CHAPTER – 8

DETERMINATION OF ASSAY OF NICORANDIL IN TABLET DOSAGE FORM BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

CHAPTER – 8

Determination of Assay of Nicorandil in tablet dosage form by using reverse phase high performance liquid chromatography

8.0 INTRODUCTION

The objective was to develop a method for determination of assay of Nicorandil in tablet dosage form. The method 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 product.

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

8.1 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].
Table 8.1.1, in next page, denotes the method received from the API supplier.

Table 8.1.1: Assay – API supplier method for <u>Drug Substance</u>

	API Supplier - method
Method	HPLC and Titration
Column	C18 250mm x 4.6mm; 5µ;
Column Temp	Not Mentioned
Mobile Phase	70 parts of 0.01M disodium hydrogen phosphate and 30 parts of methanol; adjust pH to 7.0 with o-phosphoric acid
Gradient/ Run time	Three times of the main peak
Flow Rate	1ml/min
Wavelength	215nm
Inj Vol (µL)	20

8.2 PRESENT WORK AND DISCUSSION

8.2.1 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.

8.2.2 Selection of Stationary Phase

Different reversed phase column were used as stationary phase selection during column selection. It was decided to start with the column used by the API supplier and then work on the separation of placebo and other peaks. The desired separation was achieved using Inertsil C18 (ODS 3V) 250 x 4.6 mm, 5µm.

8.2.3 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.

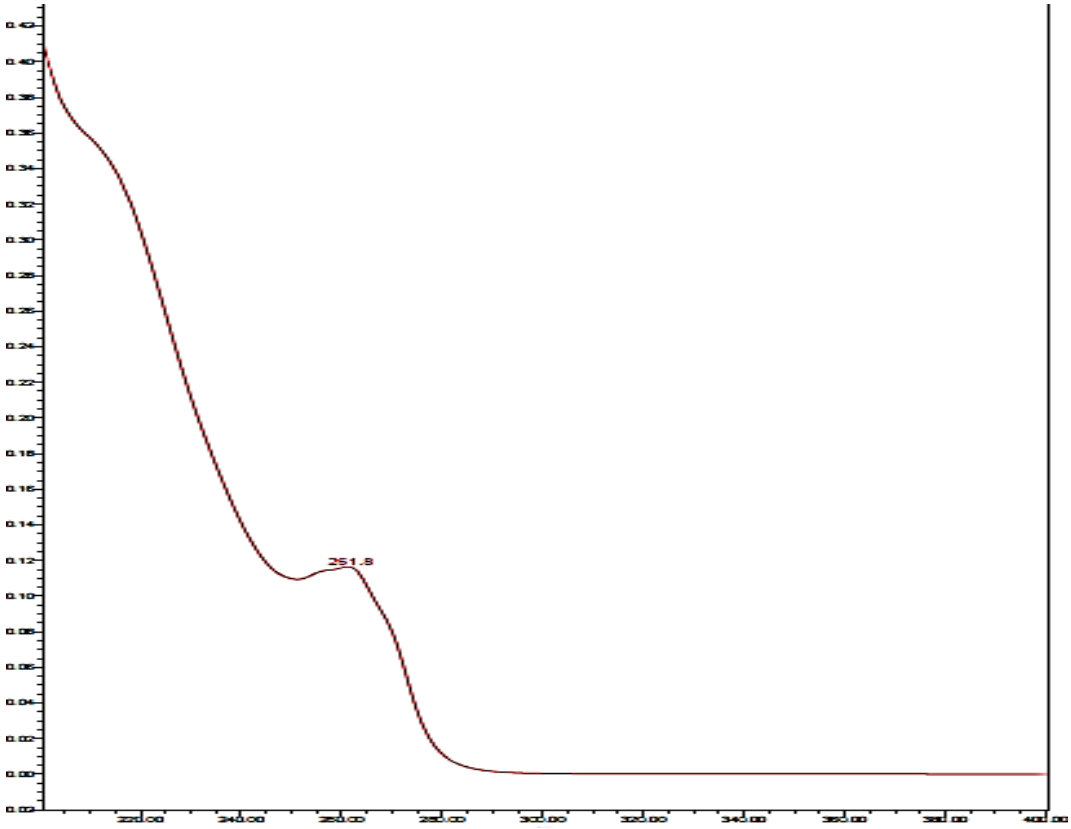


Figure: 8.2.3.1 UV spectrum of Nicorandil

8.2.4 Selection and Optimization of Mobile Phase

It was decided to move ahead with similar buffer system as used in the method for related substances since the degradants were separate from the main active using this combination of buffer and organic modifiers. However, an isocratic method was the target with a shorter run time. The final composition was an isocratic combination which was close to a level under which the Nicorandil peak elutes in the method for related substances.

