data obtained is summarized in Table. 9.4.2.1. The data demonstrate that the system suitability is within the acceptance criteria, thus the system is suitable.

Table 9.4.2.1: System suitability

Standard solution	
USP Tailing	**1.20**
USP Plates	**5549**
Area	**Standard solution**
	1274756
	1275269
	1274526
	1274750
	1274500
Mean	**1274760**
SD	**308.8498**
%RSD	**0.02**

9.4.3 Specificity:

The Specificity study included Identification of the main peak, Interference study and Peak Purity:

Injections of Blank and standard solutions were made as directed in the method; sample solution and placebo preparation were made and injected into the HPLC. The data obtained is summarized in Table 9.4.3.1. Purity angle is less than purity threshold for all components. The data demonstrate that there is no interference in blank and placebo with Nicorandil peak.

Table 9.4.3.1: Specificity (Identification and Interference)

Component	**Retention time (min)**	**USP Plates**	**USP Tailing**	**Purity angle**	**Purity threshold**
Nicorandil	5.603	5549	1.20	0.048	0.254

Chromatograms of Blank (diluent), placebo, Standard solution and Sample solution are given below under figure 9.4.3.1, 9.4.3.2, 9.4.3.3 and 9.4.3.4 respectively

Figure-9.4.3.1: Chromatogram of Blank.

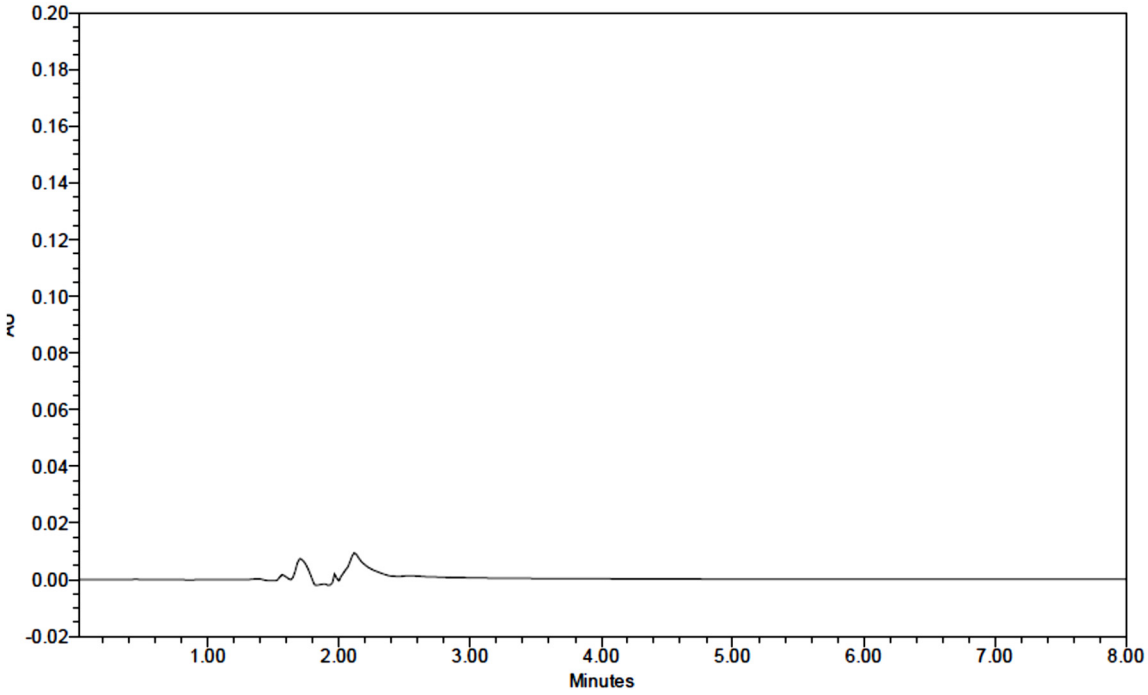


Figure-9.4.3.2: Chromatogram of Placebo.

