Simultaneous Estimation of Amlodipine and Valsartan Inpure and Pharmaceutical Dosage form by Using RP-HPLC Method

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Abstract-A simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Amlodipine and Valsartan in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved on a Symmetry C18 column (4.6 × 150 mm, 5 µm) using a mobile phase consisting of Methanol and Triethylamine (TEA) buffer (pH 4.2) in the ratio of 40:60 v/v. The flow rate was maintained at 1 ml/min, with a column temperature of 40ºC. Detection was carried out at 260 nm using a Waters HPLC system equipped with an autosampler and PDA Detector (996 model). The injection volume was 10 µl, and the total run time was 6 minutes. The method exhibited good linearity, accuracy, precision, and specificity as per ICH guidelines. It was successfully applied for the quantitative analysis of Amlodipine and Valsartan in tablet formulations, demonstrating its suitability for routine quality control applications.

Keywords: Amlodipine, RP-HPLC, simultaneous estimation, Symmetry C18 column, Validation, Valsartan.

I.INTRODUCTION

Chromatography is an effective analytical method employed to separate and examine complex mixtures of substances. This technique involves the distribution of components between a stationary phase, which is typically a solid or liquid, and a mobile phase, which can be a liquid or gas. The fundamental concept of chromatography relies on the varying interactions of mixture components with the stationary and mobile phases1. This results in components moving at different speeds, ultimately leading to their separation. Chromatography finds extensive application across various domains, such as chemistry, biochemistry, pharmaceutical analysis, environmental monitoring, and food safety. There are several forms of chromatography, including gas chromatography (GC), liquid chromatography (LC), and thin-layer chromatography (TLC), among others2. High-Performance Liquid Chromatography (HPLC) is a sophisticated and prevalent type of liquid chromatography. It is an advanced method designed to separate, identify, and quantify compounds within a mixture. HPLC has transformed analytical chemistry due to its high resolution, precision, and adaptability, establishing itself as a fundamental tool in pharmaceutical, clinical, environmental, and food testing1-3.

CHROMATOGRAPHIC PARAMETERS

Capacity factor ((k')

The capacity factor is a measure of the degree of retention time of an analyte relative(tR) to an unretained peak(t0). It is affected by solvent composition, separation, aging and temperature of separation. The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 213.

Resolution (Rs)

Resolution is ability of a column to separate Chromatographic peaks. Resolution can be improved by increasing column length, decreasing particle size, increasing temperature, changing the eluent or stationary phase.

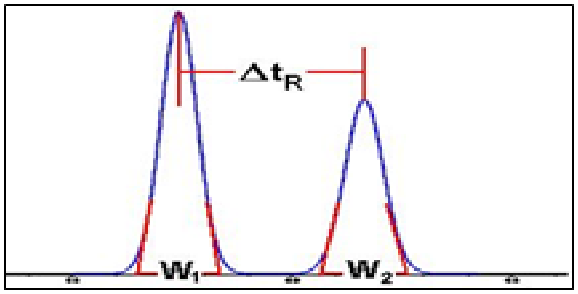

Fig-1: Resolution of peaks

$$R_S = \frac{(t_{R_1} - t_{R_2})}{0.5(t_{W_1} + t_{W_1})}$$

Where,

tR1,tR2 are retention times of the first and second adjacent bands.

tW1and tW2are baseline at bandwidth of the peaks for reliable quantitation well-separated peaks is essential for quantitation.

Rs of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc.) are desirable13.

Theoretical plate number / Efficiency (N)

A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. Smaller the band spread higher the theoretical plate indicates good column and system performance. A theoretical plate is an imaginary or hypothetical unit of a column where equilibrium has been established between stationary phase and mobile phase. Theoretical plate number is a measure of column efficiency that is, how many peaks can be located per unit run-time of the Chromatogram.

$$N = 16[t/w_b]^2$$

Where,

t =retention time for the sample peak

wb = peak width.

$$H = \frac{L}{N}$$

N is fairly constant for each peak on a Chromatogram with a fixed set of operating conditions. H (height) or HETP (height equivalent of a theoretical plate) measures the column efficiency per unit length (L) of the column.The theoretical plate number depends on elution time but in general should be > 200013.

Tailing factor (T) or Asymmetry factor (AF)

It is a measure of the symmetry of a peak.