Optimized Chromatographic Conditions:

Chromatographic conditions for assay:

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	:	20 µl
Run time	:	8 minutes
Retention time	:	approx. 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 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.

8.3 FORCED DEGRADATION STUDIES

The forced degradation studies were carried out to achieve adequate degradation of Nicorandil. They were carried out and chromatographed along with a non-stressed sample (control).

For degradation separate stock solution of the tablet and equivalent amount of placebo were prepared. They were further diluted as mentioned in the following sections.

Preparation of Sample stock solution:

10 tablets were weighed accurately and transferred into 200ml amber coloured volumetric flask. 150ml of diluent was added and sonnicated for 15 minutes. The solution was allowed to cool at room temperature. The volume was made up to the mark with diluent.

Preparation of Placebo Stock solution:

Placebo was weighed similarly and transferred into 200ml amber colour volumetric flask, 150ml of diluent and sonnicate it for 15 minutes; allow it to cool at room temperature. Make up the volume up to the mark with diluent.

8.3.1 Hydrolytic conditions: acid-, base-induced degradation.

Acid degradation

Sample preparation:

4.0 ml of above sample stock solution was transferred into 50 ml amber coloured volumetric flask. 2 ml of 5N Hydrochloric acid was added. The solution was heated on the water bath at 70°C for 3 hours; cooled, Neutralized with same volume and same strength alkali. The volume was made up with diluent and filtered through 0.45 µNylon filter.

Placebo preparation:

4.0 ml of above placebo stock solution was transferred into 50 ml amber coloured volumetric flask and treated similarly as sample.

Base Degradation

Sample preparation:

4.0 ml of above sample stock solution was transferred into 50 ml amber coloured volumetric flask. 2 ml of 0.5N Sodium hydroxide was added. The solution was heated on the water bath at 70°C for 1 minute; cooled, neutralized with same volume and same strength acid. The volume was made up with diluent and filtered through 0.45 µNylon filter.

Placebo preparation:

4.0 ml of above placebo stock solution was transferred into 50 ml amber coloured volumetric flask and treated similarly as sample.

8.3.2 Oxidative condition: hydrogen peroxide-induced degradation.

Sample preparation:

4.0 ml of above sample stock solution was transferred into 50 ml amber coloured volumetric flask. 1 ml of 50% Hydrogen peroxide was added. The solution was heated on the water bath at 70°C for 45 minutes; cooled.The volume was made up with diluent and filtered through 0.45 µNylon filter.

Placebo preparation:

4.0 ml of above placebo stock solution was transferred into 50 ml amber coloured volumetric flask and treated similarly as sample.

8.3.3 Thermal degradation study.

Sample preparation:

4.0 ml of the above sample stock solution was heated on the water bath at 70°C for 1 hour in 50 ml amber colour volumetric flask. This was allowed to cool and then diluted upto the mark with diluent. The sample solution was filtered through 0.45µ Nylon filter.

Placebo preparation:

4.0 ml of above placebo stock solution was transferred into 50 ml amber coloured volumetric flask and treated similarly as sample.

8.3.4 Photolytic degradation study.

As per guidelines for photostability testing of new drug substances and products, samples should be exposed to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200Wh/m2 to allow direct comparisons to be made between the drug substance and drug product. [19]

Sample preparation:

4.0ml of sample stock solution was exposed under UV and white light for 1.2 million lux hours in each of 50 ml of clear glass flask volumetric flask, amber colour 50 ml glass volumetric flask and flask covered with aluminium foil. The solutions were diluted to 50 ml with diluent and then filtered through 0.45µ Nylon filter.

