Dissolution Method development and validation for simultaneousdeterminationofNebivolol and Valsartanintablet dosage form

Vaishali Gohel,SejalbenPatel,UjashkumarShah

DepartmentofQualityAssuranceandPharmaChemistry,FacultyofPharmacy,NootanPharmacyCollege,SankalchandPatelUniversity, Visnagar,Gujarat,India.

*CorrespondingAuthorE-mail:**Sejupatel04@gmail.com** ,

Shahujash@gmail.com

ABSTRACT:

A selective, simple, and precise RP-HPLC dissolution methodfor estimating the concentration of Nebivolol and Valsartan in combination dosage form. This experimentalanalysis was performed on a reverse phase high performance high-performance liquid chromatography, (RP-HPLC)C18column(250mmx4.6mm,5µm)byusingMobilephaseAcetonitrile and Potassium dihydrogen Phosphate with buffer pH-3.0 in the ratio of (50:50) at a flow rate of 1.0ml/min and the detected at wavelength 282 nm. In line with ICHguidelines, thelinearity of the analytical method was discovered to be in the range of 25-75 µg/mlrange for Nebivolol. The linearity of the method for Valsartan was 400-1200µg/ml. Nebivolol and Valsartan had correlation coefficients greater than 0.990.The relative standard deviation value for system precision and method was less than 2.0%. All statistical data proves validity of the method andcan be usedforroutineanalysis ofpharmaceuticaldosageform.

KEYWORDS: Stability-indicatingRP-HPLC, Nebivolol and Valsartan, ICHguidelines, Dissolution and Validation.

INTRODUCTION:

In recent years,the pharmaceutical industry and regulatory agencies have placed a greater focus on dissolution testing.[1] The dissolution test is a commonly used method by the pharmaceutical industry to determine the effectiveness of formulations and the quality of products. The test is a required practice bymany regulatory authorities for solid orally administered dosageforms, transdermalpatches,stents,andoralsuspensions.[2]Dissolutiontestingofimmediate-releasesolid oral dosage forms, such as tablets, used to evaluatethe batch wise quality of drug products which ensureconsistentproductqualityandperformance.[3]In the treatment of newly diagnosed hypertension, Valsartan, angiotensin II type I receptor antagonist, isused.Valsartan is a potent, mighty selective and orally active antihypertensive drugNebivolol is a beta blocker with a distinctive function which differs it from other beta blockers.The advantages expected from this combination are synergistic effects such as inhibition of different physiologic pathways, blockage of the Renin-Angiotensin Aldosterone system at different points, cardio protection, and trans-inhibition of the reciprocal receptor. This drug combination is used as an antihypertensive agent.[4-5]

High performance liquid chromatography(HPLC) method provides a wide dynamic linear range,selectivity via separation and superiorsensitivity that has been used to solve a variety of analytical problems encountered during dissolution test of complex drug delivery systems.For the solid dosage form the characteristics of dissolution under physiologicalcondition influence the in vitro dissolution. Solubility and permeability of drug products and release productsarethemajorfactorswhichaffectthedissolutionofdevelopment and quality control of synthetic drugs. Thevalueofdissolutiontestenhancessignificantlywhenperformance of drug substance is evaluated as a functionof time. Dissolution test useful in QC and production batch to ensure similarities,so the dissolutionTest remains similar and is crucial for clinical trial batches;further dissolution profiling is used to support bioavailability and bio equivalence of a new pharmaceutical product.[6-8]

The literature survey reveals that both drugs Nebivolol and Valsartan have been analyzed individually and incombinationbymanyanalyticalmethodslikeHPLC,HPTLC and spectroscopic method, but no method havebeenreportedfortheestimationofNebivolol and Valsartan for dissolution in combined pharmaceuticaldosage form. This paper presents is simple, accurate and reproducible dissolution method for estimation of Nebivolol and Valsartan and its combined dosage form by using RP-HPLC method and validation of thedeveloped methods was performed according to ICHguidelines.[9-16]

EXPERIMENTAL:

Materials:

The Reference standard of NEB and its related impurityA and B, as well as Valsartan, wereobtainedasagiftsamplefromShanku Pharma.Methanol,Water,HPLCgrade of Acetonitrile, and analytical reagent grade of OrthophosphoricacidfromMerckcompanyMumbai,wereusedforthe study. And the commercially available tablet formulationof Nebivolol and Valsartan, Nebicard-V (TorrentPharma Ltd.) with label claim Nebivolol 50 mgand Valsartan 80 mg was procured from the market foruse.