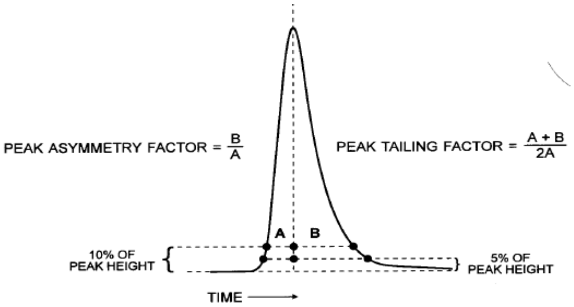

Fig-2: Peak Asymmetry

$$T = \frac{W}{2f}$$

Where, W= peak width at 5 % height,

f = distance from peak front to apex point at 5% height.

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak end and hence the calculation of the area under the peak16.

For the samples that contain acidic or basic compounds, retention may vary with both the ionic strength and pH of the buffer. If the pKa values are known for a group of compounds to be separated by RP-HPLC, and if these pKa values are different, then it is likely that a pH near (±2 units) the average pKa value of the mixture should provide good separation. The recommended additives for the reversed-phase mobile phases in the case of samples that contain acids, bases or salts are given in Table-1.313. A temperature of 40-600C appears to be convenient in reversed phase-LC, when sample component permits. Compared to ambient conditions operation at these temperatures usually doubles the column efficiency in terms of number of theoretical plates.

Documentation of analytical figures of merit

The originally determined analytical figures of merit Limit of Quantitation (LOQ), Limit of Detection (LOD), Linearity, time per analysis, cost, sample preparation etc., are documented17.

Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average ± standard deviation) from sample to sample and whether recovery has been optimized is determined. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainity.

The validity of analytical method can be verified only by laboratory studies. Therefore
Therefore documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications. High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. In now a day pharmaceutical market, many newer antihypertensive combination drugs are available to control hypertension as well as to keep society healthy and stress free. All presently available old drugs have frequent dosing produces various side effects. So, there is need to analyse such antihypertensive drugs. The aim of this review is to analysed such commonly used antihypertensive combination drugs by using reverse phase high performance liquid chromatography (RP-HPLC). Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity.The present study is to develop and validate a reliable, efficient, and sensitive Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Amlodipine and Valsartan in pure and pharmaceutical dosage forms. This method should provide a means of accurately quantifying both drugs in complex formulations, ensuring quality control and proper dosage in pharmaceuticals.The proposed method was validated as per ICH guidelines in terms of linearity, accuracy, precision, robustness and specificity study. The method has been applied to Amlodipine and valsarta formulation without the interference of excipients of the formulation. Amlodipine Chemically 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-3,5-pyridine dicarboxylic acid diethyl ester and extensively used as Calcium Channel Blocker (CCB). Valsartan Chemically N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-N-ethylethanamine and used as Antihypertensive agent.

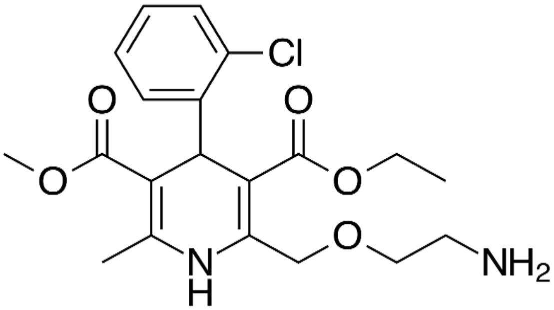

Fig-3: structure of amlodipine

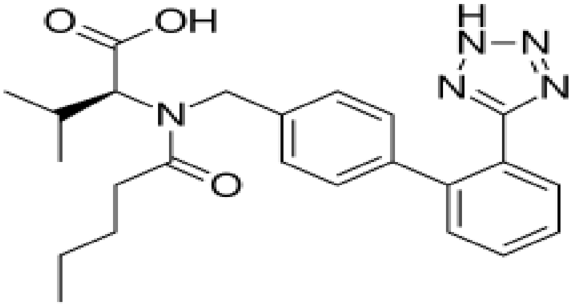

Fig-4: Stucture of valsartan

II.MATERIALS AND METHOD

HPLC Method Development:
Optimized chromatographic conditions:
Temperature : 40ºC
Column : Symmetry C18 (4.6×150mm, 5µ)
pH :4.2
Mobile phase:Methanol:TEA buffer pH 4.2 (40:60v/v)
Flow rate : 1ml/min
Wavelength : 260 nm
Injection volume : 10 µl
Run time : 6 min

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Amlodipine and Valsartan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.75 ml of Amlodipine and 1.125 ml of Valsartan from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Preparation Of Buffer And Mobile Phase:
Mobile Phase Optimization:
Initially the mobile phase tried was methanol: water with varying proportions. Finally, the mobile phase was

optimized to methanol: TEA Buffer in proportion 40:60 v/v respectively.