Placebo preparation:

4.0ml of Placebo stock solution was exposed under UV and white light for 1.2 million lux hours in each 50 ml of glass flask volumetric flask, amber colour 50 ml glass volumetric flask and flask covered with aluminium foil. These solutions were diluted to 50 ml with diluent and then filtered through 0.45µ Nylon filter.

8.3.5 Observations in forced degradation studies.

It was observed that overall Nicorandil is a highly degradable molecule. It degraded in most conditions.

Under photolytic conditions the sample in clear flask showed degradation. However it was observed that the sample stored in amber coloured flask as well as the control sample (flask wrapped in aluminium foil) showed similar degradation. It was thus concluded that the degradation observed in photostabilty was due to inherent instability of the sample solution. Thus light had little effect on degradation.

Most importantly, in the point of view of method, in all conditions the peak due to Nicorandil has been found to be pure, thus proving the specificity of the method.

A short summary of the observation is given in the table 8.3.1.

Table: 8.3.5.1 Samples injected under different stress conditions

Conditions	% Assay	Purity angle	Purity threshold
Control	101.9	0.053	0.254
Acid degradation	87.6	0.067	0.258
Base degradation	88.1	0.056	0.254
Peroxide degradation	82.5	0.057	0.255
Heat	89.3	0.054	0.252
Photolytic study			
Aluminium Covered (Control)	76.3	0.085	0.294
Amber flask	75.9	0.075	0.273
Clear glass	74.8	0.118	0.344

8.4 EXPERIMENTAL WORK

8.4.1 Instrumentation

Equipment	Make	Model
HPLC	Waters	2695Alliance Separation Module, (PDA/UV Detector) 2996/2487
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	

8.4.2 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
Sodium Hydroxide	GR	Merck
Hydrochloric acid	GR	Merck
Hydrogen peroxide	GR	Merck
Water	HPLC milli-Q	In-house

8.4.3 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

8.4.4 Solution Preparation

Preparation of Standard solution:

Accurately weigh and transfer about 80.0 mg of Nicorandil standard and transfer into a 50 ml amber colour volumetric flask, add 30 ml of diluent, sonnicate to dissolve and make up the volume with diluent. Dilute 5ml of this solution to 100ml with diluent.

Preparation of Sample solution:

Weigh accurately 10 tablets and transfer these tablets into 200ml amber colour volumetric flask, add 150ml of diluent and sonnicate it for 15 minutes; allow it to cool at room

temperature. Make up the volume up to the mark with diluent. Dilute 4 ml of this solution to 50 ml with diluent. Filter the sample the sample solution through 0.45µ Nylon filter.

Preparation of Placebo solution:

Weigh accurately placebo similar to the sample and transfer these into 200ml amber colour volumetric flask, 150ml of diluent and sonnicate it for 15 minutes; allow it to cool at room temperature. Make up the volume up to the mark with diluent. Dilute 4 ml of this solution to 50 ml with diluent. Filter the sample the sample solution through 0.45µ Nylon filter. (use for only Validation)

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 4000.

Procedure:

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

Calculation:

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

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{50} \times \frac{5}{100} \times \frac{200}{10 \text{ Tab}} \times \frac{50}{4} \times \frac{P}{LC}$$

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.

8.5 VALIDATION OF THE DEVELOPED METHOD

8.5.1 Validation parameters and acceptance criteria

The Table 8.5.1.1 summarizes the validation acceptance criteria along with the obtained results.

Table: 8.5.1.1 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 4000.	 0.21 1.03 6516
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
2.4	Forced degradation	The peak due to Nicorandil should be pure as shown on the PDA.	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 8.5.1.1: Validation Summary (continued)

Sr. No.	Parameters	Acceptance criteria	Result obtained	
4.0	Accuracy (Recovery)	Mean recovery should be in the range of 98.0%- 102.0%.	Level %	% Mean Recovery
			50	99.3
			100	98.3
			150	98.7
5.0	System Precision % RSD for Standard solution USP Tailing USP Plate count	NMT 2.0% NMT 2.0 NLT 4000	0.21 1.03 6516	
5.1	Method Precision % RSD of six determinations	NMT 2.0%.	0.16	
5.2	Intermediate Precision (Ruggedness) % RSD for Standard solution	NMT 2.0%	0.06	
	USP Tailing	NMT 2.0	1.06	
	USP Plate count	NLT 4000.	10903	
	RSD for % Assay	NMT 2.0%.	0.08	
	Difference in pooled result (Analyst-I and II)	The difference in the means should not be more than ± 2.	1.5	

