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Development and Validation of Bioanalytical Method for Determination of Nebivolol and Valsartan in Human Plasma by Using RP-HPLC

Original Paper

Kachave R. N.[1]✉, Mundhe A. G.[2]

[1]Amrutvahini College of Pharmacy, Sangamner
Sangamner, Maharashtra INDIA
[2]Amrutvahini College of Pharmacy

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Abstract

Aim: Nebivolol and valsartan are used in the treatment of hypertension. So, this study was conducted for the purpose of determining bioavailability/bioequivalence of nebivolol and valsartan in human plasma.

Materials and Methods: The chromatographic separation was performed on Symmetry C18 (150 × 4.6 mm, 5 µm) column using 0.01 N potassium dihydrogen phosphate (pH 3.0):acetonitrile (60:40) as the mobile phase at a flow rate of 1.0 mL/min and a detector wavelength of 280 nm. The retention times of nebivolol and valsartan in plasma were found to be 3.1 and 4.3 min, respectively.

Results: The method was validated statistically and by recovery studies. The linearity concentration was acceptable in the range of 0.5–10 ng/mL for nebivolol and 400–8000 ng/mL for valsartan. The lower limits of quantification were 0.5 ng/mL for nebivolol and 400 ng/mL for valsartan, which reached the levels of both drugs possibly found in human plasma. Per cent recoveries were obtained as 97.78% and 98.11% for nebivolol and valsartan, respectively.

Conclusion: The proposed method is simple, rapid, accurate, precise and gives us knowledge about the pharmacokinetics and therapeutic drug monitoring in clinical laboratories.

Keywords *Nebivolol – Valsartan – Atorvastatin – RP-HPLC – Method development – ICH Guidelines (ICH M10) – Validation*

INTRODUCTION

Nebivolol (NL) is chemically described as 1-(6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-yl)-2-{[2-(6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-yl)-2-ydroxyethyl]amino} ethan-1-ol (Figure 1a). Its molecular formula is $C_{22}H_{25}F_2NO_4$ and its molecular weight is 405.435 g/mol. Valsartan (VL) is chemically described as (2S)-3-methyl-2-[N-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl}methyl) pentanamido] butanoic acid (Figure 1b). Its empirical formula is $C_{24}H_{29}N_5O_3$ and its molecular weight is 435.52 g/mol [1-5].

VL belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes telmisartan, candesartan, losartan, olmesartan and irbesartan. ARBs selectively bind to angiotensin receptor-1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects, which include vasoconstriction, stimulation and synthesis of aldosterone and ADH, cardiac stimulation and renal reabsorption of sodium, among others. Overall, the physiological effects of VL lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity and increased excretion of sodium. NL is a racemic mixture of two enantiomers, wherein one is a β-adrenergic antagonist and the other acts as a cardiac stimulant without β-adrenergic activity. Treatment with NL leads to a greater decrease in systolic and diastolic blood pressure than atenolol, propranolol or pindolol. NL and other β-blockers are generally not first-line therapies as many patients are first treated with thiazide diuretics. They are indicated for hypertension to lower the blood pressure and reduce the risk of fatal and non-fatal cardiovascular events, primarily strokes and myocardial infarction. NL is a competitive and selective β1-receptor antagonist, has little or no effect on β2 receptors at doses <10 mg, lacks intrinsic sympathomimetic and membrane-stabilising activity at therapeutically relevant concentrations and reduces systemic vascular resistance. VL blocks the binding of angiotensin II to type 1 angiotensin receptors, causing a reduction in blood pressure, and blocks vasoconstrictor and aldosterone-secreting effects of angiotensin II. Byvalson (VL and NL tablet)

* E-mail: ramanlalkachave26@gmail.com

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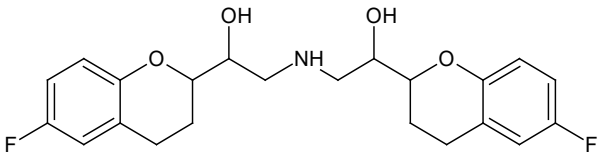


Figure 1a. Structure of Nebivolol.

