ORIGINAL RESEARCH

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"DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NEBIVOLOL AND VALSARTAN"

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ABSTRACT—In the present research work, an attempt was made for "Development and validation of RP-HPLC method for the simultaneous estimation of nebivolol and valsartan". The method of estimation was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling.

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in tablet formulation.

The proposed method was found to be linear in the range of **2.5-12.5µg/ml** for nebivolol **and 40-200µg/ml** for valsartan with **correlation coefficient 0.999.** The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (50%, 100% 150%)was ranging from **99.6 to 100.13%** for nebivolol and **99.75 to 99.875%** for valsartan**.**

Liquid chromatographic system from Shimadzu comprising of manual injector, quaternary pump for constant flow and constant pressure delivery and Photodiode array detector SP 20 connected to software LC Solutions for controlling the instrumentation as well as processing the data generated were used. The isocratic mobile phase consisted of Isopropyl alcohol : water in the ratio of 80:20v/v at a flow rate of 1.0 ml/min. A Prontosil C-18 column (4.6 x 250mm, 5µ particle size) was used as the stationary phase, 278 nm was selected as the detection wavelength for UV-PDA detector.

Precision was determined by repeatability, Intermediate precision and reproducibility of the results. The robustness of developed method was checked by changing in the deliberate variation in solvent composition as well as flow rate.

The RP-HPLC methods were developed to estimate nebivolol and valsartan was validated as per the ICH norms.

KEYWORDS: **Valsartan**, **Nebivolol, Validation, HPLC.**

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INTRODUCTION

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

There may not be a suitable method for a particular analyzes in the specific sample matrix.

- Existing methods may be too expensive, time consuming, or energy intensive, or they may not be easily automated.
- Existing methods may not provide adequate sensitivity or analyzes selectivity in samples of interest
- Existing methods may be too error, artifact, and/or contamination-prone, or they may be unreliable (have poor accuracy or precision).
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including improved analyzes identification or detection limits, greater accuracy or precision, or better return on investment.

Development and validation protocol of an operating procedure or a validation master plan for the validation is as:

- For a specific validation project define owners and responsibilities
- Develop a validation project plan
- Define the application, purpose and scope of the method
- Define the performance parameters and acceptance criteria
- Define validation experiments
- Verify relevant performance characteristics of equipment
- Qualify materials, e.g. standards and reagents for purity, accurate amounts and sufficient stability
- Perform pre-validation experiments
- Adjust method parameters or/and acceptance criteria if necessary
- Perform full internal (and external) validation experiments
- Develop SOP for executing the method in the routine
- Define criteria for revalidation
- Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine

Introduction to chromatography

Chromatography is probably the most powerful and versatile technique available to the modern analyst. In a single step process it can separate a mixture into its individual components and simultaneously provide a quantitative estimate of eachconstituent. Samples may be gaseous, liquid or solid in nature and can range in complexity from a simple blend of two entantiomers to a multi component mixture containing widely differing chemical species. Furthermore the analysis can be carried out at one extreme on a very costly and complex instrument and at the other on a simple, inexpensive thin layer plateSome Common Mistakes

Modes of Chromatography

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces orbasedon the size of the particles. Different modes of chromatography are as follows.

- Normal Phase Chromatography
- Reversed Phase Chromatography
- Reversed Phase – Ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography
- Size Exclusion Chromatography

HPLC Method Development:

High-performance liquid chromatography or commonly known as HPLC, is an analytical technique used to separate, identify or quantify each component in a mixture.The mixture is separated using the basic principle of column **chromatography** and then identified and quantified by spectroscopy.In the 1960s, the column chromatography LC with its low-pressure suitable glass columns was further developed to the HPLC with its high-pressure adapted metal columns.HPLC is thus basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.