Table 8.5.1.1: Validation Summary (continued)

Sr. No.	Parameters	Acceptance criteria	Result obtained
6.0	Stability in analytical solution	The difference should not be more than ± 2.	Sample is stable upto 24 hours, stored at 15°C
7.0	Filter compatibility	The difference between centrifuged sample and filtered sample should not be more than ± 2.	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 2%.	No significant change. Compiles
	Change in wavelength (± 5 nm)	No significant change should be in System suitability parameters. % RSD should be less than 2%.	No significant change. Compiles
	Change in Buffer pH (± 0.2)	No significant change should be in System suitability parameters. % RSD should be less than 2%.	No significant change. Compiles
	Column oven temperature (± 5°C)	No significant change should be in System suitability parameters. % RSD should be less than 2%.	No significant change. Compiles
	Mobile phase composition (± 2%) absolute	No significant change should be in System suitability parameters. % RSD should be less than 2%.	No significant change. Compiles

8.5.2 System Suitability

Single injection of Blank (Diluent) and five replicate Standard solution were made on the system. The data demonstrate that the system suitability is within the acceptance criteria, thus the system is suitable. The data obtained is summarized in Table. 8.5.2.1.

Table 8.5.2.1: System suitability

Standard solution	
USP Tailing	**1.03**
USP Plates	**6516**
Area	**Standard solution**
	1015332
	1015632
	1014845
	1019325
	1018951
Mean	**1016817**
SD	**2141.393**
%RSD	**0.21**

8.5.3 Specificity:

The Specificity study included Identification of the main peak, Interference study and Peak Purity. Peak purity of degraded peak was also studied.

Injections of standard solution, sample solution and placebo, as a directed in experimental section, were made onto the HPLC. The data demonstrate that there is no interference in blank and placebo peaks with Nicorandil peak.

Purity angle is less than purity threshold for the main peak. The data obtained is summarized in Table 8.5.3.1.

Table 8.5.3.1: Specificity (Identification and Interference)

Component	Retention time (min)	USP Plates	USP Tailing	Purity angle	Purity threshold
Nicorandil	5.663	6516	1.03	0.054	0.254

Chromatograms of Blank (diluent), placebo, Standard solution and Sample solution are given below under figure 8.5.3.1, 8.6.3.2, 8.5.3.3 and 8.5.3.4 respectively

Figure-8.5.3.1: Chromatogram of Blank.

