INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Research Article

Development And Validation Of Reverse Phase-High-Performance Liquid Chromatography Method For Nebivolol

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ARTICLE INFO

Received: 03 May 2024
Accepted: 07 May 2024
Published: 15 May 2024
Keywords:
Nebivolol, RP-HPLC, Acetonitrile, Methanol and Water.
DOI:
10.5281/zenodo.11195367

ABSTRACT

The methodology was set up for synchronous estimation of a Nebivolol by RP-HPLC system. The chromatographic conditions were viably created for the unit of Nebivolol by using Inertsil - ODS C18 (250 x 4.6 mm, 5µ), stream is 1.0 ml/min, convenient stage extent was Methanol: Water (55:45), recognizable proof wave length was 274nm.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and is one of the most important tools of analytical chemistry today. In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products. The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants. High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase.

The technique of HPLC has following features.

1. High resolution
2. Small diameter, Stainless steel, Glass column
3. Rapid analysis
4. Relatively higher mobile phase pressure
5. Controlled flow rate of mobile phase

HPLC Method Development

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeial) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.

Steps involved in Method development are

1. Understanding the Physicochemical properties of drug molecule
2. Selection of chromatographic conditions
3. Developing the approach of analysis
4. Sample preparation
5. Method optimization
6. Method validation

Materials and Methods

Instruments used:

1. HPLC – Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column
2. UV spectrophotometer (Systronics)
3. Electronic balance (SARTORIOUS)
4. Sonicator (FAST CLEAN)

Chemicals used:

- Methanol HPLC Grade
- Buffer (KH_2PO_4) HPLC Grade

Drug Profile:

NEBIVOLOL

Synonym:

Nebivolol, Nebivololum

Chemical Structure:

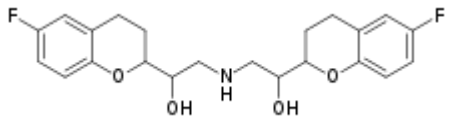

Fig.1 Chemical Structure of Nebivolol

IUPAC:

(1R)-1-[(2R)-6-fluoro-3,4-dihydro-2H-chromen-2-yl]-2-[[(2R)-2-[(2S)-6-fluoro-3,4-dihydro-2H-chromen-2-yl]-2-hydroxyethyl]amino]ethanol

Molecular Framework:

Aromatic heteromonocyclic compounds

Molecular formula:

$C_{22}H_{25}F_2NO_4$

Molecular weight:

405.4 g/mol

Monoisotopic:

405.17516460 g/mol

Physico Chemical Properties

Appearance:

Solid in nature

Solubility:

0.091g/100mL in water

Melting Point:

223.0-228.00C

pKa :

Strongest Acidic - 8.13

Strongest Basic - 8.22

log P: 4.183

EXPERIMENTAL WORK:

Stock and standard Working solution:

Nebivolol is used as working standard in this method development

Stock Solution Preparation:

Take 100 mg Nebivolol working standard in 100 ml Volumetric flask, add methanol up to the mark sonicate for 30 minutes (i.e. 1000 ppm solution).

Further Dilution (or) Trials Solution:

Take 10 ml of above solution in 100 ml Volumetric flask, add Methanol up to the mark sonicate for 10 minutes (i.e. 100 ppm solution)

Selection of Wave Length:

Scan standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Nebivolol shows λmax at 274nm.

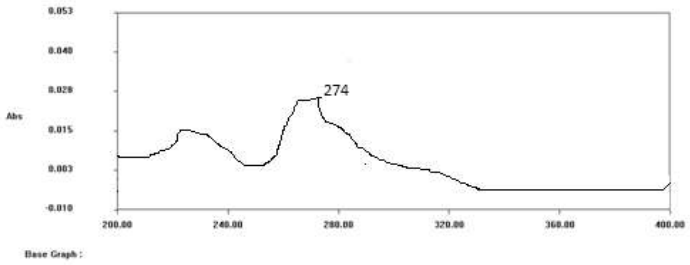

Fig.2 UV Spectrum of Nebivolol at 274nm

Development of HPLC Method:

The goal of this study was to improve the assay technique for simultaneous quantification of Nebivolol on literature surveys. As a result, the trials detailed below show how the optimization was accomplished.

Table.1 Chromatographic Conditions

Sr. No.	Trial	Mobile Phase	Name of the peak	Retention time (min)	Flow rate	Time to run	Tempo in the column
1.	1	Degassed Methanol: Water 65:35	Nebivolol	3.071	1.0ml/min	6min	Ambient
2.	2	Degassed Acetonitrile: methanol 50:50	Nebivolol	2.831	1.0ml/min	6min	Ambient
3.	3	Degassed Acetonitrile: methanol 35:65	Nebivolol	3.336	1.0ml/min	6min	Ambient

Method Validation:

System Suitability:

A Standard solution was prepared by using Nebivolol working standard as per test method and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Nebivolol retention times and peak areas.

