IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Analytical Method Development And Validation For Simultaneous Estimation Of Nebivolol Hydrochloride And Ramipril In Synthetic Mixture By HPLC Method

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Abstract:

A novel, simple, precise, and rapid HPLC method was developed for the determination of simultaneous estimations of Nebivolol Hydrochloride and Ramipril in a synthetic mixture. The developed method was validated as per the ICH guidelines. The chromatographic separation was performed on an ODS C18 column measuring 25 cm (4.6 mm x 250 mm, 5 um) with Buffer: Methanol: Acetonitrile 20:60:20, pH adjusted to 2.8 as a mobile phase. The UV detection was carried out at 212 nm, and the flow rate was set at 1 ml/min. Injection volume is 10 µl and retention time was found to be 4.702 and 3.361 for Nebivolol hydrochloride and Ramipril respectively as a result, the linearity was found to be 25–200 µg/ml for Nebivolol Hydrochloride and 25–200 µg/ml for Ramipril. The correlation coefficient was found to be 0.9997 and 0.9993 for Nebivolol Hydrochloride and Ramipril, respectively. The limit of detection and limit of quantitation for Nebivolol Hydrochloride were found to be 1.910409 and 5.7891187, respectively, and Ramipril was found to be 4.356805 and 13.20244 respectively. The interday and intraday precision and accuracy were found to be ≤ 2% RSD for this method. This method has been successfully developed and validated.

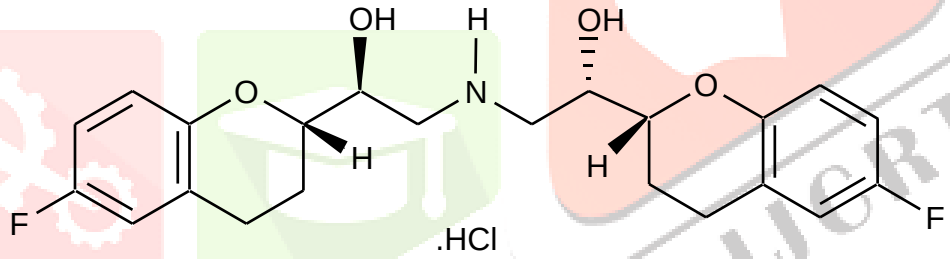
Key word: HPLC; Nebivolol Hydrochloride; Ramipril; validation; development; synthetic mixture

1. INTRODUCTION [1-4]

Nebivolol hydrochloride and Ramipril Anti-Hypertensive Drug which is used to treat high blood pressure. An abnormal elevation in the diastolic and/or systolic pressure is known as hypertension. Although it is rarely measured in humans, hypertension is also characterized by a higher mean arterial pressure. When evaluating hypertension in the past, the diastolic number was given special attention. Still, there is a correlation between elevated systolic pressure ("systolic hypertension") and a higher risk of coronary and cerebrovascular disease (e.g., stroke). As a result, we can now see the significance of noting both the systolic and diastolic pressure numbers. The most recent U.S. national guideline.

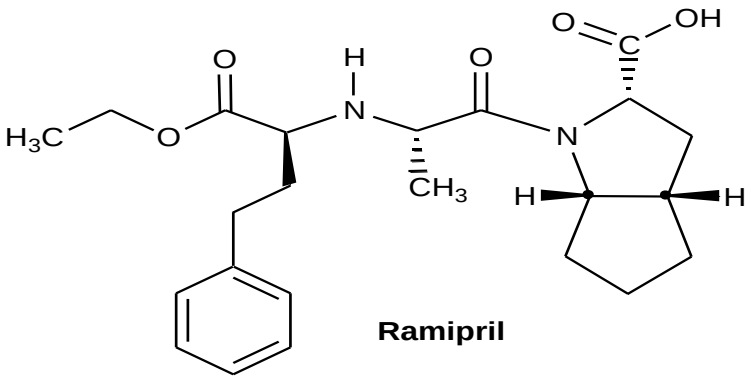
Nebivolol Hydrochloride is chemically known as 2,2'-iminobis [1-(6-fluoro-3,4-dihydro-2H-chromen-2-yl) ethanol] It has a molecular formula of $C_{22}H_{25}F_2NO_4$ with molecular weight 405.4 g/mol. The Nebivolol Hydrochloride category is Beta Blocker. It is soluble in methanol, sparingly soluble in ethanol, and very Slightly soluble in hexane. It has Melting Point between 223.0 to 228.0 °C. it is White crystalline powder and pKa is 8.1