Instrumentation:

LabIndiaprivatelimiteddissolutionapparatus,ShimadzuLC20ATHPLCchromatographicsystem,Systronic119 UV visible spectrophotometer, Shimadzu digital weighing balance(ATX224), Frontline

Ultrasonic Cleanerultra-Sonicator, AnalabScientificPvt. Ltd pH meter used for the method development.

Methods:

Selectionanddetectionofwavelength[17]

UV detection which determined by proper selection ofdetection wavelength which is used for interpretation ofsensitivity of HPLC method. It is an ideal wavelengthgives good response for the both drugs i.e. Nebivolol and Valsartan.

PreparationofMobilePhase:

ThePotassiumDihydrogenPhosphatewasPreparedwith0.05M concentration by dissolving accurately weigh of 6.8gof Potassium Dihydrogen Phosphate in 1000 ml HPLC gradewaterina1LvolumetricflaskandpHwasadjustedtopH3.0 with o-Phosphoric acid (OPA). The prepared buffer pHwas checked by using a pH meter by ultra sonicating. For 5minutes, the solution was degassed, and the obtained solutionwas filtered through a 0.45µ Millipore filter. And the mobilephaseispreparedwiththeratioofbuffer(pH3.0):Acetonitrile(50:50v/v).

Preparation of dissolution medium:Phosphate bufferpH 6.8:

Accuratelyweigh6.8gofPotassiumDihydrogeno-phosphate and 0.94g of NaOH and diluted with 1000mLof water in 1L volumetric flask. The pH of phosphatebuffer checked using calibrated pH meter and phosphatebuffershows pH 6.8.

PhosphatebufferpH7.5:

Accuratelyweigh6.8gofPotassiumDihydrogeno-phosphate and 1.56g of NaOH and diluted with 900mLof water in 1L volumetric flask. The pH of phosphatebuffer 7.5 adjusted using NaOH solution and diluted with water to produce 1000ml.

0.1N HCl:

Accurately taken 8.5mL of concentrated HCl and dilutedwithwater to make 1000mL in 1Lvolumetricflask.

StandardSolutionsPreparation:

PreparationofStandardStockSolutionofNEB (500µg/mL):

Accurately taken 50mg of NEB bulk drug into a100ml volumetric flask and dissolved with dissolutionmedium Phosphate buffer pH 6.8 up to the mark to get500µg/ml of NEB Standard Stock Solution andthen 5.0ml of this solution was transferred into a 50mlvolumetric flask and finally volume was made up with dissolution medium phosphate buffer pH6.8 toget 50µg/mlofNEBworkingstandardsolution.

PreparationofStandardStockSolutionofVAL(8000µg/mL):

Accurately taken 800mg of VAL bulk drug into a100ml volumetric flask and it dissolved in dissolution medium Phosphate buffer with pH 6.8 up to mark to obtain 8000µg/mlofstandard stockSolution of VAL and then 5.0ml of this solution was transferred into a 50ml graduated flask and the final volume was made up with dissolution medium phosphate buffer pH 6.8 up to the mark to get 800µg/mlof VALworking StandardSolution.

Preparation of TestSolution:

An intact tablet of Nebicard-V dissolved in dissolutionmedia and set dissolution condition. Withdraw 10mL ofsample by syringe at an interval of 10, 20, 30, 45 and 60minutes and filter the

solution with 0.45µ filter and thefinal filtrate collected as test solution. This test solutioninjectedinto theHPLCsystemto get thepeak.

ChromatographicSeparation:
BoththestandardsolutionsofNebivolol and Valsartan were injected 20µl in column with micro-syringe. And for appropriate minutes the chromatogramwas run with mobile phase. And at 282nm the detectionwas performed. The chromatogramwasstopped afterachieved completely separation and recorded the data related to peak like retention time, area, resolution, height,etc. by using software.

DevelopmentandOptimizationofDissolutiontestmethod:
Determination ofsolubilityindifferentDissolutionMedium:
At room temperature the solubility for NEB in different dissolution medium which determined by taking NEB and taken required quantity of solvent and it shake well for few minutes and for similarly solubility of VALin different dissolution medium which determined by taking VAL and taken necessary quantity of solvent at room temperature and it shaken well for few minutes.