Preparation of Triethylamine (TEA) buffer (pH-4.2): Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Filter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase:
Accurately measured 400 ml (40%) of Methanol and 600 ml of TEA buffer (60%) a were mixed and degassed in digital Ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the diluent.

Optimization of Column:
The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow. Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

VALIDATION PARAMETERS
System Suitability
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity Study of Drug:
Linearity
Preparation of Sample Solution:
Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Amlodipine and Valsartan sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.75 ml of Amlodipine and 1.12 ml of Valsartan above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula

Preparation Of Drug Solutions For Linearity:
Accurately weigh and transfer 10 mg of Amlodipine and 10 mg of Valsartan working standard into a 10 mL of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Preparation of Level – I to V solutions: Pippete out 0.25 ml(25 ppm), 0.50 ml(50 ppm), 0.75 ml (75 ppm), 1.00 ml(100 ppm), 1.25 ml(125 ppm) of Valsartan and 0.375 ml(37.5 ppm), 0.75 ml(75 ppm), 1.125 ml (112.5 ppm), 1.5 ml(150 ppm), 1.875 ml(187.5 ppm) of Amlodipine stock solutions take in a 10ml of volumetric flask dilute up to the mark with diluent. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision
Repeatability
The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision/Ruggedness
To evaluate the intermediate precision of the method, Precision was performed on different days by maintaining same conditions.

Procedure: On Day1 and Day2 the standard solution were injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:
For preparation of 50-150% Standard stock solution
Accurately weigh and transfer 10 mg of Amlodipine and 10 mg of Valsartan working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.375 ml(50%), 0.75 ml(100%), 1.125 ml(150%) of Amlodipine and 0.562 ml(50%), 1.125 ml(100%), 1.6875 ml (150%) of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Three replicate injections of individual concentrations (50%,

100%,150%) were injected under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Valsartan and Amlodipine and calculate the individual recovery and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded

Effect of Variation of mobile phase organic composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer was taken in the ratio and 35:65, 45:55 instead (40:60), remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Method Validation

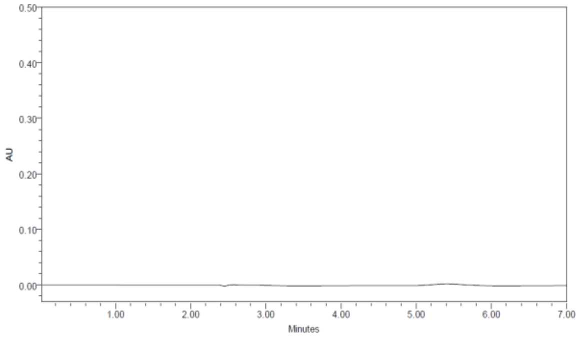

Fig.5- Blank chromatogram

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates the drugs in drug product.

The % purity of Amlodipine, Valsartan in pharmaceutical dosage form was found to be 100. 9%, 100.7% respectively. The results of standard and sample assay shown in Table-2.

Linearity

The response linearity is verified if the Correlation Coefficient is 0.99 or greater. Correlation Coefficient (r) is 0.99, and the intercept is 4195. These values meet the validation criteria and the results shown in table-4.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions the results shown in table-5.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD. The results shown in table-

Intermediate Precision:

Results of Intermediate precision for Amlodipine and Valsartan on day1&day2 shown in table-6.

Accuracy:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated. Results of Accuracy for concentration-50%,100%,150% shown in table-8.

Limit Of Detection

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

Result- Amlodipine= 2.4µg/ml, Valsartan= 2. 19µg/ml.

Conclusion:

The method showed good resolution between the peaks of Amlodipine and Valsartan, with acceptable system suitability parameters including retention time, peak symmetry, and theoretical plates. The developed RP-HPLC method is simple, accurate, and reliable for the simultaneous estimation of Amlodipine and Valsartan in pure drug and combined pharmaceutical formulations. The optimized chromatographic conditions ensure efficient separation with short run time, making the method suitable for high-throughput analysis in quality control laboratories. Its validation as per ICH guidelines further confirms its applicability for routine use in pharmaceutical analysis.