Ramipril is chemically known as 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid. It has a molecular formula of $C_{23}H_{32}N_2O$ with molecular weight 416.5 g/mol. The Ramipril category is Angiotensin-converting enzyme (ACE) inhibitors. It is soluble Poorly in water, slightly soluble in methanol and very slightly soluble in ethanol. It has Melting Point between 105°C and 112°C. it is White crystalline substance and pKa is 5.16



Nebivolol Hydrochloride

Fig. No.1: Structure of nebivolol hydrochloride



Ramipril

Fig. No.2: Structure of nebivolol hydrochloride

Literature review revealed that methods are not developed and reported for determination of nebivolol hydrochloride and Ramipril in synthetic mixture. They are developed in combination with other drug but not with each other

HPLC(High Performance Liquid Chromatography):[5]

A type of liquid chromatography is high-pressure liquid chromatography, also referred to as high-performance liquid chromatography. It is a widely used analytical method for identifying, measuring, and isolating each constituent of a mixture. Column liquid chromatography comes in a more sophisticated form with HPLC. The solvent is normally moved through the column by gravity, but the HPLC method compresses the solvent at pressures as high as 400 atmospheres, enabling the sample to be divided into different constituents according to variations in relative affinities.

2. Experimental work:

- **Reagents and Material:**

Nebivolol hydrochloride API, Ramipril API, Methanol HPLC grad, Acetonitrile HPLC grade, Double distilled Water, Potassium Hydroxide, Ortho phosphoric acid

- **Preparation of stock solution:**

Accurately weighed 100 mg of Nebivolol hydrochloride and 100mg of Ramipril in 100ml of volumetric flask, 50 ml of methanol was added and sonicated to dissolve. Volume was making up to the mark with methanol. Concentration of Nebivolol hydrochloride is 100 µg/ml and Ramipril 100 µg/ml.

Take 1ml of above solution and transferred into 10 ml volumetric flask add methanol and sonicate for 10min and diluted up to the mark with methanol to give concentration for Nebivolol hydrochloride 100 µg/ml and Ramipril 100 µg/ml.

- **Preparation of buffer:**

Dissolve 5.04 g disodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate in sufficient water to produce 1000 ml. Adjust the pH with glacial acetic acid.

❖ **The final optimized mobile phase is given below:**

Sr.no	Parameter	condition
1.	Mobile phase	Buffer: Methanol: Acetonitrile (20:60:20)(2.8)
2.	Flow Rate	1ml/min
3.	Run time	15min
4.	Volume of injection	20 µl
5.	Detection of wavelength	212nm
6.	Detector	PDA detector
7.	Column Temperature	35°C

Table No.1: Optimization of Mobile Phase

3.Method Development: [6-7]

For the analysis of novel products, new methods are being developed when there are no official methods available. Innovative techniques are created to examine current pharmacopoeia- or non-pharmacopoeia-approved items in order to save costs while improving robustness and precision. Trial runs are used to optimize and validate these procedures. With all the available benefits and drawbacks, alternative approaches are suggested and implemented to replace the current strategy in the comparative laboratory data.

Analytical chemistry, which encompasses techniques to detect, isolate, and quantify the chemical components of pharmaceutical substances, is the foundation for method development. The purpose of analytical techniques is to determine the identification, potency, purity, and physical attributes of pharmaceuticals.

4. Result and Discussion:

❖ **Method validation:** [8-11]

The analytical method must be validated before the chemical evaluation can be performed. Method validation involves carrying out a series of tests to confirm that an analytical test system is suitable for its intended use and capable of delivering relevant and reliable analytical data. A validation examination tests multiple features of a procedure to see if they can yield accurate information when applied automatically. In order to effectively evaluate method parameters, the validation test should include typical test circumstances such as product excipients. As a result, a technique validation analysis is product-specific.

