ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

A STUDY OF METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES FOR THE SIMULTANEOUS QUANTIFICATION OF AMLODIPINE AND VALSARTAN IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

Malepati Ramakrishna[1]*, Gade Anantha Lakshmi[2], J.N.Suresh Kumar[3]

1. Associate professor, Department of Pharmaceutical Analysis, Narasaraopeta institute of Pharmaceutical Sciences, Narasaraopeta -522601, Palnadu (Dist), Andhra Pradesh
2. Student, Narasaraopeta institute of Pharmaceutical Sciences, Narasaraopeta -522601, Palnadu (Dist), Andhra Pradesh
3. Principal, Narasaraopeta institute of Pharmaceutical Sciences, Narasaraopeta -522601, Palnadu (Dist), Andhra Pradesh

Corresponding author E- mail: ramakrishna0922@gmail.com

ARTICLE INFO

Key words:

Valsartan with amlodipine, RP-HPLC, precision, accuracy, and validation.

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

For the purpose of testing amlodipine and valsartan in both their pure and pharmaceutical dosage forms, a rapid and efficient reverse phase high performance liquid chromatography technique has been developed. The Kromasil, C18, ODS (4.6 mm × 250 mm) 5µm particle size was used. column with a 1 ml/min flow rate and a mobile phase consisting of a 30% v/v combination of methanol and phosphate buffer (pH-2.8). At 238 nm, the measurement was made. Amlodipine and valsartan were shown to remain in the body for 2.133 and 3.692 ± 0.02 minutes, respectively. Amlodipine concentrations between 20 and 60 µg/ml and Valsartan concentrations between 10 and 30 µg/ml are compatible with the new approach. The test method's accuracy was less than 2.0%RSD. The technique may be used to evaluate the quality of both commercial and large-scale pharmaceutical formulations.

INTRODUCTION:

The area of chemistry known as analytical chemistry1 deals with the separation, identification, and calculation of the relative quantities of the constituents of a sample of matter. It primarily focuses on the quantitative measurement of the compounds found in bulk and pharmaceutical preparation as well as the qualitative identification or detection of chemicals

1.1 INTRODUCTION TO HPLC:

A liquid mobile phase and a very finely separated stationary phase are used in HPLC, a kind of liquid chromatography. Liquid must be pressurized to a few thousand pounds per square inch in order to achieve an appropriate flow rate.

The diffusion mechanism regulates the rate of drug transfer between the stationary and mobile phases. Faster and more efficient

separation might result from reducing diffusion. Because they perform better than conventional column chromatography, which improves column chromatography into a fast, accurate, efficient, and highly resolved separation method, high performance liquid chromatography methods receive their name. The most recent research used propranolol and clonazepam to quantify the quantity of analyte in the formulation and bulk medication. Because of its low detection limit, precision, accuracy, linearity, robustness, and other benefits, the HPLC technique is favored in the area of analytical chemistry.

- Many analyses may be completed in 20 minutes or less.
- Increased sensitivity (it may be required to use alternative detectors).
- A greater range of stationary phases due to improved resolution.
- Reusable columns, which are costly but useful for many investigations.
- Perfect for low-viscosity materials.
- Simple sample handling, maintenance, and recovery.
- Automation and quantification (less labour and time) are the results of instrumentation.
- The computations are performed by the integrator.
- Suitable for considerably larger-scale preparative liquid chromatography.

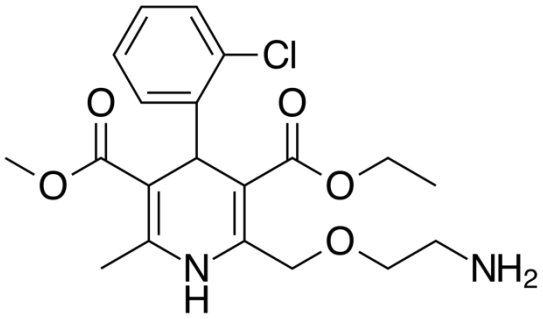
2. DRUG PROFILE

DRUG PROFILE OF AMLODIPINE

Name of the Drug: Amlodipine

Amlodipine is a synthetic dihydropyridine and calcium channel blocker with antihypertensive and antianginal properties. By preventing extracellular calcium ions from entering myocardial and peripheral vascular smooth muscle cells, amlodipine inhibits cardiac and vascular contraction. This results in the dilatation of the principal coronary and systemic arteries, a reduction in myocardial contractility, an increase in blood flow and oxygen delivery to the heart tissue, and a decrease in total peripheral resistance. This medicine may also change how multi-drug resistance (MDR) functions by inhibiting the p-glycoprotein efflux pump.