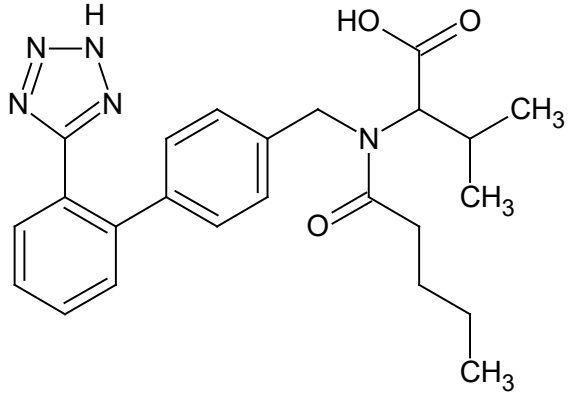


Figure 1b. Structure of Valsartan.

80 and 5 mg is a fixed-dose combination for the treatment of hypertension [1-8].

The literature survey presents different methods available for the detection of NL and VL separately and also in a combined form. Ultra-performance liquid chromatography (UPLC) method for detection of both drugs [9], an isocratic (high-performance liquid chromatography [HPLC]) method [10], an ion-pair HPLC method [11], RP-HPLC method for the estimation of both drugs [12], ultraviolet (UV) simultaneous estimation of NL and VL [13] – these all are the methods available for the detection of NL and VL.

This study aims to develop a rapid analytical technique for the estimation of NL and VL in human plasma. The proposed method is a rapid, precise, selective and sensitive RP-HPLC method that has short and simple extraction procedures that consume small amounts of solvent and biological fluid for extraction and time. This method is used in the bioavailability/bioequivalence (BA/BE) study for analysis of NL and VL in biological samples like blood, plasma and urine.

MATERIALS AND METHODS

NL and VL active pharmaceutical ingredients (APIs) were received as a gift sample from Spectrum Pharma Research solutions. HPLC-grade acetonitrile and methanol were procured from Rankem, Avantor Performance Material India Limited. Plasma used for the study was received from Deccan pathological lab, Hyderabad. Analytical grade phosphate buffer, potassium dihydrogen phosphate and orthophosphoric acid were also procured from Rankem, Avantor Performance Material India Limited.

Chromatographic conditions

Chromatographic separation was performed at 30°C of column temperature with the mobile phase consisting of 0.01 N potassium dihydrogen phosphate pH 3.0:acetonitrile in the ratio of 60:40. Separation of NL and VL was achieved on Symmetry C18 (150 × 4.6 mm, 5 µm) at a flow rate of 1.0 mL/min; the injection volume was 50 µL and detector wavelength was 280 nm.

Preparation of standard stock solution

Diluents

Based upon the solubility of the drugs, diluent was selected; 0.01 N potassium dihydrogen phosphate and acetonitrile were taken in the ratio of 50:50.

Preparation of NL stock solution (5000 ng/mL)

Take 5 mg of NL in a 100-mL volumetric flask and add diluent to give a volume of 50,000 ng/mL. Take 1 mL of the prepared solution and dilute to 10 mL with diluent to produce 5000 ng/mL.

Preparation of NL spiking solutions

From the above-mentioned NL stock solution, 0.01, 0.02, 0.03, 0.08, 0.10, 0.12, 0.16 and 0.20 mL was pipetted and transferred to eight individual 10-mL volumetric flasks and the volume was made up to the mark with diluent to produce 5, 10, 15, 40, 50, 60, 80 and 100 ng/mL. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.5, 1, 1.5, 4, 5, 6, 8 and 10 ng/mL.

Preparation of VL stock solution (400 µg/mL)

Take 40 mg of VL in a 100-mL volumetric flask and add diluent to give a volume of 400,000 ng/mL.

Preparation of VL spiking solutions

From the above-mentioned VL stock solution, 0.1, 0.2, 0.3, 0.8, 1.0, 1.2, 1.6 and 2.0 mL was pipetted and transferred to eight individual 10-mL volumetric flasks, and the volume was made up to the mark with diluent to produce 4.0, 8.0, 12, 32, 40, 48, 64 and 80 µg/mL. Calibration standards and QC samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 400, 800, 1200, 3200, 4000, 4800, 6400 and 8000 ng/mL.