During the optimization stage the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts asymmetry, capacity, elution time, detection limits, limit of Quantization, and overall ability to quantify the specific analytic of interest.Method development in HPLC is a complex process that involves a number of steps which are as follows:

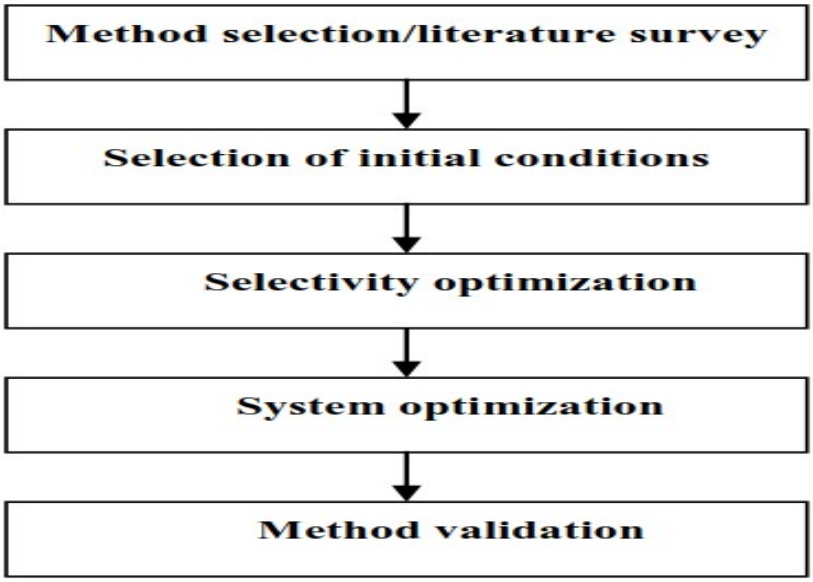


Figure 1.1: Flow Chart of HPLC Method Development

Method Optimization:

During the optimization stage the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts asymmetry, capacity, elution time, detection limits, limit of Quantitation, and overall ability to quantify the specific analyte of interest.

Parameters to be optimized

The various parameters that include to be optimized during method development

1. Mode of separation
2. Selection of stationary phase
3. Selection of mobile phase
4. Selection of detector

Selection of Stationary Phase / Column

Selection of the column is the first and the most important step in method development. The appropriate choice of separation column includes three different approaches

- Selection of separation system
- The particle size and the nature of the column packing
- The physical parameters of the column i.e. the length and the diameterSome of the important parameters considered while selecting chromatographic columns are:
- Length and diameter of the column
- Packing material
- Shape of the particles
- Size of the particles
- % of Carbon loading
- Pore volume
- Surface area
- End capping

Selection of Mode of Separation

In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A second factor is the nature of the matrix.

Selection of Mobile Phase

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from each other and from analyte peak.

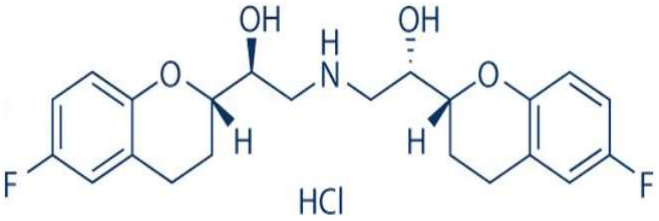
DRUG PROFILE

1. Nebivolol

Description

Nebivolol is a highly cardioselectivevasodilatory beta-1 receptor blocker used in treatment of hypertension. In most countries, this medication is available only by prescription.

Structure



Molecular Weight : 405.44

Chemical Formula : $C_{22}H_{25}F_2NO_4$

Toxicity : The most common signs and symptoms associated with nebivololoverdosage are bradycardia

and hypotension. Other important adverse events reported with nebivolol overdose include

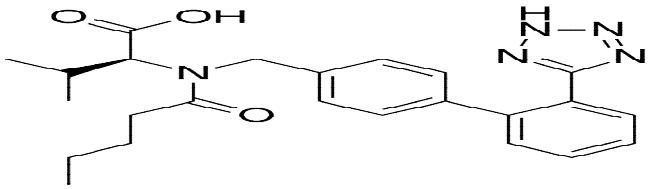
cardiac failure, dizziness, hypoglycemia, fatigue and vomiting.

IUPAC Name : 1-(6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2-{[2-(6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2-hydroxyethyl]amino}ethan-1-ol

2. Valsartan

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure.