Table – 1. Peak Results for Optimized Condition of standard and sample

S. No.	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amlodipine(Standard)	2.781	2774027	299752		1.2	6314
2	Valsartan(Standard)	4.048	2533532	210321	4.6	1.3	5521
3	Amlodipine(sample)	2.773	2770123	282157		1.6	5011
4	Valsartan(sample)	4.065	2522041	251068	3.3	1.5	5947

Table-2.Peak results for assay standard of Amlodipin&Valsartan

Drug	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count	
Amlodipine	Injection-1	2.767	2762937	357421		1.3	6344.7	Mean area: 2771306 Std. Dev: 7321.9 % RSD: 0.26
	Injection-2	2.795	2774613	388745		1.3	6344.2	
	Injection-3	2.768	2776429	364121		1.3	6344.2	
Valsartan	Injection-1	4.029	2534375	210326	4.6	1.3	5937.7	Mean area: 2535604 Std.Dev: 10085.82 % RSD: 0.397768
	Injection-2	4.067	2526189	226741	4.7	1.3	5008.8	
	Injection-3	4.030	2546248	231494	4.7	1.3	5990.7	

Table -3. Peak results for Assay samples

S.No.	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Amlodipine	2.764	2732203	294531		1.3	6314	1
2	Valsartan	4.012	2507543	216321	4.6	1.3	5954	1
3	Amlodipine	2.767	2751843	286473		1.3	6369	2
4	Valsartan	4.016	2509101	216354	4.6	1.3	5944	2
5	Amlodipine	2.764	2744776	312684		1.3	6329	3
6	Valsartan	4.013	2515628	206571	4.6	1.3	5990	3

Table-4.Chromatographic Data For Linearity Study

Drug	Concentration µg/ml	Average Peak Area
Amlodipine	37.5	892464
	75	1866364
	112.5	2777423
	150	3709213
	187.5	4601317
Valsartan	25	920032
	50	1752782
	75	2521426
	100	3326009
	125	4217393

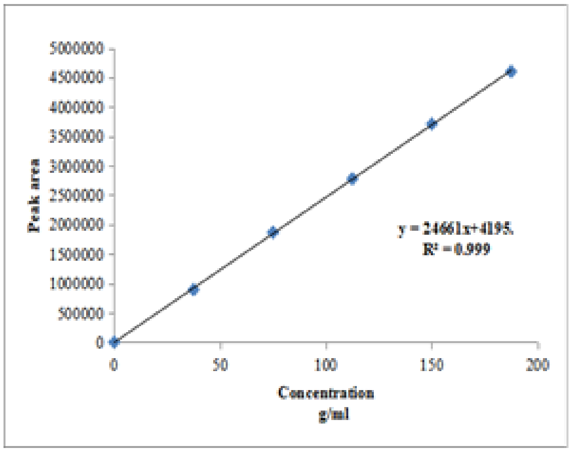

Fig-6. calibration graph forAmlodipine

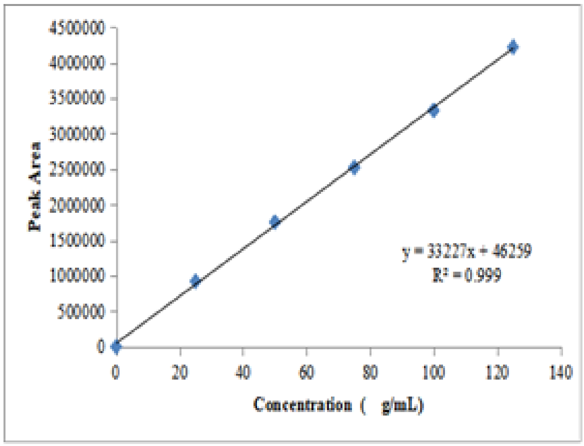

Fig-7.calibration graph for Valsartan

Table-5.:Results of method precision for Amlodipine and Valsartan

Drug	Name	Rt	Area	Height	USP plate count	USP Tailing	
Amlodipine	Injection-1	2.766	2766870	294578	6684	1.3	Mean 2776159 Std. Dev 8969.6 % RSD 0.32
	Injection-2	2.774	2771971	286541	6347	1.3	
	Injection-3	2.770	2771958	302657	6674	1.3	
	Injection-4	2.772	2780299	293412	6451	1.3	
	Injection-5	2.771	2789695	283154	6678	1.3	
Valsartan	Injection-1	4.025	2534539	193240	5761	1.3	Mean 2545221 Std. Dev 9330.0 %RSD 0.37
	Injection-2	4.040	2539247	201647	5489	1.3	
	Injection-3	4.032	2544661	193472	5367	1.3	
	Injection-4	4.041	2548839	196475	5845	1.3	
	Injection-5	4.036	2558822	201394	5347	1.3	