Chemical Structure:



IUPAC Name: 3-O-ethyl 5-O-methyl 2-(2-aminoethoxy methyl)-4-(2-chloro phenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate

Molecular Formula: $C_{20}H_{25}ClN_2O_5$

Molecular Weight: 408.876g/mol

Pka Value (Strongest Basic): 9.45

Melting Point: 199-201^{0}C

Log P: 1.64

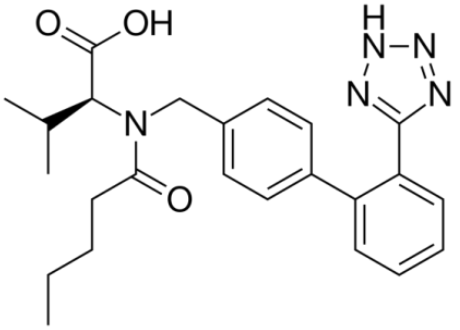
Bioavailability: Following oral administration, bioavailability is 60 to 65% and plasma concentrations rise gradually to peak 6 to 8h after administration.

DRUG PROFILE FOR VALSARTAN

Name: Valsartan

Description: Valsartan is an orally active nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive properties. Valsartan selectively and competitively blocks the binding of angiotensin II to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland, preventing AT II-mediated vasoconstriction, aldosterone synthesis and secretion, and renal reabsorption of sodium, and resulting in vasodilation, increased excretion of sodium and water, a reduction in plasma volume, and a reduction in blood pressure.

Chemical Structure:



Molecular Weight: 435.5188g/mol

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

IUPAC Name: (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid

In order to reduce the risk of both fatal and nonfatal cardiovascular events, primarily myocardial infarctions and strokes, valsartan is used to treat hypertension. Additionally, heart failure (NYHA class II–IV) and left ventricular dysfunction or failure after myocardial infarction may be treated with angiotensin-converting enzyme inhibitors (ACEIs) when their usage is inappropriate.

3. REVIEW OF LITERATURE

Nagamani P. et al. (2020): Reported an RP-HPLC method for simultaneous estimation of amlodipine besylate and celecoxib in tablet dosage forms. The method was validated according to ICH guidelines and showed satisfactory accuracy, precision, linearity, and recovery.

Tejaswini Rajaram Mandale et al. (2020): Developed a UV spectrophotometric method for simultaneous estimation of amlodipine besylate and celecoxib. Validation studies confirmed good linearity, precision, accuracy, and recovery, indicating its suitability for routine analysis.

Chiranjeevi P. et al. (2022): Established an RP-HPLC method for simultaneous estimation of amlodipine besylate and celecoxib in fixed-dose tablets. The validated method demonstrated acceptable accuracy, precision, robustness, and recovery.

Somsubhra Ghosh et al. (2013): Developed an RP-HPLC method for simultaneous estimation of valsartan and aliskiren hemifumarate. The method showed good specificity, accuracy, precision, linearity, and robustness for routine pharmaceutical analysis.

4. AIM AND OBJECTIVES

The literature analysed the physical and chemical properties of amlodipine and valsartan as well as various analytical methods used both on their own and in combination with other amlodipine and valsartan.

According to a study of the literature, amlodipine and valsartan may be measured simultaneously using a single RP-HPLC procedure.

Given the need for a suitable RP-HPLC method for routine analysis of these drugs in formulations, efforts were made to develop a simple, accurate, and precise analytical method for the simultaneous estimation of amlodipine and valsartan and to extend it for their determination in formulation.