1.LINEARTIY: The linearity study was recorded in concentration 25, 50, 100, 150, and 200 µg/ml Nebivolol Hydrochloride and 25, 50, 100, 150, and 200 µg/ml Ramipril. and then its linearity by plotting calibration curve of peak area versus concentration.

Nebivolol Hydrochloride		Ramipril	
Conc.(µg/ml)	**Area**	**Conc.(µg/ml)**	**Area**
25	350800	**25**	349890
50	733606	**50**	682338
100	1596071	**100**	1289664
150	2479537	**150**	1920540
200	3282491	**200**	2473220

Table No.2: Linearity of Nebivolol Hydrochloride and Ramipril

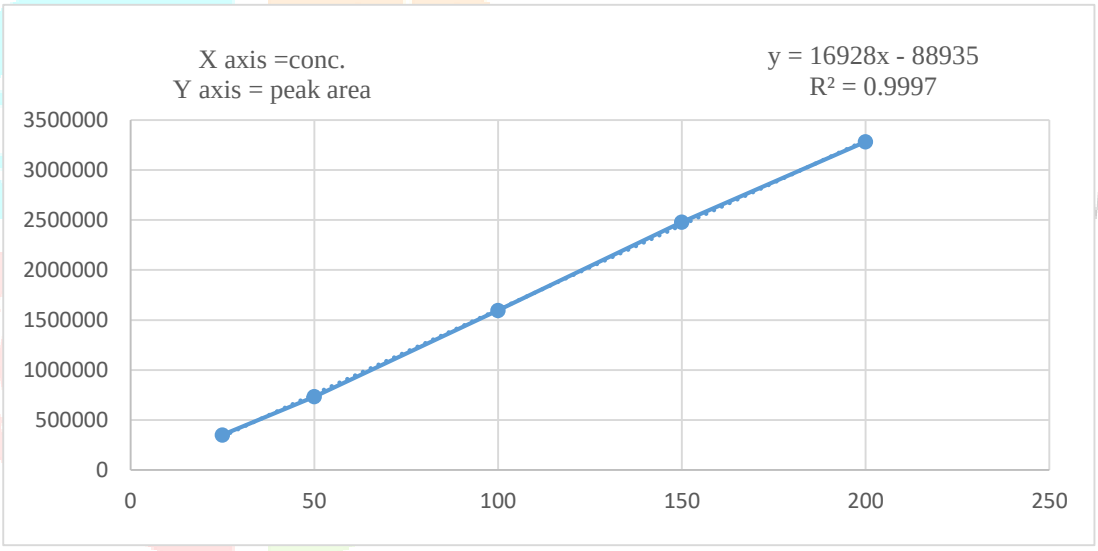


Fig. No.3: Calibration Curve of Nebivolol Hydrochloride HPLC method

Regression Equation Data Y= mx+c	
Slope(m)	16928
Intercept(c)	- 88935
Correlation Coefficient	0.9997