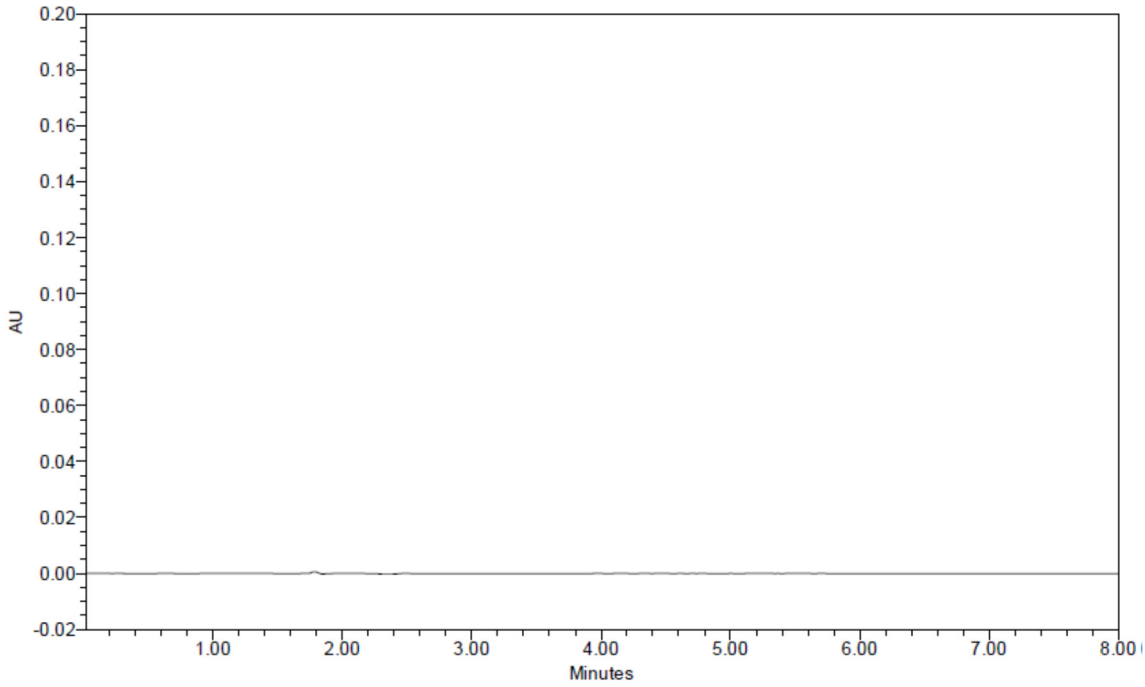


Figure-8.5.3.2: Chromatogram of Placebo.

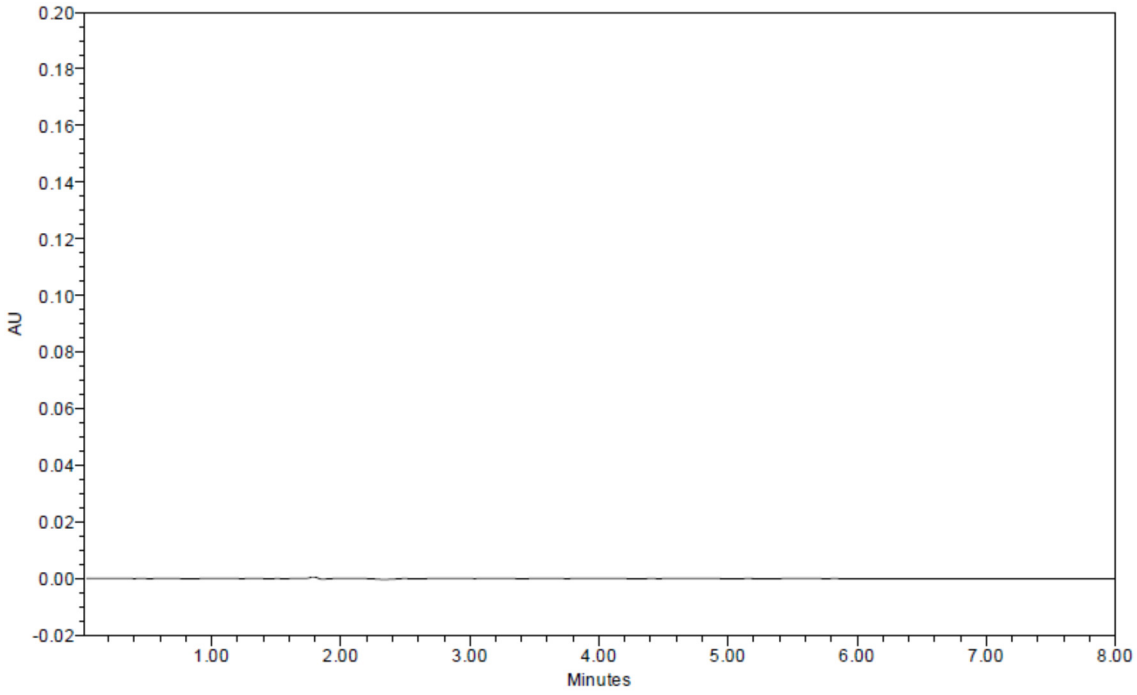


Figure-8.5.3.3: Chromatogram of Standard solution.