Optimizationofdissolutionmediumatdifferentconditions:
USPDissolutionApparatus:
USP Dissolution Apparatus 2 (Paddle) was selected tocalculate % release of Nebivolol and Valsartan drugintabletdosageform.Theresultof%dissolutionNebivolol and Valsartan drug in tablet dosage formwasobservedandrecorded.

Dissolution Medium of USP DissolutionApparatus: The Phosphate Buffer(pH6.8) was selected as dissolution medium due to high solubility of Nebivolol and Valsartan drug in Phosphate Buffer(pH 6.8). A media volume of 900ml was kept constant and%release of Nebivolol and Valsart andrug in table tdosage form wascalculated using Phosphate Buffer(pH6.8).

Speed of USP Dissolution Apparatus:
The USP Dissolution Apparatus2 (Paddle) was rotated at 50,75 and 100RPM to calculate %release of Nebivolol and Valsartan drug in tablet dosage form.It can be concluded that the % release of Nebivolol and Valsartan drug using USP Dissolution Apparatus 2(Paddle)at 100RPM was found tobe optimum as compared to other Revolution per Minute conditions. Therefore 100RPM was selected as one of the optimizeddissolutionapparatus speed.

TemperatureofUSPDissolutionApparatus:
The USP Dissolution Apparatus2 (Paddle) was maintained a ttemperature 37±0.5°Ctocalculate%releaseofNebivolol and Valsartandrugintabletdosage form.

Optimizationofdissolutiontest:
The dissolution studies was performed by using a USP dissolution apparatus2 by subjecting tablets in dissolution medium which containing 900mL of dissolution media of phosphate buffer with pH 6.8 using paddle dissolution test apparatus with it stirring speed of 100rpm at temperature of 37±0.5°C. and aliquots of 10ml were withdrawn manually at an intervals of 10, 20,30, 45 and 60 minutes and its same volume of fresh medium was replaced at 37±0.5°C to maintain the constant volume.And finally sample was filtered through filter paper and which was analyzed by RP-HPLCmethod.

MethodValidation[18-19]
The developed RP-HPLC method was validated for Nebivolol and Valsartan according ICH guidelines.The parameters

validatedareSpecificity,Systemsuitability,Precision,range,Linearity,Robustness,Limitof quantitation(LOQ),Limitof detection(LOD).

Systemsuitability:
It is used to authenticate the reproducibility and resolution of the system for the analysis being performed. The System Suitability was calculated from different parameters like the Theoretical plates for the analyte peak and tailing factor for analyte peak.

Linearity andRange:
The linearity of a method is measured to see how well a calibration plot of response vs.Concentration approximates a straight line.The linearity of Nebivolol and Valsartan were evaluate by analysis of combined standard solution in range of 25-75 µg/mlofNEBand400-1200µg/mlof VAL respectively. Suitable aliquots of the standard stock solutions of NEB transferred into series of 10ml volumetric flasks respectively to get concentration levels of 25.0µg/ml, 37.5µg/ml, 50.0µg/ml,62.5µg/ml and75.0µg/ml. Similarly concentration levels of 400µg/ml,600µg/ml, 800µg/ml, 1000µg/ml and 1200µg/ml from standard stock solution of VAL prepared. The obtained graph of peak area verses respective concentration was plotted.

Precision:
System precision was performed by injecting five replicates of a standard solution of NEB(50.0µg/ml) as well as standard solution of VAL(800.0µg/ml) and chromatograms were recorded and areas of peaks were measured to calculate results of repeatability.Method precision was performed by injecting sample solution of Nebivolol and Valsartan six times and areas of peaks measured% dissolution and%RSD was calculated.

LOQandLOD:
LimitofquantitationandLimitofDetectionwasestimated from the 3 set of calibration curves which wasused to determine method linearity. The LOQ and LODwere calculatedwithbelow formula.

LOQ=10 * SD/slope of calibration curveLOD=3.3*SD/slopeofcalibrationcurve

Robustness:
The robustness studywas carriedout to evaluate the effect of small but considered variations in the chromatographic conditions,which havebeen described in the Chromatographic conditions section. The factors chosen for thisstudy,whichwerecriticalsourcesofvariability in the operating procedures such as pH of mobile phase changed(±0.2) and Temperature was changed(±2.0ºC)were identified.In all these experiments,themobile-phase components were not changed and their effect observed on system suitability for standard preparation.