Preparation of atorvastatin (internal standard [IS]) spiking solutions

Fifty milligrams of atorvastatin was weighed, dissolved and diluted to 100 mL by diluent (500 µg/mL). From this stock solution, 2 mL was pipetted, diluted to 100 mL with diluent and used as a spiking solution (10 µg/mL).

EXTRACTION PROCEDURE

Protein precipitation method

Preparation of calibration standard and QC samples

Take 750 µL of plasma and add 500 µL of IS, 250 µL of NL and 250 µL of VL from the spiking solutions into a centrifuging tube; add 1 mL of acetonitrile. Mix by cyclomixer for 15 sec. Then vertex for 2 min and finally centrifuge for 5 min at 3200 rpm. After centrifugation, collect the supernatant, filter it and directly inject 50 µL into HPLC.

Preparation of patient's samples

Take 1250 µL of the patient's sample and add 500 µL of IS into a centrifuging tube; add 1 mL of acetonitrile. Mix by cyclomixer for 15 sec. Then vertex for 2 min and finally centrifuge for 5 min at 3200 rpm. After centrifugation, collect the supernatant, filter it and directly inject 50 µL into HPLC.

Method validation

Method validation for an analytical method is a process used to verify/confirm whether the newly developed method is suitable for its intended purpose as per the ICH guidelines [14-19]

Parameters involved in bioanalytical validation are as follows:

1. System suitability test
2. Selectivity
3. Matrix factor evaluation
4. Precision and accuracy
5. Linearity
6. Recovery
7. Stability

1. System suitability test

System suitability test was performed before each batch sample analysis to ensure the reproducibility of the chromatographic system. This test was performed by running six injections of the diluted drug and IS in the linear region of the calibration curve and measuring the percentage of relative standard deviation (% RSD).

2. Selectivity/specificity

To establish the selectivity of the method, possible interferences at the retention time (RT) of NL, VL and IS due to endogenous plasma components were checked during validation. Selectivity was performed by testing six lots of K_2EDTA blank plasma, and the analyte detection of extracted plasma has good selectivity of both drugs and IS.

3. Matrix factor evaluation

The combined effect of all components of the sample other than the analyte on the measurement of quantity. Three blank specimens from each of not less than six batches of the matrix under screening were extracted. For the matrix effect, LQC and HQC spiking dilutions were spiked, and IS dilutions were also spiked in the previously extracted blank specimens.

4. Precision and accuracy

The intra-day and inter-day accuracy and precision were assessed by analysing six replicates at four different QC levels like LLOQ, LQC, MQC and HQC. Accuracy and precision method performance was evaluated by six replicate analyses for NL at four concentration levels, that is, 0.5 ng/mL (LLOQ), 1.5 ng/mL (LQC), 5 ng/mL (MQC) and 8 ng/mL (HQC), and for VL at 400 ng/mL (LLOQ), 1200 ng/mL (LQC), 4000 ng/mL (MQC) and 6400 ng/mL (HQC). The intra-day and inter-day accuracies of plasma samples were assessed and excellent mean % accuracies were calculated. The precision of the method was expressed in terms of % RSD, and accuracy was expressed as a percentage of theoretical concentration (observed concentration × 100/theoretical concentration).

5. Linearity

The calibration curve in a bioanalytical method is a linear relationship between concentration (independent variable) and response (dependent variable) using a least-squares method. This relationship is built to predict the unknown concentrations of the analyte in a complicated matrix. The linearity sample should consist of a blank sample, a zero sample and six to eight non-zero samples covering the expected range, including LLOQ.

6. Recovery

Recovery was determined by comparing the peak areas obtained from prepared plasma samples with those extracted from blank plasma spiked with the same amount of NL and VL standards at three QC concentration levels.