The primary objective of the proposed effort is:

to develop a new, simple, sensitive, accurate, and economical analytical method for the simultaneous assessment of valsartan and amlodipine in both their pure and pharmaceutical dosage forms.to confirm that the recommended method complies with USP and ICH requirements for the planned analytical application, which is to use the recommended method for amlodipine and valsartan dosage form analysis

5. METHODOLOGY & INSTRUMENTS

Table-4: Instruments used

S. No	Instruments & Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes & Burettes	Borosil
6	Beakers	Borosil
7	Ultra sonicator	Labman

CHEMICALS USED:

Table-5: Chemicals used

S.No	Chemical	Brand Name
1	Amlodipine (Pure)	Synpharma Research Lab, Hyderabad
2	Valsartan (Pure)	Synpharma Research Lab, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Acetic Acid	Merck

Rp-HPLC METHOD DEVELOPMENT:

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 0.4ml of Amlodipine and 0.2ml of Valsartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

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.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (pH-2.8) in proportion 30:70% v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like Symmetry, X terra and ODS column. Kromasil, C_{18}, ODS (4.6mm×250mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

6. RESULTS AND DISCUSSION

Optimized Chromatographic Conditions:

Instrument used	Waters Alliance 2695 HPLC with PDA Detector 996 model
Temperature	Ambient
Column	Kromasil, C_{18}, ODS (4.6mm×250mm) 5µm particle size
Mobile phase	Methanol and Phosphate Buffer (pH-2.8) (30:70% v/v)
Flow rate	1.0ml/min
Wavelength	238nm
Injection volume	20µl
Run time	6minutes

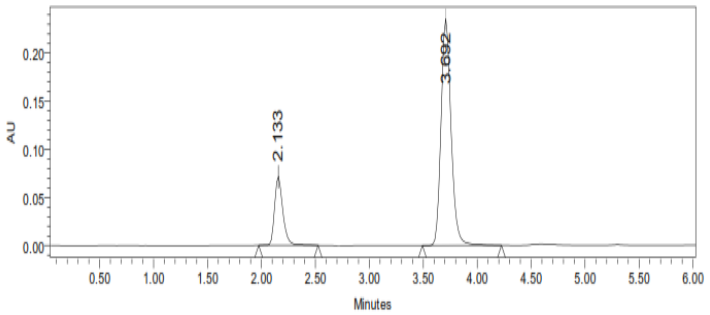


Figure-14: Optimized Chromatogram (Standard)

Table-16: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Amlodipine	2.133	526389	86756	1.56	5679	
2	Valsartan	3.692	1687285	367532	1.79	8685	9.8

Observation: From the above chromatogram it was observed that the Amlodipine and Valsartan peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

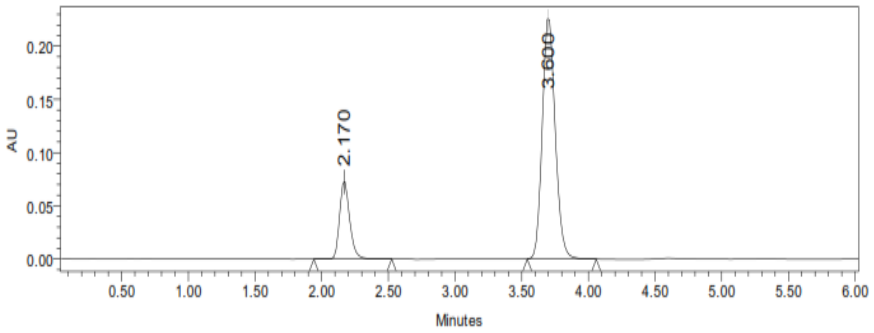


Figure-15: Optimized Chromatogram (Sample)

Table-17: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Amlodipine	2.170	534514	87568	1.61	5786	
2	Valsartan	3.600	1796854	375424	1.82	8769	10.01

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank:

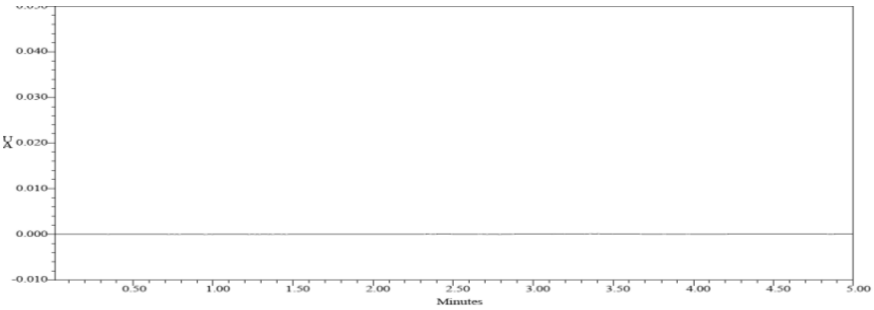


Fig-16: Chromatogram showing blank Solution (Mobile Phase Preparation)