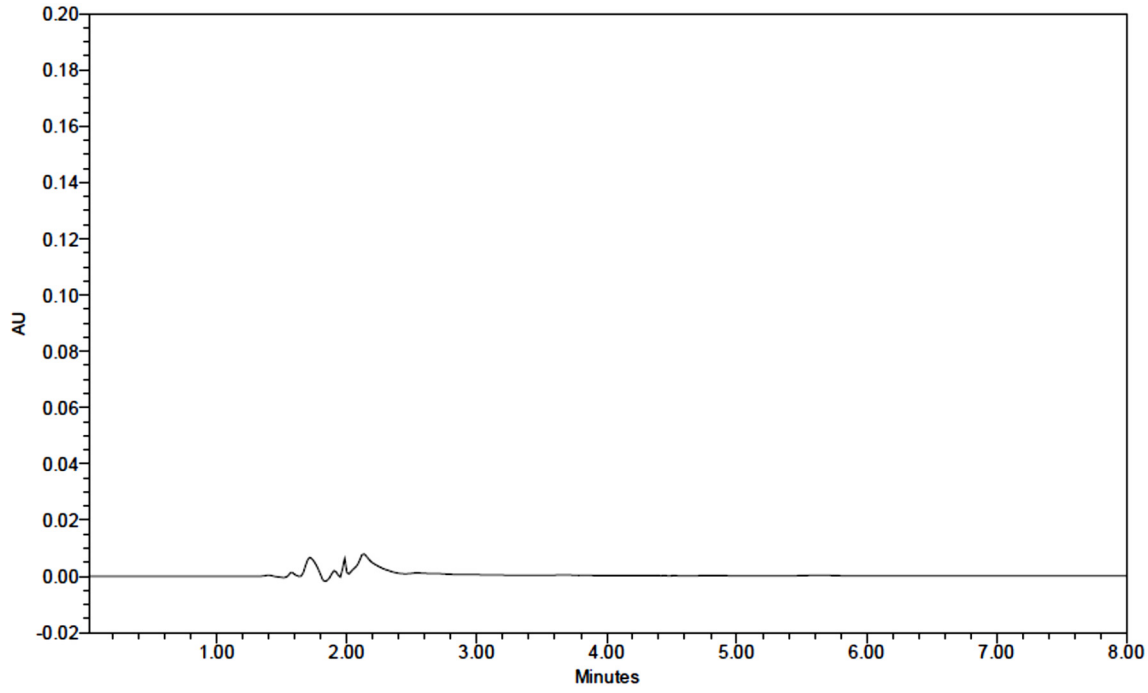


Figure-9.4.3.3: Chromatogram of Standard solution.