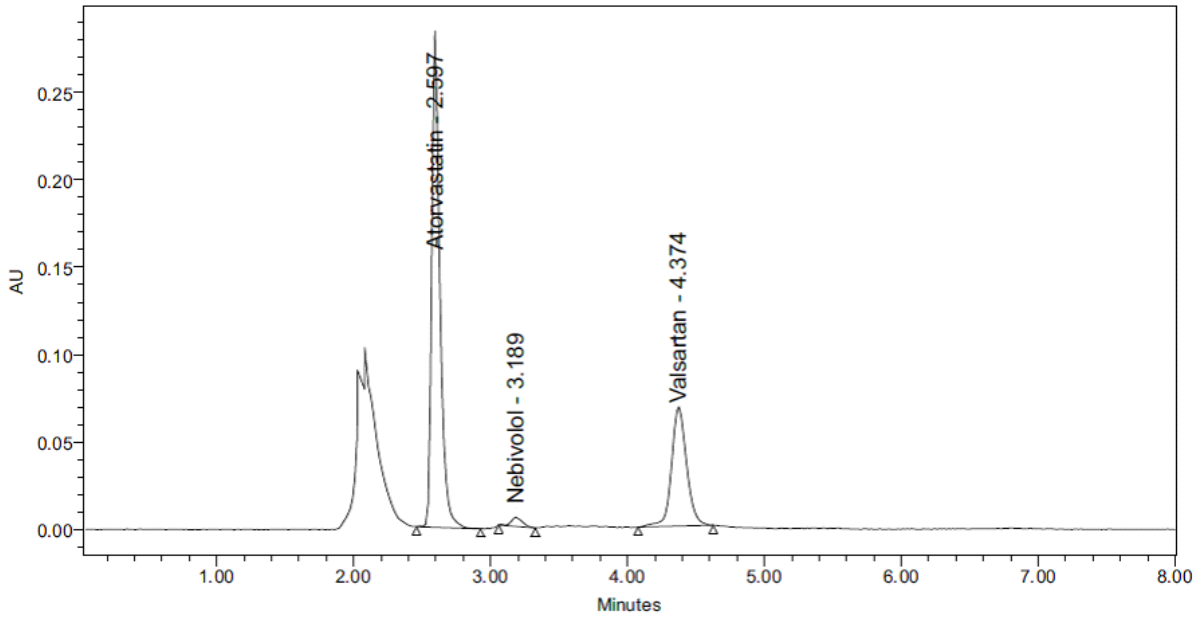


Figure 2. Chromatogram of Optimized Chromatographic Method.

7. Stabilities

Long-term stock solution stability for NL: In bench-top stability, six replicates of LQC and HQC samples (1.5 and 8 ng/mL) were analysed after 9 h of storage at room temperature in the laboratory bench. The % mean stability was calculated. **Long-term stock solution stability for VL:** In bench-top stability, six replicates of LQC and HQC samples (1200 and 6400 ng/mL) were analysed after 9 h of storage at room temperature in the laboratory bench. The % mean stability was calculated.

Matrix samples' stability at -20°C ± 5°C and -70°C ± 5°C after for 37 days of storage

Long-term stock solution stabilities for NL and VL were determined at LQC and HQC concentration levels after a storage period of 37 days at -20°C and -70°C in the deep freezer. The % means stabilities of NL and VL were calculated.

RESULTS

Optimisation of chromatographic method

Chromatographic separation of NL and VL by RP-HPLC method was performed by various mobile phase trials. The ideal separation of NL and VL was obtained by using a mobile phase consisting of 0.01 N potassium dihydrogen phosphate (pH 3.0):acetonitrile in the ratio of 60:40. A representative chromatogram of the optimised chromatographic method is shown in Figure 2.

System suitability test

All the system suitability parameters were within the range and satisfactory as per the ICH guidelines. The % coefficient of variation (CV) for system suitability test was in the range of 1.0 ± 0.0316 for RT of NL, 1.18 ± 0.0512 for RT of VL and 0.23% for the area ratio (analyte area/IS area) of atorvastatin. Full results of the system suitability test are shown in Table 1.

Selectivity

Evaluation of selectivity was performed by testing six batches of K_2EDTA blank plasma. The analysis of extracted blank plasma showed that the developed method has good selectivity for both drugs and IS. Representative chromatograms are shown in Figure 3 of standard blank and blank with IS sample using pooled plasma.

Matrix factor evaluation

The matrix effect plays a vital role in the assessment of pharmacokinetic studies. It was expressed as an IS-normalised matrix factor and it varied from 0.90 to 0.99, which was close to 1, which indicates that there was no analytical signal suppression or enhancement in plasma samples. Results are shown in Table 2.