Results of system suitability for Amlodipine

S.No.	Peak Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Amlodipine	2.152	526358	86598	5695	1.56
2	Amlodipine	2.157	526548	86254	5652	1.57
3	Amlodipine	2.141	526854	86598	5627	1.56
4	Amlodipine	2.133	526598	86245	5692	1.57
5	Amlodipine	2.166	524874	86521	5641	1.56
Mean			526246.4			
Std. Dev.			787.353			
% RSD			0.149617			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table-19: Results of system suitability for Valsartan

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Valsartan	3.674	1682821	1686958	8659	1.56	9.8
2	Valsartan	3.631	1682726	1685745	8675	1.57	9.9
3	Valsartan	3.625	1687361	1685421	8692	1.56	9.8
4	Valsartan	3.692	1682811	1685242	8642	1.57	9.8
5	Valsartan	3.629	1683816	1685364	8635	1.58	9.8
Mean			1683907				
Std.			1982.03				
% RSD			0.117704				

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

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 quantitate Amlodipine and Valsartan in drug product.

Assay (Standard):

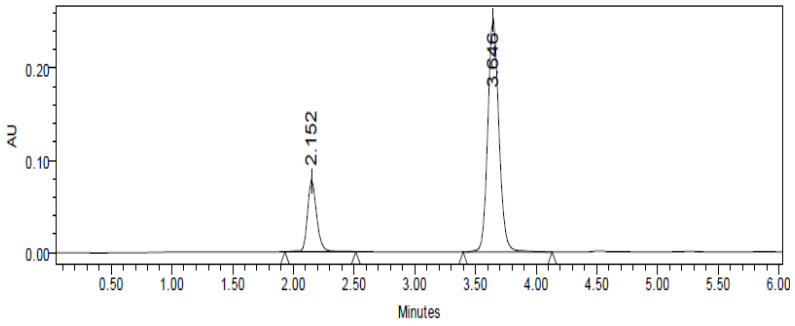


Fig-22: Chromatogram showing assay of standard injection -1

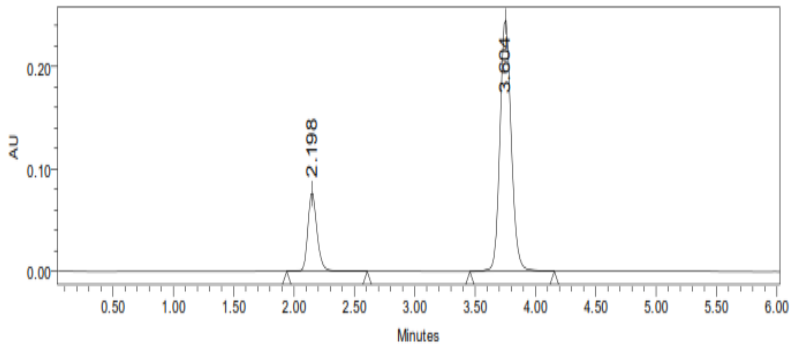


Fig-23: Chromatogram showing assay of standard injection -2

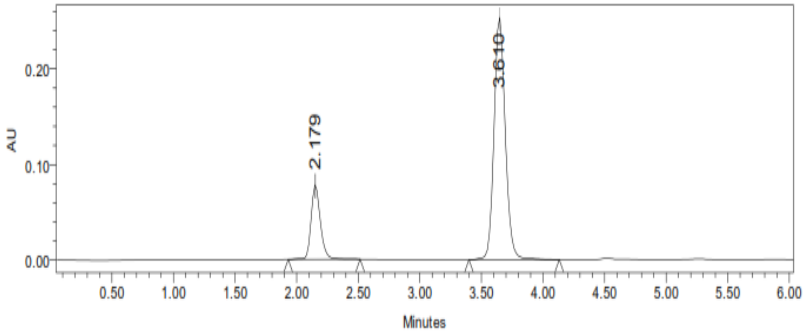


Fig-24: Chromatogram showing assay of standard injection -3

Table-20: Peak results for assay standard of Amlodipine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Amlodipine	2.152	526358	86598	1.56	5698	1
2	Amlodipine	2.198	526584	86784	1.57	5687	2
3	Amlodipine	2.179	529658	86253	1.56	5639	3