Table 6-: Results of Intermediate precision

Drug	Day	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution	
Amlodipine	Day1	Injection-1	2.781	2715421	294651	6647	1.3		Mean area: 2764338 Std. Dev 25974 % RSD 0.9
		Injection-2	2.780	2778540	284123	6781	1.3		
		Injection-3	2.782	2754247	274561	6984	1.3		
		Injection-4	2.780	2780545	281241	6475	1.3		
		Injection-5	2.782	2777021	286471	6647	1.3		
		Injection-6	2.774	2780254	294512	6489	1.3		
Valsartan	Day1	Injection-1	4.048	2506927	211541	5495	1.4	4.6	Mean area: 2511662 Std. Dev 14572.01 % RSD 0.5
		Injection-2	4.050	2504522	206141	5694	1.4	4.6	
		Injection-3	4.049	2541270	198641	5785	1.4	4.7	
		Injection-4	4.050	2507885	206741	5947	1.4	4.6	
		Injection-5	4.049	2504587	209487	5742	1.4	4.6	
		Injection-6	4.040	2504780	193481	5914	1.4	4.6	
Amlodipine	Day2	Injection-1	2.764	2781856	294651	6647	1.3		Mean area: 2775009 Std. Dev 16222.05% RSD 0.5
		Injection-2	2.759	2761510	284123	6781	1.3		
		Injection-3	3.015	2748811	274561	6984	1.3		
		Injection-4	2.773	2790831	281241	6475	1.3		
		Injection-5	2.765	2785112	286471	6647	1.3		
		Injection-6	2.764	2781932	294512	6489	1.3		
Valsartan	Day2	Injection-1	4.015	2536301	211541	5495	1.4	4.6	Mean area:

		Injection-2	4.007	2541972	206141	5694	1.4	4.6	2537131 Std. Dev 9370.087% RSD 0.36
		Injection-3	4.323	2521259	198641	5785	1.4	4.7	
		Injection-4	4.065	2537081	206741	5947	1.4	4.6	
		Injection-5	4.020	2549869	209487	5742	1.4	4.6	
		Injection-6	4.015	2536301	193481	5914	1.4	4.6	

Table-7.Robustness results of amlodipine&Valsartan

Drug	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
amlodipine	Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
	Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
	More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
	Less organic phase	2888515	3.326	6298	1.4
	More organic phase	2541550	2.416	6287	1.2
Valsartan	Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
	Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
	More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5
	Less organic phase	2754017	5.318	5309	1.4
	More organic phase	2215870	3.233	5580	1.51

Table 8. Results of Accuracy for concentration 50%,100%,150%

Concentration of drug(%)	Drug	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
50%	Amlodipine	2.799	1387991	294712		1.3	6914	1
	Valsartan	4.105	1303532	216471	4.6	1.3	5614	1
	Amlodipine	2.779	1383988	283147		1.3	6745	2
	Valsartan	4.079	1302671	217465	4.6	1.3	5694	2
	Amlodipine	2.775	1375831	281364		1.3	6347	3
	Valsartan	4.043	1314767	203471	4.6	1.3	5647	3
100%	Amlodipine	2.781	2771991	294612		1.3	6874	1
	Valsartan	4.048	2506681	216169	4.6	1.3	52614	1
	Amlodipine	2.780	2783988	283247		1.3	6845	2
	Valsartan	4.050	2506927	217415	4.6	1.3	5364	2
	Amlodipine	2.782	2775831	281364		1.3	6674	3
	Valsartan	4.049	2518275	203261	4.6	1.3	5496	3
150%	Amlodipine	2.777	4237191	324622		1.3	6921	1
	Valsartan	4.032	3819721	296749	4.6	1.3	5631	1
	Amlodipine	2.773	4034381	334695		1.3	6864	2
	Valsartan	4.021	3804001	291461	4.6	1.3	5318	2
	Amlodipine	2.765	4163055	376212		1.3	6691	3
	Valsartan	4.009	3710275	284612	4.6	1.3	5491	3

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