AnalysisofMarket formulation:
An intact tablet dissolved in dissolution media and setdissolution condition. After interval of 10 minutes, 20minutes, 30 minutes, 45 minutes and 60 minutes 10mLsample withdrawn from the cylinder by syringe and sample filtered with0.45micronmembrane filter and the finalf Filtrate collected as test solution.The testsolution was injected concentration of 20µL and area of resulting which measured at 82nm.

RESULTS AND DISCUSSION:

Wavelength determination:

UVspectraofNebivolol and Valsartanweretakenin Methanol solvent and its λmax was observed usingSystronic119

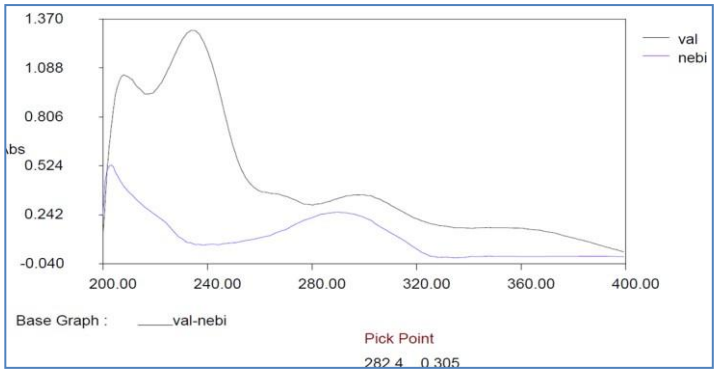


Figure No.1 Overlay UV Spectrum of Nebivolol and Valsartanshowing SelectionofWavelength Detection.

At282nmdetectionwavelengthNebivolol and Valsartan both drug give higher absorbance. Hence λmax 282nmhas beenselectedasdetection wavelength.

Optimisedchromatographicconditions: The chromatographic trials have been taken for the effect of different mobile phase compositions on the separation of Nebivolol and Valsartan.Method development process was carried out by examining different conditions like mobile phase compositionslikeWater: Methanol, Water: Acetonitrile, phosphate bufferpH 3.0: Acetonitrilewith different ratios were used.

TheNebivolol andValsartan were found show in gasignificantUVabsorbanceat282nm,sothiswavelength was chosen forUV detection.

By use of aC18columnitwasfoundthatthemobilephaseconsistingofBuffer(PhosphateBuffer,pH3.0):Acetonitrile provided well defined peak shape with goodresolution.The peaks withretentiontime(RT)4.293minutesand7.003minutesforNEBandVAL. The representative chromatograms (FigureNo. 2) which show significant amount of resolution andthe goodpeak shapeswithselectedmobile phase.

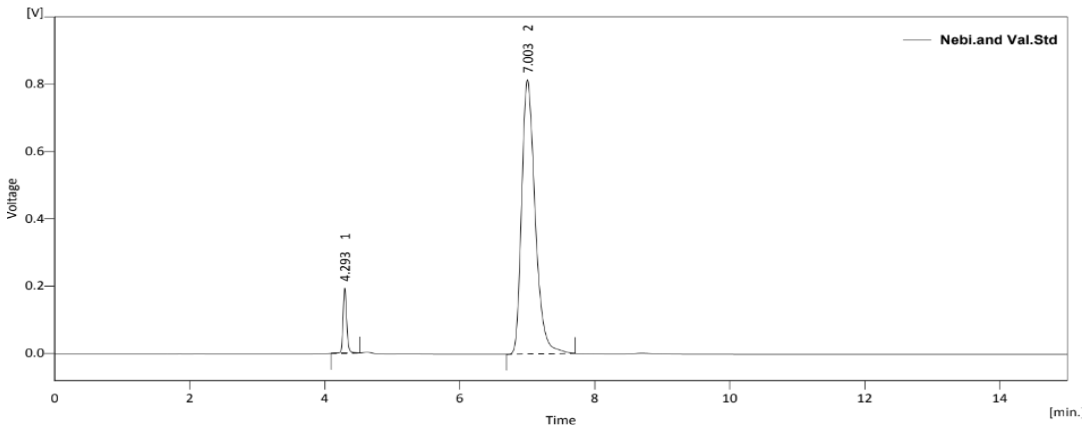


Figure No.2: Chromatogram of Nebivolol and Valsartan inBuffer (pH3.0):Acetonitrile

ThefinaloptimizedchromatographicconditionforNebivolol and Valsartan and having Stationary phase used BDS Hypersil C18column with size of 250mm×4.6 mm, 5 µm particle size, and

utilized mobile phase was PhosphateBuffer (pH3.0):Acetonitrile(50:50v/v)with flowrate is1ml/min,at 282 nm wavelength detection for15min run time and its injection volume is20µl.