Precision and accuracy

The intra-day and inter-day accuracies of plasma samples were assessed and excellent mean % accuracies for NL and

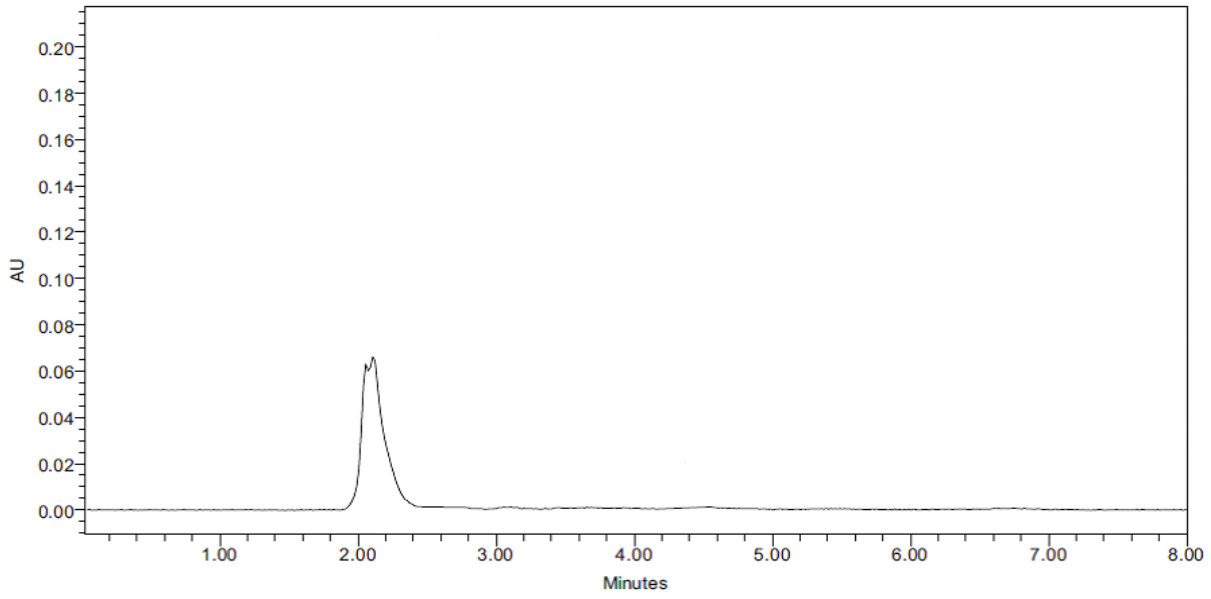


Figure 3. Extracted standard blank sample.

Table 1. Observation of optimised method chromatogram.

System suitability parameters	Atorvastatin	Nebivolol	Valsartan
RT	2.597	3.189	4.374
Area	97,579	5671	11,302
USP plate count	9792.8	9135.0	8011.3
USP tailing	1.4	1.4	1.1
USP resolution	-	4.6	6.9
Area ratio	-	0.05686	0.3545
SD	-	0.0316	1.00
% CV	-	0.0512	1.18

Table 2. Matrix factor evaluation of nebivolol and valsartan.

Parameters	Nebivolol		Valsartan	
	HQC	LQC	HQC	LQC
n	18	18	18	18
Mean	7.9592	1.4937	6400.5556	1190.4444
SD	0.07785	0.01768	44.02035	30.91016
% CV	0.98	1.18	0.69	2.60
% Mean accuracy	99.49	99.58	100.01	99.20
No. of QC failed	0	0	0	0

Table 3. Intra-day precision and accuracy of nebivolol and valsartan.

Parameters	Nebivolol				Valsartan			
	HQC	MQC	LQC	LLOQ QC	HQC	MQC	LQC	LLOQ QC
n	6	6	6	6	6	6	6	6
Mean	7.9513	4.9662	1.4943	0.4985	6403.1667	4017.5000	1196.000	396.5000
SD	0.05211	0.10071	0.01707	0.01320	10.60974	107.61924	11.6666	8.06846
% CV	0.66	2.03	1.14	2.65	0.17	2.68	0.97	2.03
% Mean accuracy	99.39	99.32	99.62	99.70	100.05	100.44	100.44	99.13

Table 4. Inter-day precision and accuracy of nebivolol and valsartan.