Table-21: Peak results for assay standard of Valsartan

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Valsartan	3.646	1687589	365879	1.80	8659	1
2	Valsartan	3.604	1685987	365854	1.79	8697	2
3	Valsartan	3.610	1685974	369854	1.80	8675	3

Assay (Sample):

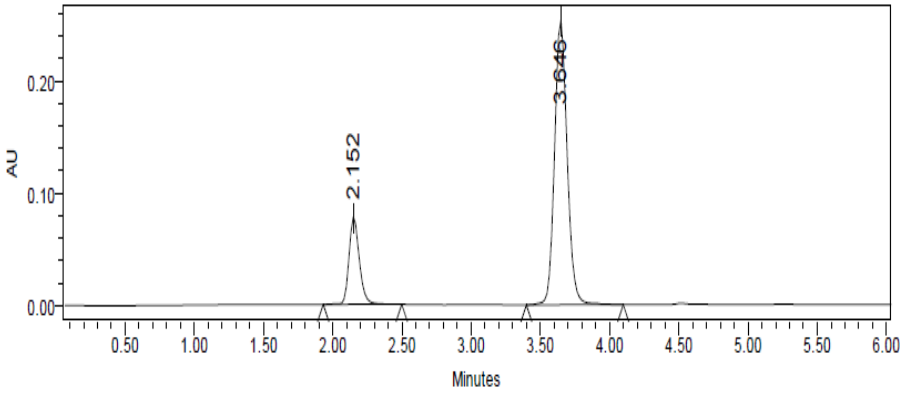


Fig-25: Chromatogram showing assay of sample injection-1

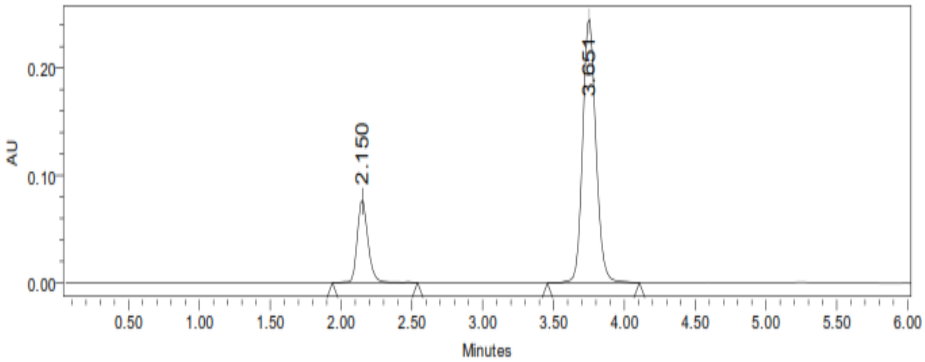


Fig-26: Chromatogram showing assay of sample injection-2

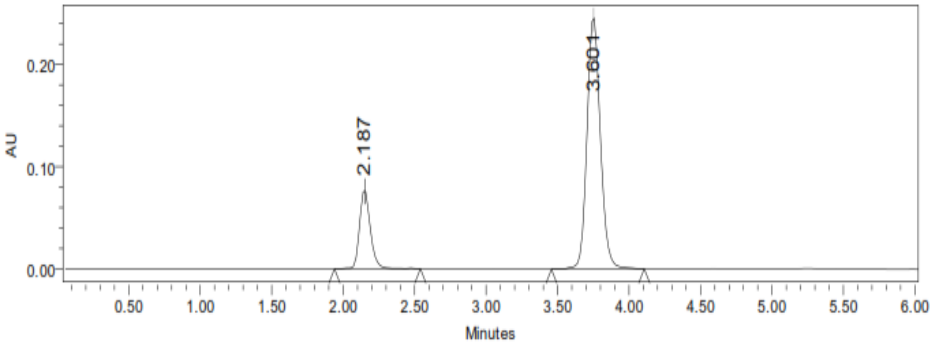


Fig-27: Chromatogram showing assay of sample injection-3

Table-22: Peak results for Assay sample of Amlodipine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Amlodipine	2.152	536859	87584	1.58	5789	1
2	Amlodipine	2.150	532654	87965	1.59	5784	2
3	Amlodipine	2.187	532685	87465	1.58	5769	3