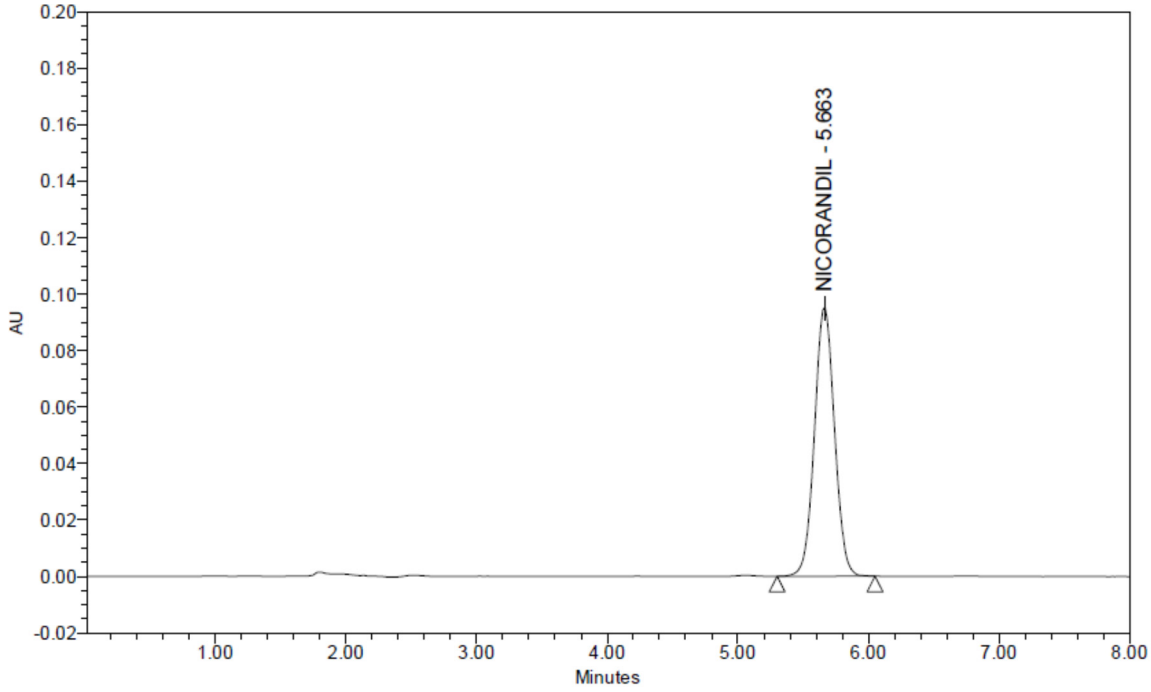
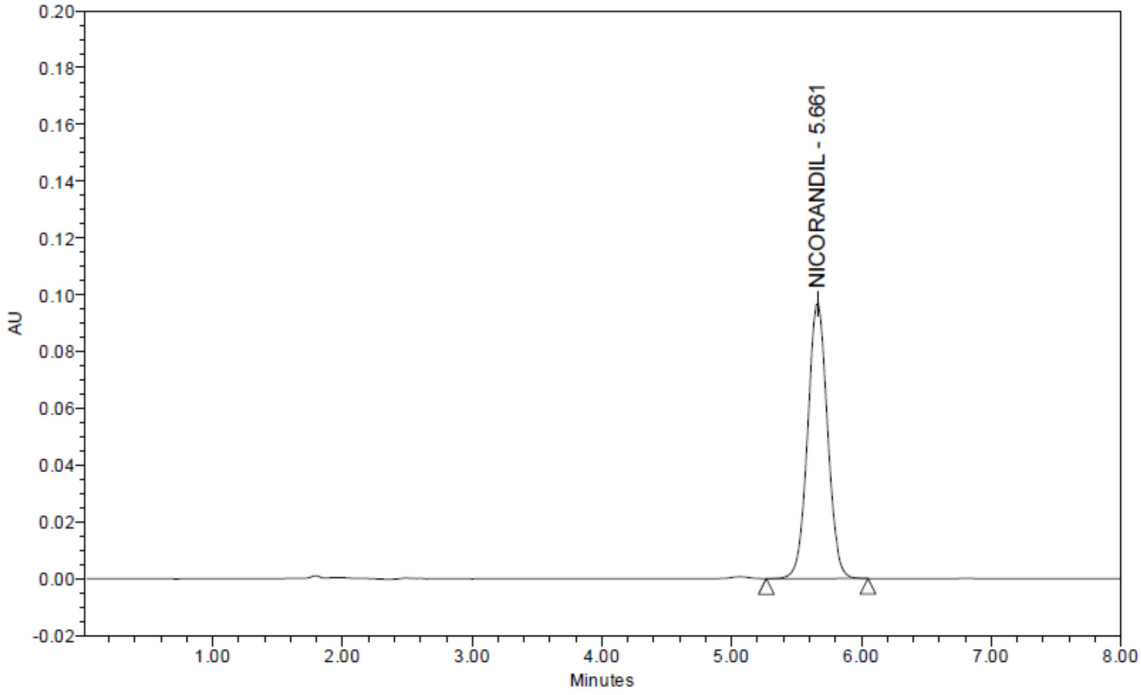


Figure-8.5.3.4: Chromatogram of Control Sample solution.