Optimiseddissolutionconditions:

Solubility of Nebivolol and Valsartanin differentDissolutionMedium:

ThedataforSolubilityofNebivolol and Valsartanin different Dissolution Medium is given in below table,ThesolubilityofNebivolol and Valsartanindifferent dissolution media concludes that the PhosphateBufferpH6.8wastheDissolutionMediumforNebivolol and Valsartan dissolution test and also ensuredSinkCondition.

TableNo.1:SolubilityofNebivolol and ValsartanindifferentDissolutionMedium

DissolutionMedium	Solubility(mg/ml)	Sink Condition(Solubility>0.25mg/ml)	Solubility(mg/ml)	Sink Condition(Solubility>0.033mg/ml)
	Nebivolol		Valsartan	
Water	0.025mg/ml	No	0.00225mg/ml	No
0.1NHCl	0.1mg/ml	No	0.001mg/ml	No
pH7.5	0.025mg/ml	No	0.0225mg/ml	No
pH6.8	≥0.25mg/ml	Yes	≥0.033mg/ml	Yes

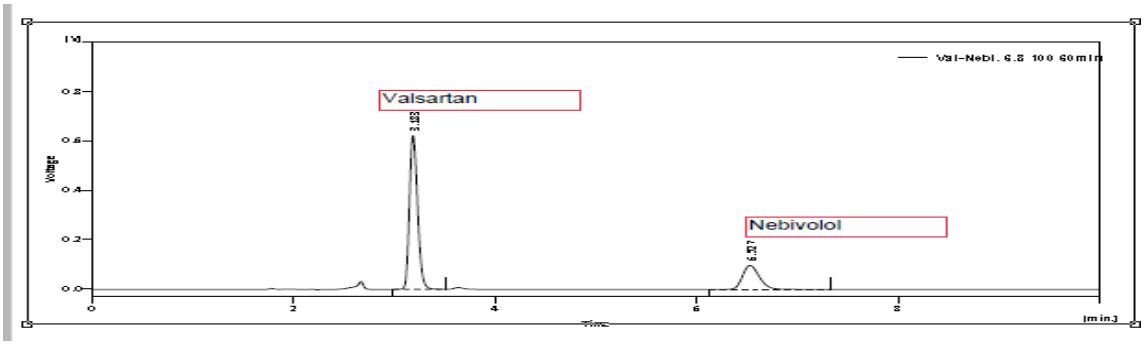
TableNo.2:%dissolutionwithpaddleapparatusatvariousspeedofrotation(rpm)indissolutionmediumpH6.8

Time(Minutes)	%DissolutionatpH6.8					
	50RPM		75RPM		100RPM	
	Nebivolol	Valsartan	Nebivolol	Valsartan	Nebivolol	Valsartan
10	33.525	6.449	47.073	13.190	57.385	21.922
20	46.178	15.800	61.423	28.379	71.596	36.940
30	60.351	38.184	77.830	48.835	84.814	60.110
45	71.886	44.065	85.024	62.866	93.201	81.563
60	82.163	60.408	94.094	74.102	100.225	94.013

Optimizationofdissolutionparameters:

Differentdissolutionsparameterswereperformedforoptimizeddissolutionparameter.Likemaximum %releaseofdrugitstrialsweretakenbyusingUSPApparatusIIi.e.paddletypeatdifferentspeedofrotation (rpm) 50, 75 and 100. Based on the solubility ofNebivolol and Valsartan, phosphate buffer pH 6.8wasselectedassuitabledissolutionmedia(900mL).

ItwasobservedthatdrugreleaseinUSPtype-II(Paddle)apparatusshowsmaximumreleaseforbothdrugs at 100 rpm. Hence dissolution parameters havebeen optimized. The optimized condition for dissolutionmethod development includes USP dissolution apparatusII (paddle), 900 mL of phosphate buffer with pH 6.8 asdissolutionmediumat100rpmdissolutionapparatuspaddle speedat temperatureof37±0.5^{o}C.