Table-23: Peak results for Assay sample of Valsartan

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Valsartan	3.646	1698568	378562	1.81	8759	1
2	Valsartan	3.651	1698574	375847	1.80	8795	2
3	Valsartan	3.601	1698547	376584	1.81	8745	3

$$\%\ \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Amlodipine and Valsartan in pharmaceutical dosage form was found to be 99.89%

LINEARITY

Chromatographic Data for Linearity Study of Amlodipine

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

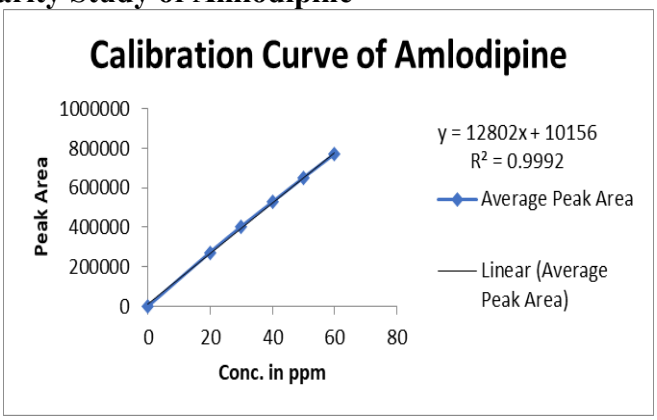


Fig-33: Calibration Curve of Amlodipine

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Amlodipine is a straight line.

Y = mx + c

Slope (m) =12802

Intercept (c) = 10156

Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA:

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

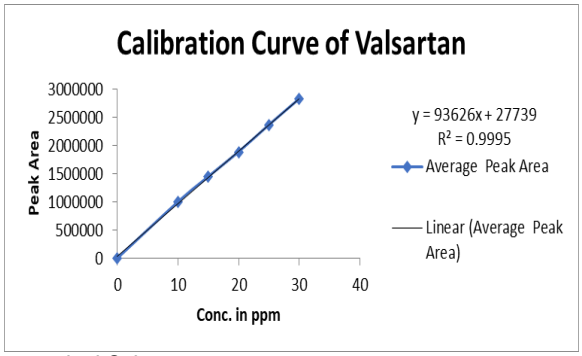
CONCLUSION:

Correlation Coefficient (r) is 0.99, and the intercept is 10156. These values meet the validation criteria.

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF VALSARTAN:

Table-25: Chromatographic Data for Linearity Study of Valsartan

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845



LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Valsartan is a straight line.

$$Y = mx + c$$

Slope (m) = 93626

Intercept (c) = 27739

Correlation Coefficient (r) = 0.99

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 27739. These values meet the validation criteria.

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.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