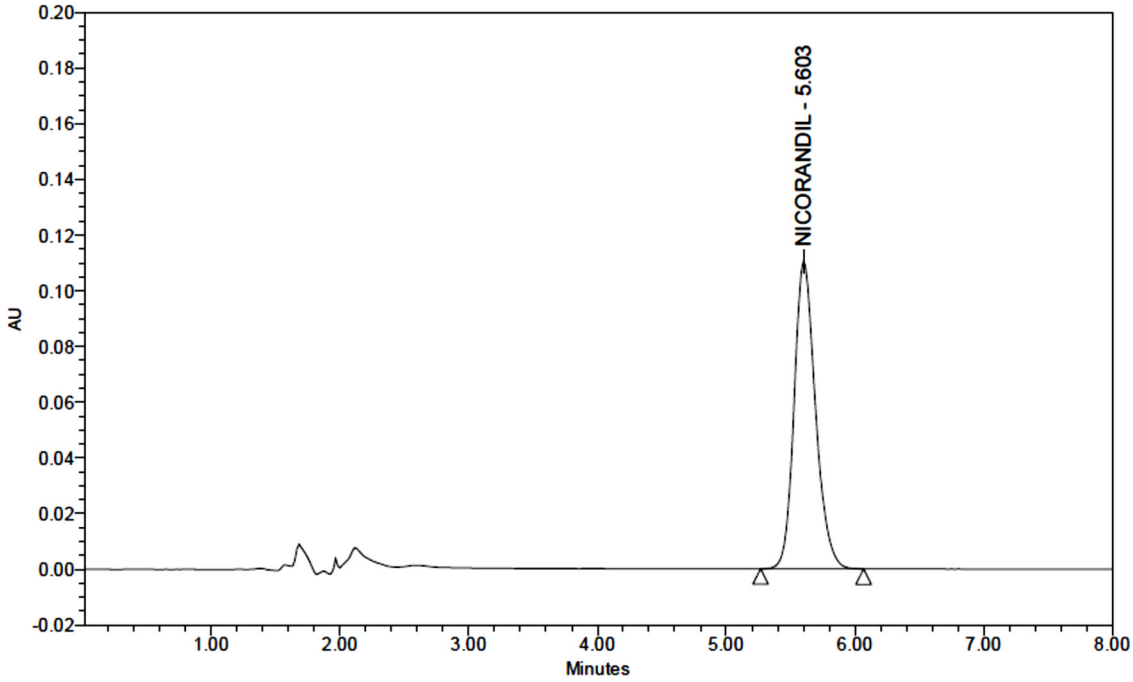
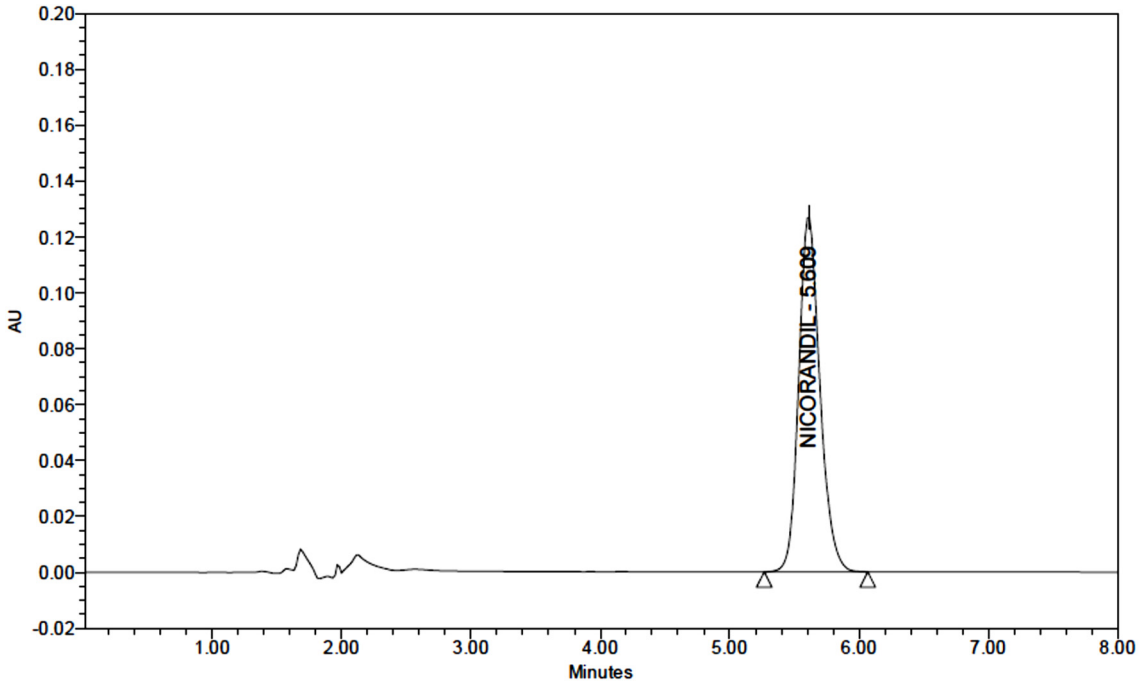


Figure-9.4.3.4: Chromatogram of Control Sample solution.