Parameters	Nebivolol				Valsartan			
	HQC	MQC	LQC	LLOQ QC	HQC	MQC	LQC	LLOQ QC
n	6	6	6	6	6	6	6	6
Mean	7.9238	4.9362	1.4907	0.4977	6394.3333	3999.0000	1194.8333	393.5000
SD	0.08962	0.08039	0.01770	0.01799	15.83246	70.11419	11.72035	6.05805
% CV	1.13	1.63	1.19	3.61	0.25	1.75	0.98	1.54
% Mean accuracy	99.05	98.72	99.38	99.53	99.91	99.98	99.57	98.38

Table 5. Batch-to-batch precision and accuracy of nebivolol and valsartan.

Parameters	Nebivolol				Valsartan			
	HQC	MQC	LQC	LLOQ QC	HQC	MQC	LQC	LLOQ QC
n	18	18	18	18	18	18	18	18
Mean	7.9577	4.9657	1.4917	0.4981	6398.5000	3993.7222	1194.17	396.1667
SD	0.07478	0.08347	0.01596	0.01470	11.92254	79.93071	11.1596	7.10634
% CV	0.94	1.68	1.07	2.95	0.19	2.00	0.93	1.79
% Mean accuracy	99.47	99.31	99.44	99.61	99.98	99.84	99.44	99.04

VL were obtained with range varying from 99.32% to 99.70% and from 99.13% to 100.44% for intra-day accuracy and from 99.31% to 99.61% and from 99.84% to 99.98% for inter-day accuracy, respectively. The precision (% CV) of the analytes in the plasma samples was calculated and found to be 0.66%–2.65% and 0.17%–2.68% for intra-day accuracy and 0.94%–2.95% and 0.19%–2.00% for inter-day accuracy, respectively. Results are shown in Tables 3–5.

Linearity

Calibration was found to be linear over the concentration range of 0.5–10 ng/mL for NL and 400–8000 ng/mL for VL. The coefficient of determination (r^2) value was found to be consistently greater than 0.999 in all the cases shown in Figure 4a and b. Linearity of results shows an excellent correlation between the peak area ratios for each concentration of analytes; results are shown in Table 6.

Recovery

The overall % mean recoveries for NL and VL were found to be 97.96% and 98.11%, respectively. The recoveries were obtained for NL and VL at three QC concentration levels. The overall % mean recovery for atorvastatin (IS) was found to be 99.00%. The results are shown in Table 7.

Stabilities

Long-term stock solution stability for NL

The % mean stability of six replicates of LQC and HQC samples (1.5 and 8 ng/mL) was analysed after 9 h of storage at room temperature on the laboratory bench. These were calculated and found to be 99.51% for LQC and 99.92% for HQC. The result is shown in Table 8.

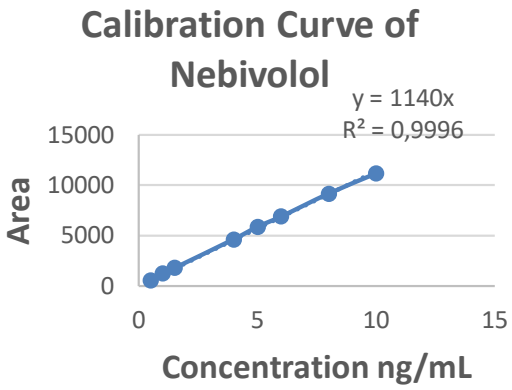


Figure 4a. Calibration Curve of Nebivolol.

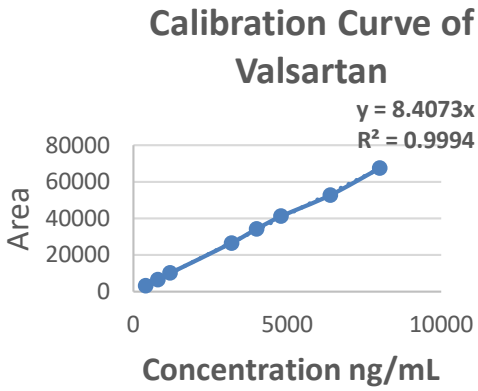


Figure 4b. Calibration Curve of Valsartan.