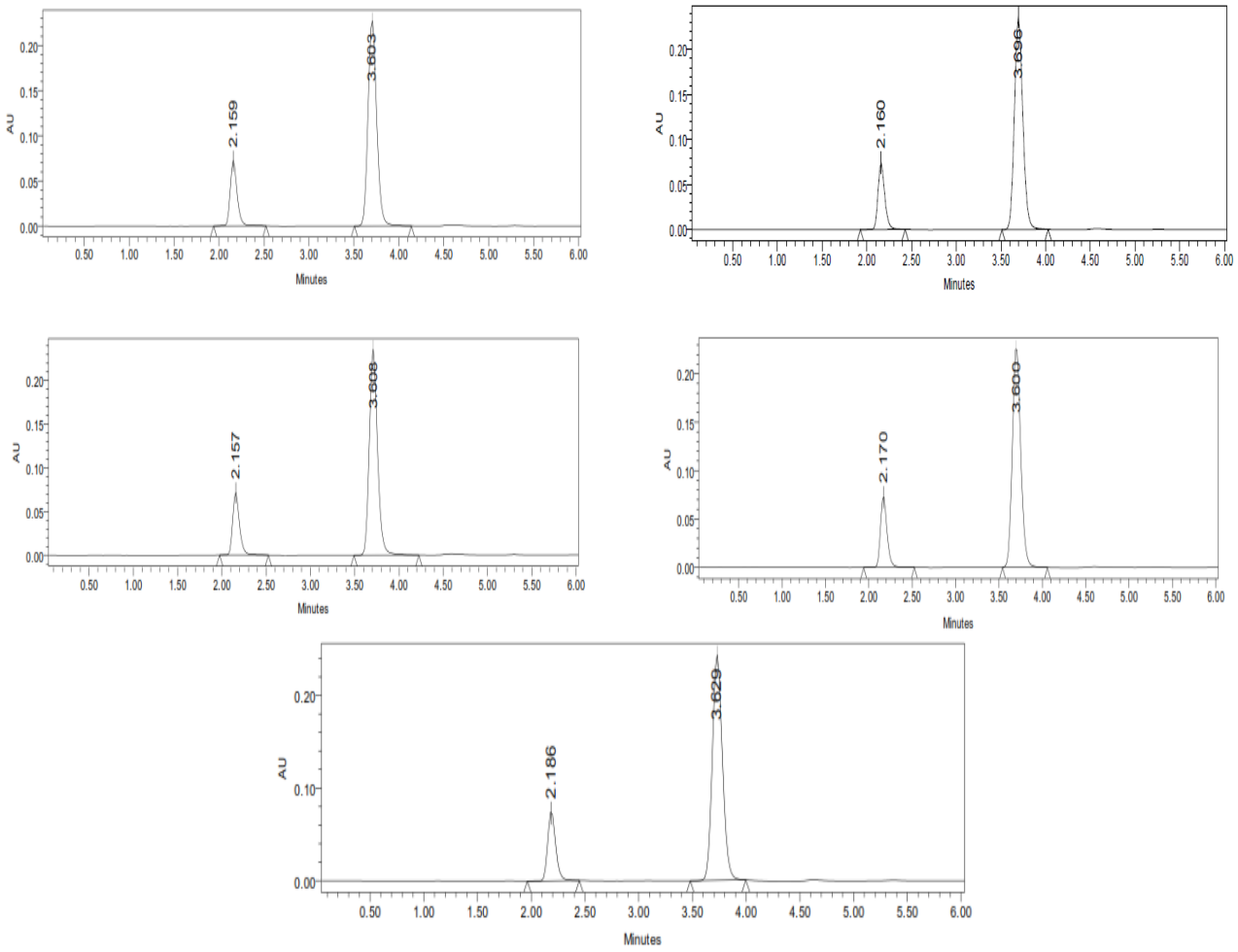


Fig-(35-39): Chromatograms showing precision injection-(1-5)

Table-26: Results of repeatability for Amlodipine:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Amlodipine	2.157	526358	86598	5689	1.56
2	Amlodipine	2.159	524856	86542	5687	1.57
3	Amlodipine	2.186	526985	86578	5684	1.56
4	Amlodipine	2.160	528654	86354	5689	1.56
5	Amlodipine	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

Acceptance Criteria:

- %RSD for sample should be NMT 2
- The %RSD for standard sol is below 1, which is within the limits hence method is precise.

Table-27: Results of Repeatability for Valsartan:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Valsartan	3.603	1687589	367859	8659	1.79
2	Valsartan	3.608	1685987	368547	8679	1.80
3	Valsartan	3.600	1685987	367985	8645	1.80
4	Valsartan	3.696	1685754	365874	8695	1.79
5	Valsartan	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std.Dev			749.493			
%RSD			0.044447			

The accuracy results for Amlodipine

% Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	%Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

Table-36: The accuracy results for Valsartan

%Concentration	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Results for Robustness Amlodipine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table-38: Results for Robustness Valsartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

Acceptance Criteria: The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

7. STABILITY STUDIES

Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	526389	0	100%	100%
2	Acidic	371683.27	29.39	70.61	100%
3	Basic	411794.11	21.77	78.23	100%
4	Oxidative	480645.79	8.69	91.31	100%
5	Thermal	327045.48	37.87	62.13	100%
6	Photolytic	477118.99	9.36	90.64	100%

Table-45: Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1687285	0	100%	100%
2	Acidic	1359614.25	19.42	80.58	100%
3	Basic	1445497.05	14.33	85.67	100%
4	Oxidative	1644427.96	2.54	97.46	100%
5	Thermal	1297353.43	23.11	76.89	100%
6	Photolytic	1632954.42	3.22	96.78	100%

The specificity of the approach may be shown by applying stress conditions using acid, alkaline, peroxide, thermal, UV, and water degradations. The drug's principal peak was tested for peak purity after the sample was treated to these conditions, revealing that the approach effectively extracted the pure active ingredient from the degradation products.