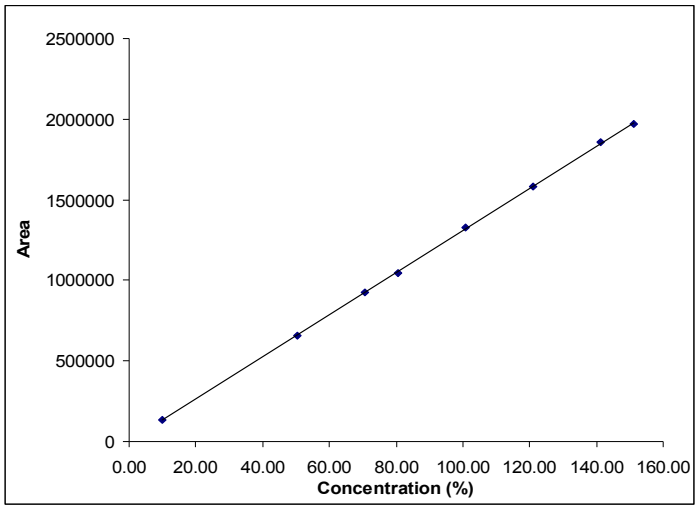
9.4.4 Linearity and Range:

The Linearity of response was determined by preparing different concentrations of standard stock solution ranging from 10% to 150% of the working concentration. The data is summarized in Table 9.4.4.1. The data shows that the response is found to be linear; Correlation coefficient is more than 0.999. The Y-intercept is also within the set criterion. The graphical depiction is included in Figure 9.4.4.1

Table 9.4.4.1: Linearity of Nicorandil

Level	Concentration (%)	Response		
		1	2	Mean
1	10.08	136076	137568	136822
2	50.39	657264	656801	657033
3	70.54	925481	925613	925547
4	80.62	1046850	1048645	1047748
5	100.78	1326519	1325539	1326029
6	120.93	1578085	1578714	1578400
7	141.09	1857148	1856206	1856677
8	151.16	1968083	1965993	1967038
CORRELATION COEFFICIENT (r)				**0.9999**
SLOPE				**13056**
Y-INTERCEPT				**2760**
MEDIAN (AREA)				**1186888**
LIMIT OF Y-INTERCEPT ± 5% OF MEDIAN				**59344**

Figure 9.4.4.1: Linearity of Nicorandil



9.4.5 Accuracy:

The standard solution was spiked into the placebo at three different levels, 50%, 100% and 150% from three different standard stock solutions and each level in duplicate were injected. From the amount added and the amount found, the percentage recovery was calculated. The mean recovery was calculated. The results obtained were summarized in Table 9.4.5.1 and 9.4.5.2. The data shows that the percentage mean recovery at each level is within the acceptance criteria.

Table 9.4.5.1: System suitability

Standard solution	
USP Tailing	**1.20**
USP Plates	**5549**
Area	**Standard solution**
	1274756
	1275269
	1274526
	1274750
	1274500
Mean	**1274760**
SD	**308.85**
%RSD	**0.02**

Table 9.4.5.2: Percentage Recovery

Level %	**Response**	**% Recovery**	**Mean recovery %**
50 %	707999	101.0	100.4
	711335	100.0	
	722542	100.2	
100 %	1389495	99.1	99.2
	1416838	99.6	
	1427183	99.0	
150 %	2064875	98.1	97.6
	2080639	97.5	
	2101730	97.2	

9.4.6 Precision

9.4.6.1 System Precision:

Single injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. Please refer to Table 9.4.2.1. All the data were acceptable as per the system suitability requirements.

9.4.6.2 Method Precision:

Six independent sample solutions were prepared and injected on the HPLC. The data obtained is summarized in Table 9.4.6.2.1. The data shows that % RSD is within the acceptance criteria.

Table 9.4.6.2.1: Method precision

Tablet No.	% Release
1	102
2	104
3	100
4	100
5	102
6	99
Mean	**101**
SD	**1.835**
% RSD	**1.82**

9.4.6.3 Intermediate Precision (Ruggedness):

Same procedure of system precision and method precision was followed by another Analyst on a different instrument and on a different day. The data demonstrate that the system complied with system suitability requirements. The data obtained from Analyst-II are summarized in Table 9.4.6.3.1 and 9.4.6.3.2. The data shows that percentage RSD is within the acceptance criteria.