Table 6. Observation table for linearity of nebivolol and valsartan.

Nebivolol			Valsartan		
Final conc. in ng/mL	**AUC**	**Area ratio**	**Final conc. in ng/mL**	**AUC**	**Area ratio**
0.5	570	0.006	400	3415	0.0350
1	1235	0.013	800	6724	0.0689
1.5	1785	0.018	1200	10,160	0.1041
4	4643	0.048	3200	26,678	0.2731
5	5880	0.060	4000	34,216	0.3506
6	6902	0.071	4800	41,162	0.4217
8	9176	0.094	6400	52,728	0.5402
10	11,175	0.115	8000	67,420	0.6910
Parameters	**Nebivolol**		**Valsartan**		
Correlation coefficient	0.998		0.9994		
Regression equation	y = 1140x		8.4073x		
Linearity range	0.5–10 ng/mL		400–8000 ng/mL		

Table 7. Recovery study of nebivolol and valsartan.

Parameters	**Nebivolol**						**Valsartan**					
	Unextracted (HQC)	**Extracted (HQC)**	**Unextracted (MQC)**	**Extracted (MQC)**	**Unextracted (LQC)**	**Extracted (LQC)**	**Unextracted (HQC)**	**Extracted (HQC)**	**Unextracted (MQC)**	**Extracted (MQC)**	**Unextracted (LQC)**	**Extracted (LQC)**
n	6	6	6	6	6	6	6	6	6	6	6	6
Mean	9445	9253	5965	5782	1886	1857	54,658	53,310	35,376	34,761	10,665	10,507
SD	59.17	70.20	53.57	66.05	12.32	31.02	657.59	400.50	416.36	210.54	143.61	70.53
% CV	0.62	0.75	0.89	1.14	0.65	1.67	1.20	0.75	1.17	0.88	1.34	0.67
% Mean recovery	97.96		96.93		98.46		97.54		98.26		98.52	
Overall % mean recovery	97.784						98.112					

Table 8. Long-term stock solution stability of nebivolol and valsartan.

Parameters	Nebivolol		Valsartan	
	HQC	LQC	HQC	LQC
n	6	6	6	6
Mean	7.9938	1.4927	6391.6667	1193.5000
SD	0.01950	0.02082	21.42584	20.08731
% CV	0.24	1.39	0.34	1.68
% Mean accuracy	99.92	99.51	99.87	99.46

Table 9a. Matrix samples' stability of nebivolol and valsartan at -20°C ± 5°C for 37 days.

Parameters	Nebivolol				Valsartan			
	HQC (comparison sample)	HQC (stability sample)	LQC (comparison sample)	LQC (stability sample)	HQC (comparison sample)	HQC (stability sample)	LQC (comparison sample)	LQC (stability sample)
n	6	6	6	6	6	6	6	6
Mean	7.9930	7.8872	1.4872	1.4898	6394.5000	6391.1667	1193.1667	1188.5000
SD	0.02481	0.15394	0.02729	0.01393	23.57753	18.50856	20.48821	18.98157
% CV	0.31	1.95	1.83	0.94	0.37	0.29	1.72	1.60
% Mean accuracy	99.91	98.59	99.14	99.32	99.91	99.86	99.43	99.04
% Mean stability	98.68		100.18		99.95		99.61	

Long-term stock solution stability for VL

The % mean stability of six replicates of LQC and HQC samples (1200 and 6400 ng/mL) was analysed after 9 h of storage at room temperature on the laboratory bench. These were calculated and found to be 99.51% for LQC and 99.92% for HQC. The result is shown in Table 8.

Matrix samples' stability at -20°C ± 5°C and -70°C ± 5°C after 37 days of storage

The % mean stability of NL was found to be 100.18% (LQC) and 98.68% (HQC) at 20°C ± 5°C and 99.65% (LQC) and 100.33% (HQC) at 70°C ± 5°C. The % mean stability of VL was found to be 99.61% (LQC) and 99.95% (HQC) at 20°C ± 5°C and 99.51% (LQC) and 100.23% (HQC) at 70°C ± 5°C. The results are shown in Tables 9a and b.