Specificity:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Precision:

Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision:

Prepared five sample preparations individually using single as per test method and injected each solution.% relative standard deviation of individual Nebivolol, from the five units should be not more than 2.0%.The assay of Nebivolol should be not less than 98% and not more than 102.0%.

Intermediate precision:

The study was conducted by as per test method. The individual assays of Nebivolol should not be less than 98% and not more than 102% and %RSD of assay should be NMT 2.0%.

Repeatability:

Table.2 Data of Repeatability

	System precision			Method precision			Intermediate precision		
	Inj	**Peak Areas of Nebivolol**	**% Assay**	**Inj**	**Peak Areas of Nebivolol**	**% Assay**	**Inj**	**Peak Areas of Nebivolol**	**% Assay**
Con 40 ppm	1	1146923	99.65	1	1152293	99.55	1	1139272	98.80
	2	1143596	99.08	2	1146923	99.88	2	1140892	99.54
	3	1158293	99.98	3	1147283	99.40	3	1136601	99.98
	4	1147283	100.04	4	1152490	99.56	4	1141067	100.02
	5	1152490	100.16	5	1139272	99.85	5	1136024	101.08
Statistical Analysis	**Mean**	1149717	99.78	**Mean**	1147652	99.64	**Mean**	1138771	99.88
	SD	5754.015	0.435569	**SD**	5381.38	0.208254	**SD**	2359.88	0.829385
	% RSD	0.500	0.436	**% RSD**	0.469	0.209	**% RSD**	0.207	0.830

In this validation study the accuracy can be determined by calculating the following concentration such as 50%, 100% and 150%. The assay of Nebivolol should not be less than 98% and not more than 102.0%. The obtained results are shown in table.

Table.3 Data of Accuracy

Concentration % of spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	Statistical Analysis of % Recovery	
50% Injection 1	20	19.85	99.25	MEAN	99.88
50% Injection 2	20	19.96	99.80	**%RSD**	0.67
50% Injection 3	20	20.12	100.6		
100 % Injection 1	40	39.74	99.35	MEAN	99.81
100 % Injection 2	40	40.08	100.2	%RSD	0.399
100% Injection 3	40	40.24	100.6		
150% Injection 1	60	59.04	98.40	MEAN	99.19
150% Injection 2	60	59.62	99.36	**%RSD**	0.72
150% Injection 3	60	59.89	99.81		

Linearity:

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. The limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

Limit of Detection and Limit of Quantitation (LOD and LOQ):

From the linearity plot the SLOD and LOQ are calculated:

LOD = 3.3 σ/S

3.3×2431.578/31282 = 0.25

LOQ = 10 σ/S

10×2431.578/31282 = 0.77

Table.4 Data of Linearity

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	31282
20	523467	y-Intercept	11218

30	829544	Correlation Coefficient	0.999
40	1139272	---	---
50	1448018	---	---
60	1728926		
70	2089505	---	---
80	2407574	---	---

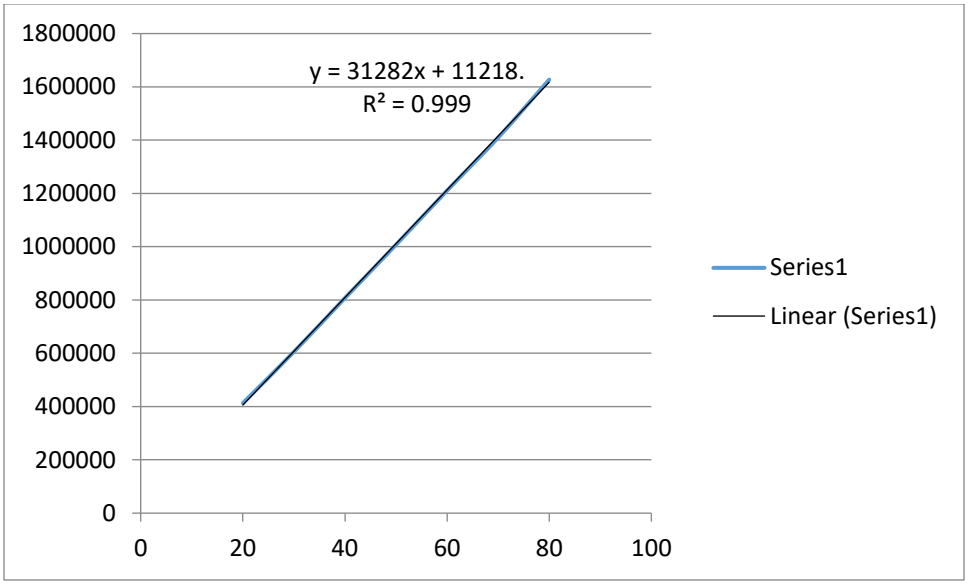

Fig.3 Linearity Plot (Concentration Vs Response)

Ruggedness:

System variability:

System variability study was conducted on HPLC system, under similar conditions at different times. Five samples were prepared and each was analyzed as per test method. The results obtained on HPLC system shows that the assay test method are rugged for System variability. The % relative standard deviation of Nebivolol from the five sample preparations should not be more than 2.0%. The % assay of Nebivolol should be between 98.0%-102.0%.