Table No.3: Regression equation data by HPLC method

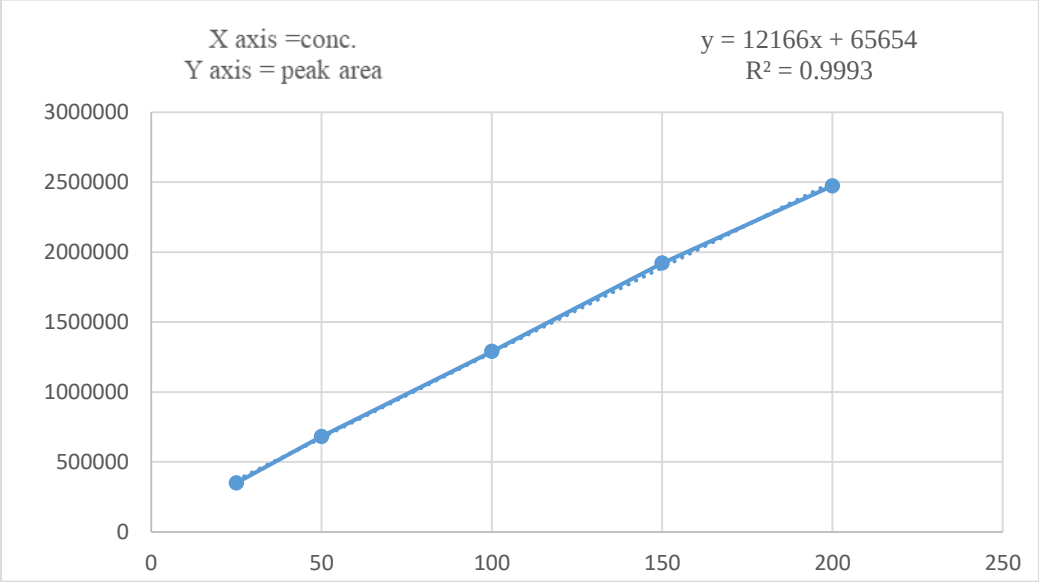


Fig. No.4: Calibration Curve of Ramipril HPLC method

Regression Equation Data Y=mx+c	
Slope(m)	12166
Intercept(c)	65654
Correlation Coefficient	0.9993

Table No.4: Regression equation data by HPLC method

The data obtained in the calibration curve when subjected to linear regression analysis showed a linear relationship between peak area and concentrations in the range 25, 50, 100, 150, and 200 µg/ml for Nebivolol Hydrochloride and Ramipril. The linear equation was y = 16928x - 88935 and y = 12166x + 65654 for Nebivolol Hydrochloride and Ramipril respectively. the correlation coefficient was found to be 0.9997 and 0.9993 of Nebivolol Hydrochloride and Ramipril respectively.

2.REPEATABILITY:

Repeatability is determined by taking solution of nebivolol hydrochloride and Ramipril containing 100 µg/ml and 100 µg/ml respectively which is then analysed six times. The %RSD was found to be 0.3236223for nebivolol hydrochloride and 0.92292 for Ramipril. Both the solution has %RSD value less than ±2, hence we can say that the method is precise.

Drug	Concentration (µg/ml)	Mean area ± SD	%R.S.D
Nebivolol Hydrochloride	100	1607350±14834.56	0.92
Ramipril	100	1303045±15778.62	1.21

Table No.5: Repeatability Studies by HPLC Method

a) Intraday:

The Intraday Precision was calculated by Analyzing solution containing concentration 25,100, and 200 %RSD for Nebivolol hydrochloride and 25,100, and 200 %RSD for Ramipril and three replicate each on same day using developed HPLC method and %RSD value is calculated. The %RSD was found to be 0.3-0.04 for nebivolol hydrochloride and 1.1-0.1 for Ramipril. Both the solution has %RSD value less than ±2, hence we can say that the method is precise.

Precision		Intraday precision	
Drugs	**(%)**	**Mean area ± SD**	**%RSD**
Nebivolol hydrochloride	25	349273.7 ± 3921.613	1.12
	100	1612218± 19619.96	1.22
	200	3288274 ± 4223.054	0.13
Ramipril	25	350923.3 ± 1095.699	0.31
	100	1298959 ± 11423.17	0.88
	200	2490558±24519.82	0.98

Table No.6: Intraday Precision data for Nebivolol Hydrochloride and Ramipril

b) Interday:

The Interday Precision was calculated by Analyzing solution containing concentration 25,100, and 200 %RSD for Nebivolol hydrochloride and 25,100, and 200 %RSD for Ramipril and three replicate each on different day using developed HPLC method and %RSD value is calculated. The %RSD was found to be 0.3-0.04 for nebivolol hydrochloride and 0.6-0.2 for Ramipril. Both the solution has %RSD value less than ±2, hence we can say that the method is precise.