Structure



Molecular Weight : 435.53

Chemical Formula : $C_{24}H_{29}N_5O_3$

IUPAC Name : (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl) pentanamido]butanoic acid

Route of Elimination : 83% of absorbed valsartan is excreted in feces and 13% is excreted in urine, primarily as unchanged drug

Clearance : 2 L/h [IV administration]4.5 L/h [heart Failure patients receiving oral administration 40 to 160 mg twice a day]

RESULT

Table : 1.1 INSTRUMENT USED

HPLC	Shimadzu LC20 equipped withRheodyne injector with a 20-microlitre loop, PDA detector, LC Solutions software and Hypersil C-18 column (4.6 x 250 mm, 5µm particle size)
Balance	Wensar
Sonicator	Labman

Table : 1.2 CHEMICALS USED

Chemicals/Reagents	Grade	Company
Isopropyl alcohol	HPLC	Merck
Methanol	HPLC	Merck
Water	HPLC	Milli-Q
Acetonitrile	HPLC	Merck
DMSO	LR	SD Fine
Ortho phosphoric acid	AR	SD Fine

Table : 1.3 Solubilityof nebivolol and valsartan in Different Solvents

SOLVENT	SOLUBILITY	
	Nebivolol	Valsartan
Water	Sparingly soluble	Sparingly soluble
Methanol	Freely Soluble	Soluble
Isopropyl alcohol	Soluble	Soluble
Acetonitrile	Soluble	Soluble
DMSO	Freely Soluble	Freely soluble

LINEARITY AND CALIBRATION GRAPH

The representative chromatogram obtained from injecting the standard solution and elution of the drug.

Table 1.4: Linearity of Nebivolol

Concentration (mg/ml)	Area under curve						Mean
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	
0	0	0	0	0	0	0	0
2.5	21235	21301	21225	21257	21271	21230	21253.2
5	42515	42480	42450	42501	42612	42597	42525.8
7.5	63657	63781	63718	63815	63799	63701	63745.2
10	84995	85018	84962	84930	84687	84540	84855.3
12.5	106008	106250	106165	106089	106318	106170	106167
CorrelCoeff (r2)	0.9997	0.9997	0.9998	0.9997	0.9997	0.9998	0.99973
Slope (m)	137.4	136.7	137.2	137.5	137	136.6	137.067
Intercept (c)	21.83	21.13	23.19	22.42	23.91	23.47	22.6583