Table.5 Data of system variability (sample)

Sr. No	Peak area	Assay % of Nebivolol
1	1146923	99.65
2	1143596	99.08
3	1158293	99.98
4	1147283	100.04
5	1152490	100.16
Mean	1149717	99.78
%RSD	0.500	0.437

Robustness:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates 1.0 ml/min and 1.2 ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0 ml/min and 1.2 ml/min flow. Nebivolol was resolved from all other peaks and the retention

times were comparable with those obtained for mobile phase having flow rates 1.0ml/min. The Tailing Factor of Nebivolol standards should be NMT 2.0 for Variation in flow.

Table. 6 Data for Effect of variation in flow rate:

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1139272	1.238915		1146923	1.251658		1152293	1.262464
	1140892	1.230637		1143596	1.245435		1146923	1.251658
	1136301	1.240858		1158293	1.262464		1147283	1..237018
	1141067	1.238995		1147283	1.237018		1152490	1.239010
	1136024	1.241073		1152490	1.239010		1139272	1.238915
Avg	1138711	1.236496	**Avg**	1149717	1.247117	**Avg**	1148852	1.245813
SD	2431.578	0.005254	**SD**	5754.015	0.010328	**SD**	7076.841	0.010984
%RSD	0.213538	0.424907	**%RSD**	0.500472	0.008282	**%RSD**	0.615992	0.00881712

Marketed Sample Analysis:

Drug name	Brand name	Company
Nebivolol	Nebicip -5	Cipla

$$\% \text{ Assay} = \frac{\text{Amount found(x)}}{\text{Amount added}} \times 100$$

X=y-c/m

Table.6 Data for Marketed Sample

Injection	Peak Areas of Nebivolol	%Assay
1	1139524	99.50
2	1139863	99.63
3	1139007	99.03
4	1139241	99.59
5	1139034	99.77
Mean	1139334	99.504
SD	361.0813	0.282276
% RSD	0.031692	0.283684

FTIR

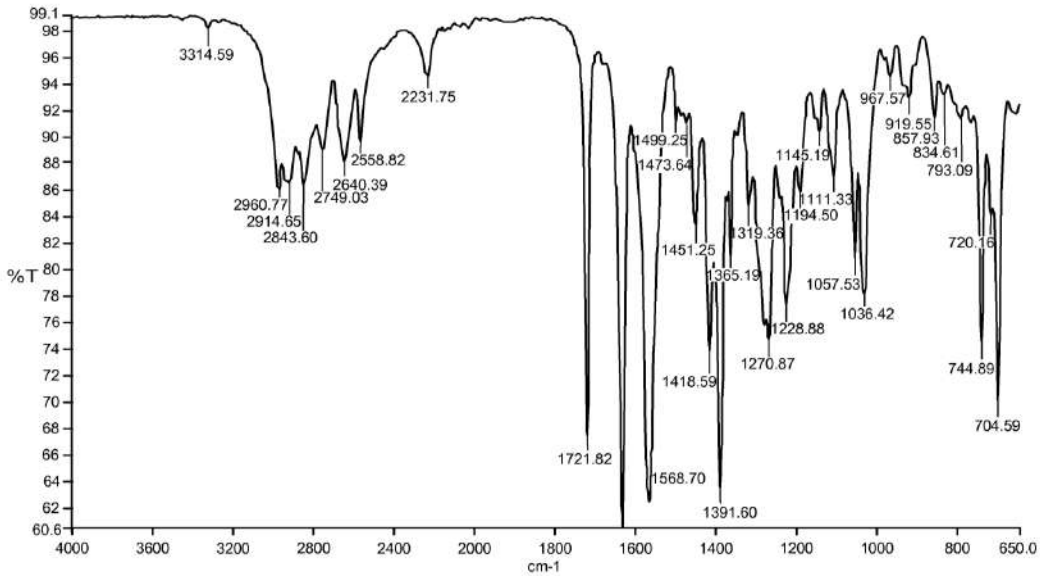

Fig.4 FTIR Spectra for Nebivolol

SUMMARY AND CONCLUSION:

Different parameters were studied to create the analytical approach. For starters, the maximum absorbance of Nebivolol was discovered to be 274nm. The injection volume was set at 20µl, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol: Water (55:45) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase. The accuracy of both the system and the procedure was determined to be precise and within the range. The correlation coefficient and curve fitting were discovered during the linearity investigation. The analytical approach of drug was shown to be linear throughout a range of 20-70ppm of the target concentration. The developed method was found to be rapid, accurate, precise, specific, robust and economical. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

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HOW TO CITE: S. P. Kumbhar, S. S. Patil , C. V. Panchal, P. M. Jadhav, Development And Validation Of Reverse Phase-High-Performance Liquid Chromatography Method For Nebivolol, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 5, 673-680. https://doi.org/10.5281/zenodo.11195367