Forced degradation:

The purity angle was less than purity threshold, for Nicorandil peak, under all degradation conditions. The peak was pure and thus the method is stability indicating with respect to forced degradation studies.

8.5.4 Linearity and Range:

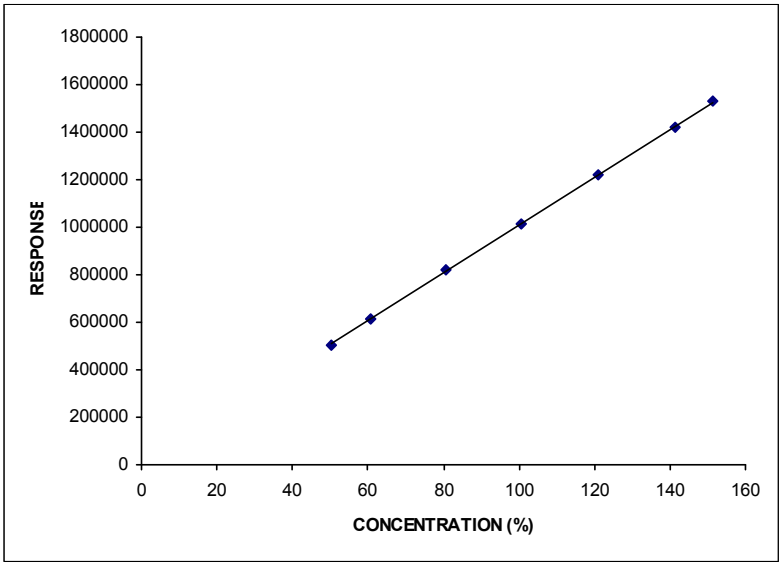
The Linearity of response was determined by preparing different concentrations of standard stock solution ranging from 50% to 150% of the working concentration. The data summarized in Table 8.5.4.1. The data shows that the response is found to be linear; Correlation coefficient is more than 0.999.

Table 8.5.4.1: Linearity of Nicorandil

Level	Concentration (%)	Response		
		1	2	Mean
1	50.4	505916	506842	506379
2	60.5	610927	610295	610611
3	80.7	820284	822170	821227
4	100.8	1011959	1010566	1011263
5	121.0	1219807	1218936	1219372
6	141.2	1418572	1416673	1417623
7	151.2	1526856	1529545	1528201
CORRELATION COEFFICIENT (r)				**0.9999**
SLOPE				**10066**
Y-INTERCEPT				**1491**
MEDIAN (AREA)				**1011263**
LIMIT OF Y-INTERCEPT ± 5% OF MEDIAN				**50563**

Graph of concentration v/s area is given in Figure 8.5.4.1

Figure 8.5.4.1 Linearity for Nicorandil.



8.5.5 Accuracy:

The standard solution was spiked into the placebo at three different level, 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 results obtained are summarized in Table 8.5.5.1. The data shows that the % mean recovery at each level is within the acceptance criteria.

Table 8.5.5.1: % Recovery

Level %	Mean response	% Recovery	Mean recovery %
50 %	465395	99.5	99.3
	506176	99.1	
	552018	99.3	
100 %	916639	98.0	98.3
	1004660	98.4	
	1097134	98.7	
150 %	1378761	98.3	98.7
	1512626	98.7	
	1651417	99.0	

8.5.6 Precision

8.5.6.1 System Precision:

Single injection of Blank (Diluent) and five replicate injections of standard solution were made on the system. Please refer to Table 8.5.2.1. All the data were acceptable as per the system suitability requirements.

8.5.6.2 Method Precision:

Six sample solutions were prepared and injected in duplicate on the HPLC. The data obtained is summarized in Table 8.5.6.2.1. The data shows that % RSD is well within the acceptance criteria.