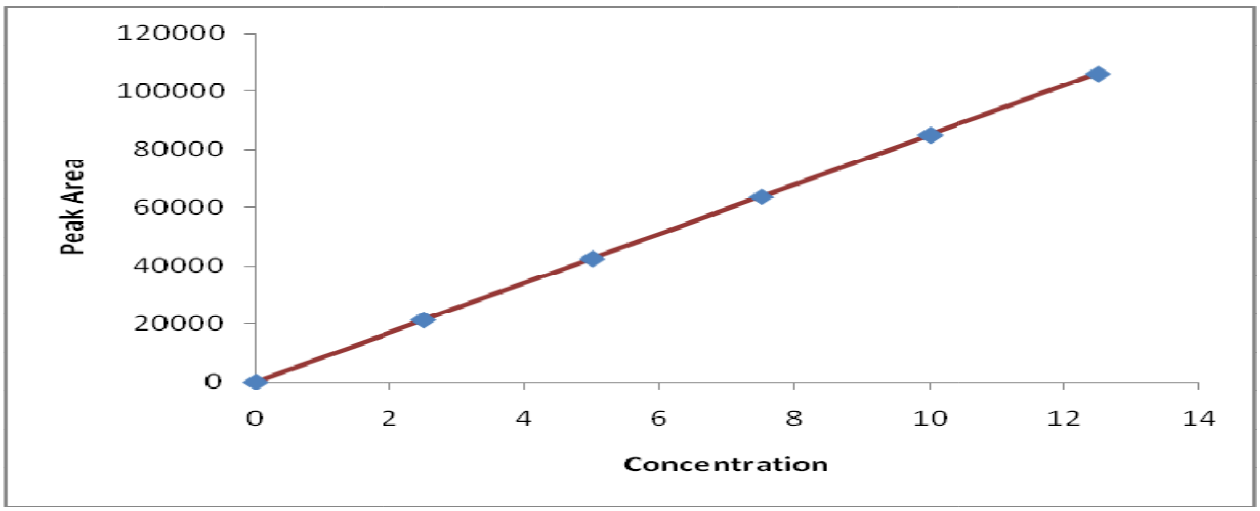


Figure 1.2 : Calibration Curve of Nebivolol

Table 1.5: Linearity of Valsartan

Concentration (mcg/ml)	Area under curve						Mean
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	
0	0	0	0	0	0	0	0
40	127410	127806	127350	127542	127626	127380	127519
80	250838.5	250632	250455	250756	251410.8	251322.3	250902
120	379395.7	380134.8	379759.3	380337	380242	379658	379921
160	497220.8	497355.3	497027.7	496841	495419	494559	496404
200	636048	637500	636990	636534	637908	637020	637000
CorrelCoeff (r2)	0.9998	0.9998	0.9996	0.9997	0.9996	0.9998	0.99972
Slope (m)	5160	5148	5134	5186	5190	5108	5154.33
Intercept (c)	317	335	313	311	327	330	322.167