Table 9.4.6.3.1: System suitability

Standard solution	
USP Tailing	**1.29**
USP Plates	**9067**
Area	**Standard solution**
	1233831
	1235665
	1235842
	1235366
	1234839
Mean	**1235109**
SD	**809.1133**
%RSD	**0.07**

Table 9.4.6.3.2: Intermediate precision

Tablet No.	% Release
1	102
2	97
3	100
4	104
5	101
6	101
Mean	**101**
SD	**2.317**
% RSD	**2.29**

The pooled data obtained from twelve independent samples by Analyst-I and Analyst-II is summarized in Table 9.4.6.3.3. The data shows that % difference is not more than ± 5.

Table 9.4.6.3.3: Pooled data

	Analyst I	**Analyst** II
% Release	102	102
	104	97
	100	100
	100	104
	102	101
	99	101
Mean	**101**	**101**
% Difference between two means	**0.0**	

9.4.7 Stability in Analytical solution:

The Sample solution kept at auto sampler temperature for 24 hours and was injected on to the HPLC time to time. The data obtained are summarized in Table 9.5.1. The data shows that % difference upto 24 hrs is less than ± 5. Thus the sample solution is stable for 24 hours at 15°C.

Table 9.5.1: Stability in analytical solution

Time	**% Release**	**% Difference**
Initial (control)	102	-
2 hrs	102	0
6 hrs	103	1
12hrs	102	0
24hrs	103	1

9.4.8 Filter compatibility:

The Sample solution was centrifuged and used as control for this study. Samples filtered through different filter were also injected on to the HPLC.

The data shows that % difference is not more than ± 5. Thus all filters tested were compatible with the sample. The data obtained are summarized in Table 9.6.1.

Table 9.6.1: Filter compatibility

Filter	% Release	% Difference
Centrifuged	103	-
Glass Filter	103	0
SS on line	102	1
Nylon Filter	103	0
Nylon + Glass	103	0
PVDF	103	0

9.4.9 Robustness:

The changes in system suitability parameters and results, when deliberate controlled changes were made to the method, were studied in robustness. No significant changes in system suitability parameters or results were observed during robustness study proving the method to be considerable robust.

The data obtained are summarized in Table 9.7.1.

Table 9.7.1: Robustness.

Changes in parameters	Values	Retention time of Nicorandil	USP Plates	USP Tailing	% RSD of standard area	% Release	% Difference
Control	As per method	5.603	5549	1.20	0.02	101	-
Flow rate	1.3	6.456	5927	1.21	0.08	101	0.0
	1.7	4.966	5239	1.18	0.07	101	0.0
Wavelength	257	5.603	5540	1.20	0.11	101	0.0
	267	5.604	5519	1.20	0.12	101	0.0
Mobile phase composition (Buffer: ACN+MeOH)	68:32	5.862	5609	1.20	0.04	101	0.0
	72:28	6.074	5610	1.20	0.10	101	0.0
Column temperature	25°C	5.879	5121	1.18	0.12	100	1.0
	35°C	5.381	6000	1.20	0.12	101	0.0
Buffer pH	6.2	5.459	5622	1.21	0.10	101	0.0
	6.6	5.527	5794	1.15	0.09	101	0.0
Speed of rotation (rpm)	48	5.616	5597	1.20	0.04	101	0.0
	52	5.610	5574	1.19	0.03	101	0.0

9.4.10 Conclusions:

- The method has been shown to be specific for Nicorandil tablets.
- The method has been shown to be Linear, precise and accurate across the suitable analytical range and stability indicating.
- Solution has been shown to be stable for at least 24 hours when stored at 15°C.
- The method has been shown to be robust towards deliberate minor changes in the method parameters of both HPLC and Dissolution.
- The method can be used in quality control laboratory for release of production batches.