FigureNo.3:Chromatogramof%dissolutionofNebivolol and ValsartanwithPaddleApparatusat100RPMafter60minutestimeinterval

MethodValidation:

The proposed method was validated with the aspect of Specificity, Precision,Systemsuitability, Limitofquantitation(LOQ),Limitofdetection(LOD),Robustness, Linearity and range.

System Suitability:

The System Suitability was calculated from different parameters like retentiontime, theoretical plates, resolution, tailing factor. System suitability parameters observed for NEB have a retention time of 10.153,theoreticalplatesper column of 7180, and tailing factor of 1.380. The system suitability parameters observed forVAL have a retention time of 4.037, theoretical plates per column7681,and tailing factorof 1.232. The resolution observed 8.189.

Specificity:

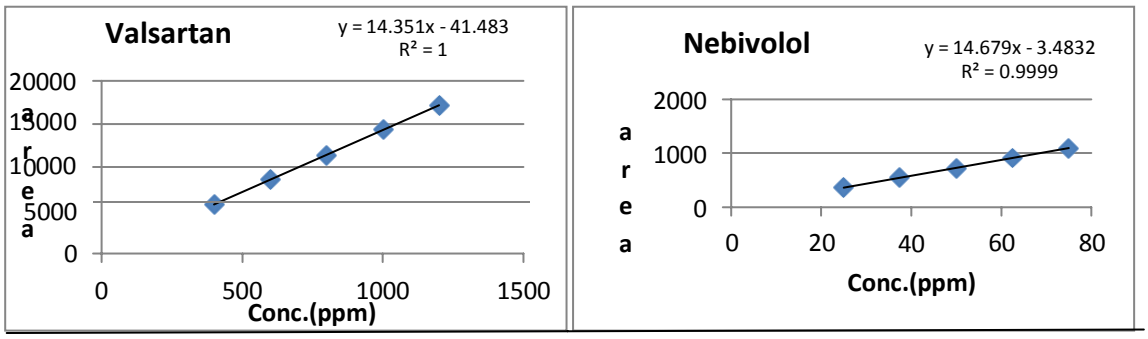
ThespecificityofthechromatographicmethodwasdeterminedtoensureseparationofNebivolol and Valsartan.TheChromatogramsofNebivolol and Valsartan sample did not show any interference withthe Chromatogram of Nebivolol and Valsartan blanksolution, SothattheMethod wasdeveloped.

Linearity andRange:

ForNebivolol and Valsartanthelinearitywasevaluated by analysis of combined standard solution inrange of 25-75µg/ml and 400-1200µg/ml respectivelyand its Correlation co-efficient for calibration curve ofNebivolol and Valsartanwas found to be NLT 0.999respectively.

Table No.3:LinearityData forNebivolol and Valsartan

Sr. No.	LinearityLevel	Concentration(µg/ml)		Area	
		Nebivolol	Valsartan	Nebivolol	Valsartan
1	50%	25	400	365.551	5708.907
2	75%	37.5	600	545.961	8579.03
3	100%	50	800	726.589	11394.852
4	125%	62.5	1000	916.714	14326.282
5	150%	75	1200	1097.643	17185.87



FigureNo.4PlotsofcalibrationcurvesofNebivolol and Valsartan

Precision:

SystemPrecision:

Performed system precision by injecting five replicatesof a standard solution of NEB (50.0µg/ml) as wellasstandardsolutionofVAL(800.0µg/ml)andchromatograms were recorded and areas of peaks weremeasured to calculate results of repeatability. The dataforsystemprecisionofpeakareameasurementforNebivolol and Valsartanshowsthatthe%RSDvaluesobservedwithin acceptancelimit of NMT 5%.

MethodPrecision:

Performed the method precision by injecting samplesolution of Nebivolol and Valsartan six times and areas of peaks measured % dissolution and % RSD wascalculated. The data for method precision of peak area measurement for Nebivolol and Valsartan shows that the % RSD values observed within acceptance limitof NMT5%.Hencethemethodisprecise.

LOQandLOD:

The limit of quantitation and Limit of detection for both the drugs were estimated using the linearity data.Forf ive times Calibration curve was repeated and interceptsof the standard deviation (SD) was calculated. The Limitof detection for NEB observed 0.017 µg/ml and forVAL observed 3.240 µg/ml. However the limit ofquantitation for NEB observed 0.051 µg/ml

andfor VALobserved9.820 µg/ml.