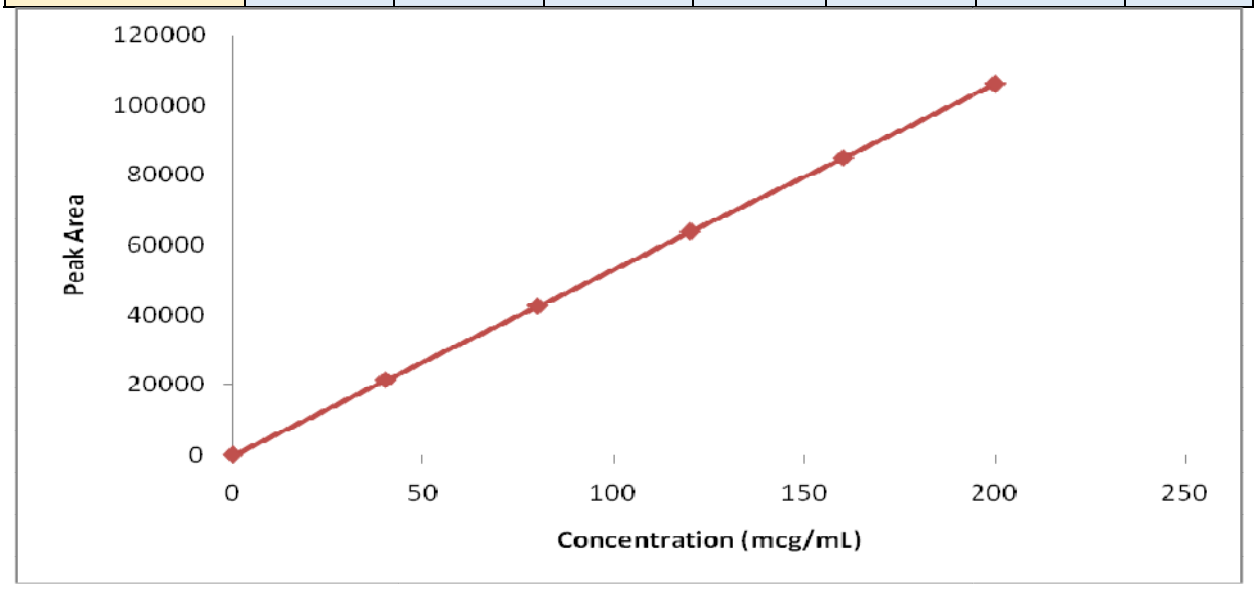


Figure 1.3: Calibration Curve of Valsartan

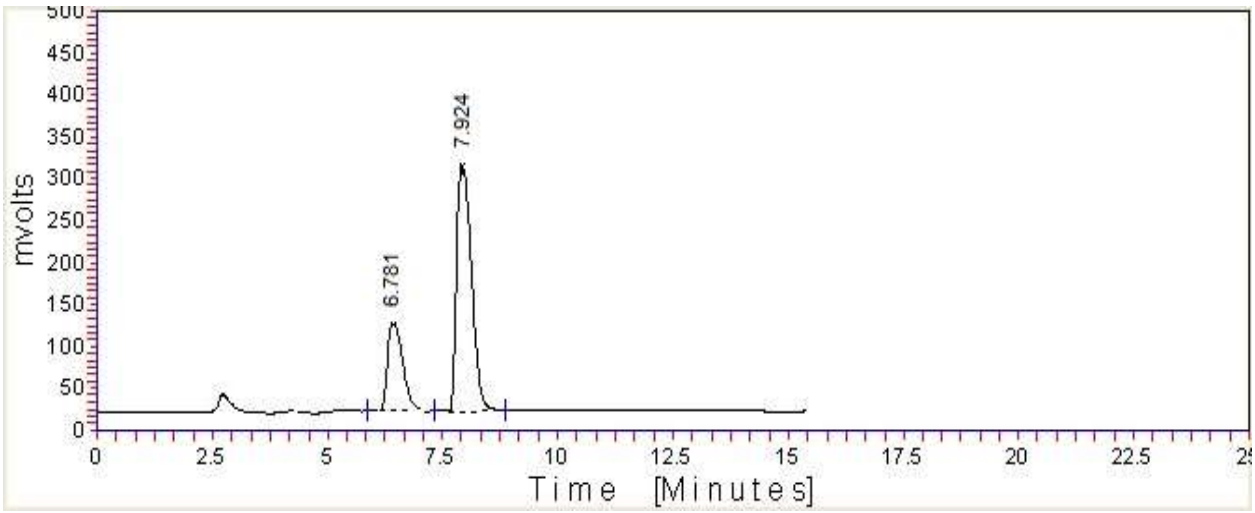


Figure 1.4 : Chromatogram of Nebivolol and Valsartan

SUITABILITY PARAMETERS

Separation variableswere set and mobile phase was allowed to saturate the column at 0.8 ml/min. After complete saturation of column, three replicates of working standard of nebivolol5µg/ml and valsartan 80 µg/ml were injected. Peak report and column performance report were recorded for all chromatogram. The result of system suitability parameter is reported in Table 1.6.

Table 1.6: System Suitability Parameters

System suitability Parameter →	Nebivolol				Valsartan			
	RT	AUC	Theoretical plates	Tailing factor	RT	AUC	Theoretical plates	Tailing factor
Rep-1	6.781	42501	3560	1.21	7.924	250845	6721	0.76
Rep-2	6.892	42547	3570	1.22	7.925	251170	6698	0.81
Rep-3	6.795	42620	3550	1.1	7.924	251318	6784	0.84
Mean	6.823	42556	3562	1.163	7.924	251111	6734	0.80
S.D.	0.001	1.12	8.377	0.045	0.001	3.12	6.718	0.025
% R.S.D.	0.009	0.115	0.235	3.87	0.009	0.215	0.203	2.04

VALIDATION OF DEVELOPED METHOD

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration (Table 1.7).

Table 1.7: Response Ratio Data for Linearity of Nebivolol and Valsartan

Drug	Concentration (µg/ml)	Mean AUC (n=3)	Response Ratio
Nebivolol	2.5	21253.17	8501.27
	5	42525.83	8505.17
	7.5	63745.17	8499.36
	10	84855.33	8485.53
	12.5	106166.67	8493.33
Valsartan	40.00	127519.00	3187.98
	80.00	250902.42	3136.28
	120.00	379921.19	3166.01
	160.00	496403.70	3102.52
	200.00	637000.00	3185.00

Table 1.8: Results of assay of marketed formulation

Brand name	Label Claim	Amount found (mg)*	% Assay	Standard deviation	% RSD
Nevigard-V	Nebivolol-5mg	4.99	99.97	0.037	0.037
	Valsartan-80mg	79.98	99.98	0.07	0.07

*Average of six replicate values

SUMMARY AND CONCLUSION

In the present research work, an attempt was made for "**Development and validation of RP-HPLC method for the simultaneous estimation of nebivolol and valsartan".** The method of estimation was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling.

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in tablet formulation.

The proposed method was found to be linear in the range of **2.5-12.5µg/ml** for nebivolol **and 40-200µg/ml** for valsartan with **correlation coefficient 0.999.** The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (50%, 100% 150%) was ranging from **99.6 to 100.13%** for nebivolol and **99.75 to 99.875%** for valsartan**.**

The result obtained shows the developed method to be **New** with the use of a comparative low cost and greener solvent, **Rapid**, **Simple**, **Accurate** (the value of SD and % RSD less than 2), P**recise** and it can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form.

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