Table 8.5.6.2.1: Method precision

Sample No.	Response			% Assay
	1	2	Mean	
1	1033771	1033660	1033716	101.9
2	1035164	1036606	1035885	102.1
3	1032371	1030681	1031526	101.7
4	1034842	1037523	1036183	102.1
5	1033528	1034255	1033892	101.9
6	1036091	1035955	1036023	102.1
Mean				**102.0**
SD				**0.163**
% RSD				**0.16**

8.5.6.3 Intermediate Precision (Ruggedness):

Same procedure of system precision and method precision is followed by another Analyst on different instrument and on different day. The data obtained from Analyst-II are summarized in Table 8.5.6.3.1 and 8.5.6.3.2. The data demonstrate that the system complies. Thus the system was suitable and precise and % RSD is within the acceptance criteria.

Table 8.5.6.3.1: System suitability

Standard solution	
USP Tailing	**1.06**
USP Plates	**10903**
Area	**Standard solution**
	1005475
	1005336
	1003891
	1004791
	1004887
Mean	**1004876**
SD	**622.088**
%RSD	**0.06**

Table 8.5.6.3.2: Intermediate precision

Spl. No.	**Response**			**% Assay**
	1	**2**	**Mean**	
1	1009818	1007835	1008827	100.4
2	1008268	1009462	1008865	100.4
3	1008388	1007781	1008085	100.4
4	1009015	1009284	1009150	100.5
5	1008269	1011088	1009679	100.5
6	1011232	1009764	1010498	100.6
Mean				**100.5**
SD				**0.082**
% RSD				**0.081**

The pooled data obtained from twelve independent samples by Analyst-I and Analyst-II is summarized in 8.5.6.3.3. The data shows that % difference is not more than ± 2.

Table 8.5.6.3.3: Pooled data

	Analyst I	**Analyst** II
% Assay	101.9	100.4
	102.1	100.4
	101.7	100.4
	102.1	100.5
	101.9	100.5
	102.1	100.6
Mean	**102.0**	**100.5**
% Difference between two means	**1.5**	

8.5.7 Stability in Analytical solution:

The sample solution was kept at sample temperature for 24 hours and was injected on to the HPLC time to time. The data obtained are summarized in Table 8.5.7.1. The data shows that % difference up to 24 hrs is less than ± 2, so sample solution is stable (at least) up to 24 hours at 15°C stored condition.

Table 8.5.7.1: Stability in analytical solution

Time	**% Assay**	**% Difference**
Initial (control)	101.9	-
2 hrs	101.8	0.1
6 hrs	102.0	0.1
12 hrs	102.3	0.4
18 hrs	102.5	0.6
24 hrs	102.3	0.4

8.5.8 Filter Compatibility:

The Sample solution centrifuged and filtered through different filters was injected on to the HPLC. The data obtained are summarized in Table 8.5.8.1. The data shows that % difference is not more than ± 2. Thus all filters tested were compatible with the sample.

Table 8.5.8.1 Filter compatibility

Filter	% Assay	% Difference
Centrifuged	101.7	-
Glass Filter	102.4	0.7
Nylon Filter	101.6	0.1
Nylon + Glass	102.0	0.3
PVDF	102.1	0.4

8.5.9 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 8.5.9.1.

Table 8.5.9.1: Robustness.

Changes in parameters	Values	Retention time of Nicorandil	USP Plates	USP Tailing	% RSD of standard area	% Assay	% RSD
Control	As per method	5.663	6516	1.03	0.21	101.9	-
Flow rate	1.3	6.525	6848	1.04	0.20	102.1	0.14
	1.7	5.010	6144	1.03	0.17	102.9	0.69
Wavelength	257	5.663	6516	1.03	0.22	101.8	0.07
	267	5.663	6521	1.03	0.20	101.7	0.14
Column temperature	25°C	5.936	5941	1.03	0.30	103.0	0.76
	35°C	5.435	7212	1.02	0.20	102.4	0.35
Buffer pH	6.2	5.505	10642	1.06	0.21	99.6	1.61
	6.6	5.366	10412	1.05	0.11	100.7	0.84
Mobile Phase composition Buffer: ACN+ MeOH	68:32	4.952	6310	1.05	0.20	100.9	0.70
	72:28	6.302	6816	1.04	0.09	99.8	1.47

8.5.10 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.
- The method can be used in quality control laboratory for release of production batches.