Robustness:

The robustness study was performed to evaluate the effect of small but deliberate variations. And chromatographic factors as mobile phase pH (± 0.2) and temperature of Mobile phase was changed(±2ºC) without changing the mobile phase components and their effect observed on system suitability for standard preparation.The results shows the effect of changes was found to bethe % RSD and within theacceptance criteria valuesobserved withinstandard limit of not more than 5%.Hence the method is robust.

TableNo.4systemprecisiondataandMethodprecisiondataforEstimationofNebivolol and Valsartan

SystemPrecisionData							
Conc.(µg/ml)	**Area**	**Mean Area ± S.D.(n=3)**	**%R.S.D**	**Conc.(µg/ml)**	**Area**	**Mean Area ± S.D.(n=3)**	**%R.S.D**
Nebivolol				**Valsartan**			
50.00 µg/ml	745.257	370.149±2.709	0.617 %	800.00 µg/ml	11296.759	11388.51±0.79	0.79%
	753.986				11384.782		
	752.282				11483.985		
MethodPrecisionData							
Area	**%Dissolution**	**MeanArea ± S.D.(n=6)**	**%R.S.D**	**Area**	**%Dissolution**	**MeanArea ± S.D.(n=6)**	**%R.S.D**
Nebivolol				**Valsartan**			
1118.012	100.6243	1121.982 ±7.059	0.629 %	3722.393	100.3781	3701.134 ±22.085	0.599 %
1128.964	101.6100			3696.041	99.66744		
1113.737	100.2395			3678.500	99.19443		
1124.155	101.1772			3733.428	100.6756		
1130.835	101.7784			3694.235	99.61874		
1116.188	100.4601			3682.209	99.29445		

TableNo.5:RobustnessdataforNebivolol and Valsartan

Sr.No.	**Area at pH(+2.0)**	**% DissolutionatpH(+2.0)**	**Area atpH(-2.0)**	**% Dissolutionatp H (-2.0)**
Nebivolol				
1	742.871	97.389	710.767	101.559
2	744.254	100.950	704.921	95.913
3	745.582	97.213	709.038	102.078
%RSD	0.182	2.910	0.424	4.031
Valsartan				
1	11632.316	94.807	11109.866	100.699

2	11616.862	100.953	11121.373	98.165
3	11634.555	95.843	11118.583	98.352
%RSD	0.083	3.269	0.054s	2.351

Analysis of Market formulation and % dissolution ofNebivolol and Valsartan:

Relevancyofthepproposedmethodwasestimatebyanalyzing the commercially available Tablet formulationofNebicard-V.DuringTheresults ofassayand%dissolutionarecalculated.TheaverageareaofNEB observed 1121.982 however % dissolution ofNEBobserved100.98%.TheaverageareaofVALobserved3701.134 however%dissolutionof VAL observed 99.80 %. The results indicatethat the developed method is simple, precise, accurate,and rapid. It can be used in the regular quality controltestofformulationin industries.

CONCLUSION:

There is no analytical work has been available regardingdissolutionRP-HPLCmethodforNebivolol and Valsartan in a literature. It is the new efforts in a area of research has been made to validate and develop dissolution method viaRP-HPLC.Conclusively,thedissolution method via RP HPLC-method described in this paper is specific, sensitive,rapid and easy to perform.The proposed dissolution test method was successfully validated in terms of specificity, precision,linearity and robustness as per ICH guidelines. It can be concluded that the proposed method can be employed for routine dissolution analysis of Nebivolol and Valsartan in pharmaceutical tablets.

ABBREVIATIONS:

RP HPLC:ReversePhaseHighPerformanceLiquidChromatography; pH:PotentialofHydrogen; mm:millimetre; ml: Milliliters; M: Molar; µm: Micrometer;nm: nanometer; LOQ: Limit of quantitation; LOD: Limitofdetection; ICH:InternationalConferenceonHarmonization; NLT: Not less than; NMT: Not morethan; %RSD:Relativestandarddeviation; min:Minutes;Rs:Resolution; SD:Standard deviation;°C:Degree Celsius; mg: Milligrams; µg: Microgram; v/v:Volume/volume; %: Percentage; NEB: Nebivolol; VAL: Valsartan; UV:Ultraviolet; pvt:private; g:gram; ppm:partspermillion; fig: figure.

CONFLICTS OF INTERESTS:

The authors declare no conflicts of interest.

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