Precision		Interday precision	
Drugs	**(%)**	**Mean area ± SD**	**%RSD**
Nebivolol hydrochloride	25	353605.3± 2286.354	0.65
	100	1613200 ± 18679.94	1.16
	200	3299447± 54802.83	1.66
Ramipril	25	355652.7 ± 1216.173	0.34
	100	1311396± 18552.26	1.41
	200	2499039±22331.92	0.89

Table No.7: Interday Precision data for Nebivolol Hydrochloride and Ramipril

3. ACCURACY:

Accuracy of method is performed at three different percentage i.e,50%,100%,150%. The percentage recovery for Nebivolol Hydrochloride and Ramipril was found to be in a range of 99-102%

Level	Target Conc (µg/ml)	Spiked Conc (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	%Recovery
50%	50	50	100	1623101	101.1363	101.1363
	50	50	100	1602243	99.90418	99.90418
	50	50	100	1597861	99.64532	99.64532
100%	50	100	150	2478504	151.6682	101.1121
	50	100	150	2489487	152.317	101.5447
	50	100	150	2490537	152.379	101.586
150%	50	150	200	3270019	198.4259	99.21296
	50	150	200	3290384	199.629	99.81448
	50	150	200	3272463	198.5703	99.28515

Table No.8: Recovery Data for Nebivolol Hydrochloride by HPLC Method

Level	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. (µg/ml)	Area	Conc. Found (µg/ml)	%Recovery
50%	50	50	100	1297041	101.2154	101.2154
	50	50	100	1298634	101.3464	101.3464
	50	50	100	1299555	101.4221	101.4221
100%	50	100	150	1928317	153.104	102.0693
	50	100	150	1929910	153.2349	102.1566
	50	100	150	1921177	152.5171	101.6781
150%	50	150	200	2480997	198.5322	99.26611
	50	150	200	2482790	198.6796	99.3398
	50	150	200	2483711	198.7553	99.37765

Table No.9: Recovery Data for Ramipril by HPLC Method

4. ROBUSTNESS:

The changes are made in different parameters like flow rate, wavelength and mobile phase and %RSD value is calculated. all the parameters %RSD value was found to be less than 2, hence we can say the method is robust.

Drugs	Wavelength	Mean area ± SD	%RSD
Nebivolol Hydrochloride	208	1590782 ±10955.64	0.69
	212	1590828±16325.74	1.03
	216	1590859 ± 19097.27	1.20
Ramipril	208	1281750±10911.07	0.85
	212	1281769±12973.32	1.01
	216	1273168±11426.92	0.90

Table No.10: Robustness study of Nebivolol Hydrochloride and Ramipril (change in wavelength)

Drugs	Flowrate	Mean area ± SD	%RSD
Nebivolol Hydrochloride	0.9 ml/min.	1598617 ±7110.533	0.44
	1ml/min.	1590828±16325.74	1.03
	1.1ml/min.	1604294± 26509.09	1.65
Ramipril	0.9 ml/min.	1272738±11986.04	0.94
	1ml/min.	1281769±12973.32	1.01
	1.1ml/min	1285195±6039.216	0.47

Table No.11: Robustness study of Nebivolol Hydrochloride and Ramipril (change in flow rate)

Drugs	Mobile phase	Mean area ± SD	%RSD
Nebivolol Hydrochloride	BMA(15:60:25)	1593291 ±10740.12	0.67
	BMA(20:60:20)	1590828±16325.74	1.03
	BMA(15:65:20)	1606974± 23725.96	1.48
Ramipril	BMA(15:60:25)	1270589±8952.272	0.70
	BMA(20:60:20)	1281769±12973.32	1.01
	BMA(15:65:20)	1284335±7255.877	0.56

Table No.12: Robustness study of Nebivolol Hydrochloride and Ramipril (change in mobile phase)

5.LOD: The Limit of Detection for Nebivolol Hydrochloride and Ramipril were found to be 1.910409 and 4.356805 respectively.

Limit of Detection: (Nebivolol Hydrochloride)

LOD: $LOD=3.3\times\sigma/S$

= 3.3× 9834.986/16988.75

LOD = 1.910409

Where, σ = Standard deviation of Y intercept

S = Slope

Limit of Detection: (Ramipril)

LOD: $LOD=3.3\times\sigma/S$

= 3.3× 15755.25/11933.59

LOD = 4.356805

Where, σ = Standard deviation of Y intercept

S = Slope

6.LOQ: The Limit of Quantitation for Nebivolol Hydrochloride and Ramipril were found to be 5.789117 and 13.20244 respectively.