DISCUSSION

After studying literature about NL and VL, the method development was started according to hydrophobic and hydrophilic interaction of the drugs. The absorbance spectra of both drugs were measured, overlayed and the wavelength of optimal sensitivity of determination was chosen (280 nm). The IS was selected based on the presence of chemical properties similar to the compounds of interest. Atorvastatin was chosen as the IS. After selecting the wavelength, the next step was the selection of the extraction procedure by observing the % recovery. The protein precipitation method was selected as the extraction procedure for better recovery. After selection of the extraction method, the method development process started with a flow rate of 1.0 mL/min at a column temperature 30°C, mobile phase 0.1% orthophosphoric acid (pH 2.2):acetonitrile (50:50), column Agilent C18 (150 mm × 4.6 mm, 5 µm) and injection volume 50 µL. In these parameters, VL was not eluted. So, further trial was carried out. In the second trial, the mobile phase and its ratio were changed to 0.01 N potassium dihydrogen phosphate (pH 3.0):acetonitrile (55:45) and other chromatographic conditions were the same as first trial. In the second trial, the resolution between atorvastatin and NL was unacceptable. So, third trial was carried out using 0.1% orthophosphoric acid (pH 2.2):acetonitrile (60:40) mobile phase and Symmetry C18 (150 mm × 4.6 mm, 5 µm) column. VL was not eluted in this trial. In the fourth trial, mobile phase 0.01 N potassium dihydrogen phosphate (pH 3.0):acetonitrile (70:30) and column Agilent C18 (150 mm × 4.6 mm, 5 µm) were used. In

Table 9b. Matrix samples' stability of nebivolol and valsartan at -70°C ± 5°C for 37 days.

Parameters	Nebivolol				Valsartan			
	HQC (comparison sample)	HQC (stability sample)	LQC (comparison sample)	LQC (stability sample)	HQC (comparison sample)	HQC (stability sample)	LQC (comparison sample)	LQC (stability sample)
n	6	6	6	6	6	6	6	6
Mean	7.9880	8.0145	1.4953	1.4902	6386.5000	6401.5000	1194.5000	1188.6667
SD	0.02458	0.05858	0.02273	0.01646	22.29574	21.37054	17.14351	14.17980
% CV	0.31	0.73	1.52	1.10	0.35	0.33	1.44	1.19
% Mean accuracy	99.85	100.18	99.69	99.34	99.79	100.02	99.54	99.06
% Mean stability	100.33		99.65		100.23		99.51	

this trial, NL United States Pharmacopeia (USP) plate count was low and RTs were unnecessarily high. So, in the next trial, 0.01 N potassium dihydrogen phosphate (pH 3.0):acetonitrile (60:40) was used as the mobile phase and Symmetry C18 (150 × 4.6 mm, 5 µm) was used as the column. All peaks eluted with good peak shape and RT. The tailing tests were also passed. So, this method is used as an optimised method for the separation of NL and VL from human plasma. After optimisation was completed, the method was evaluated for various validation parameters as per the ICH M10 guideline.

CONCLUSION

A simple, accurate and precise method was developed for the estimation of NL and VL in human plasma using atorvastatin as the IS. RTs of NL and VL were found to be 3.146 and 4.346 min, respectively. The % CV of NL and VL was found to be 1.0% and 0.58%, respectively. Per cent recoveries were obtained as 98.28% and 98.11%, respectively. The linearity concentrations were in the range of 0.5–10 ng/mL for NL and 400–8000 ng/mL for VL. The lower limits of quantification were 0.5 ng/mL for NL and 400 ng/mL for VL, which reached the levels of both drugs possibly found in human plasma. Further, the reported method was validated as per the ICH guidelines and found to be well within the acceptable range. The proposed method is simple, rapid, accurate, precise and appropriate for pharmacokinetic studies and therapeutic drug monitoring in clinical laboratories.

LIST OF ABBREVIATIONS

ABBREVIATIONS	FORMS
NL	Nebivolol
VL	Valsartan
RP	Reverse phase
HPLC	High-performance liquid chromatography
BA	Bioavailability
BE	Bioequivalence
API	Active pharmaceutical ingredient
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
QC	Quality control
RT	Retention time
USP	United States Pharmacopeia
CV	Coefficient of variation
IS	Internal standard
SD	Standard deviation

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