Limit of Quantitation: (Nebivolol Hydrochloride)

LOQ: $LOQ=10\times\sigma/S$

= 10 × 9834.986/16988.75

LOQ = 5.789117

Where, σ = Standard deviation Y intercept

S = Slope

Limit of Quantitation: (Ramipril)

LOQ: $LOQ = 10 \times \sigma / S$

= 10 × 15755.25/ 11933.59

LOQ = 13.20244

Where, σ = Standard deviation Y intercept

S = Slope

7. Specificity:

The specificity of the method was determined by analyzing standard drugs and sample of Nebivolol Hydrochloride and Ramipril. The excipient present in the synthetic mixture does not interference in the result. So, the results suggested that proposed method is specific.

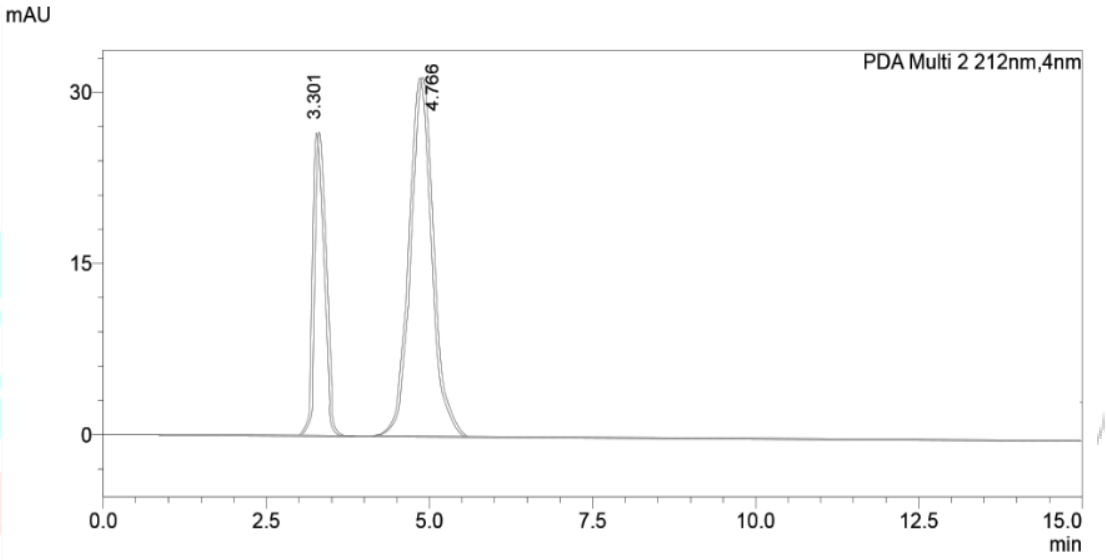


Fig.No.5: Chromatogram of Standard and Test from Synthetic Mixture of Nebivolol Hydrochloride and Ramipril

Conclusion:

The relevance of establishing HPLC procedures is enormous in the field of drug discovery and the pharmaceutical sector. These created RP-HPLC techniques have shown to be very precise, reliable, and simple. The statistical analysis has demonstrated that the validation parameters fulfill the necessary standards after rigorous validation in compliance with ICH recommendations, indicating their high level of reliability. The approaches' ability to do away with preparatory steps like extraction simplifies the analytical process is an added benefit. According to the results above, every parameter has been verified to fulfill the pre-established acceptance criteria and has been deemed satisfactory.

Thus, we conclude that the suggested approach has been demonstrated to be adequate, simple, precise, and accurate.

Abbreviations: HPLC: High Performance Liquid Chromatography; ICH: International Council for Harmonization; OPA: Orthophosphoric Acid; LOD: Limit of Detection; LOQ: Limit of Quantitation; SD: Standard Deviation RSD: Relative Standard Deviation; R2 -Correlation coefficient; µL – Microliter; mL-Millilitre; µg – Microgram; Mg – Milligram; G–Gram; BMA-buffer: methanol: acetonitrile.

Acknowledgement:

I would like to express my sincere gratitude to P. S. G. V. P. M's College of Pharmacy for providing all the necessary requirements.

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