Acid Degradation Studies: 1 ml of 2N HCl was added to 1 ml of Amlodipine and Valsartan stock, and the mixture was refluxed for 30 minutes at 60 ºC. The final volume of 40µg/mL and 20µg/mL solutions was achieved by neutralising the resultant solution with 1 ml of 2N NaOH. A 0.45µm membrane filter should be used to filter the solution when it has cooled to room temperature. To assess the stability of the substance, a 20µl sample was injected into the HPLC system, and the chromatograms were recorded.

8. CONCLUSION

For the purpose of quantitatively measuring amlodipine and valsartan in pharmaceutical dosage forms and bulk pharmaceuticals, the present research developed a simple, sensitive, accurate, and precise RP-HPLC approach.

This method was simple since diluted samples are used right away without any further chemical derivatisation or purification procedures.

Amlodipine was found to be readily soluble in methanol and acetonitrile and to be slightly soluble in water. Since valsartan dissolves in organic solvents including ethanol, dimethyl formamide (DMF), and DMSO, the solvent should be removed using an inert gas. Valsartan dissolves at a rate of around 0.5 mg/ml in ethanol and 20 mg/ml in DMSO and DMF.

Methanol and phosphate buffer (pH-2.8) (30:70% v/v) were found to be the mobile phase. The solvent system used in this process turned out to be economical. Since the %RSD results were within 2, the method was found to be accurate.

The results of the RP-HPLC procedure were positive, as shown by the tables. The RP-HPLC procedure is more precise, accurate, and sensitive than spectrophotometric methods.

This method may be used to regularly determine amlodipine and valsartan in pharmaceutical dose forms and bulk pharmaceuticals.

The specificity of the approach was therefore confirmed by a stability study. During the peak purity study, it was found that the peak threshold was larger than the angle, and neither analyte showed a flag. A degradation study found that amlodipine and valsartan only degraded in hot, acidic environments. Tables 44 and 45 provide the results, whereas figures 65 and 68 display the chromatograms.

9. REFERENCES

1. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method

development and transfer. Lc Gc N. Am, 75, 2002, 49-50.
2. Synder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643-712.
3. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), P1-7.
4. A. Braith Wait and F. J. Smith, Chromatographic Methods, , Kluwer Academic Publisher, (1996), PP 1-2.
5. Andrea Weston and Phyllisr.Brown, HPL C Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
6. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWil ey & Sons, Inc., , (2007), PP 15-23.
7. Chromatography,(online). URL:http://en.wikipedia.org/wiki/Chrom atography.
8. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
9. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
10. D. H. Shewiy, et al., Journal Pharmaceut. Biomed. Anal, **66,** 2012, 11-23.
11. M. D. Rockville, General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopoeial Convention (USP), USP 31, 2009.
12. FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER), 2000.
13. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modeling in HPLC method development. J Adv Res, 3**,** 2012, 53-63.
14. Swartz M, Murphy MB. New Fronties in Chromatography. Lab, 37, 2005, 22-27.
15. Dolan JW. Peak tailing and resolution. Lc GC N. Am, 20, 2002, 430-436.
16. Chan CC, Leo YC, Lam H. Analytical method validation and Instrument Performance Validation. Vol-I, Wiley Interscience, 2004.
17. Shabir GA, Lough WJ., et al., Evaluation and application of best practice in analytical method validation. J Chromatogr RT, **30,** 2007, 311-333.
18. Miller JN, Miller JC, Statistics and chemometrics for analytical chemistry Harlow, Pearson Prentice Hall, 2005, 263.
19. Huber L. Validation of HPLC methods. Bio Pharm. Int, 12, 1999, 64-66.
20. European-commission Quality control procedures for pesticide residues analysis. Report number SANCO/10232/2006.
21. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, IFPMA, Geneva, 2005.
22. Nagamani P*, et al., Development and Validation of RP-HPLC Method for Estimation of Amlodipine Besylate and Celecoxib in Pharmaceutical Formulation, Journal of Drug Delivery & Therapeutics, 2020; 10(6):31-36.
23. Tejaswini Rajaram Mandale, et al., Development and Validation of Analytical Method for Simultaneous Estimation of Amlodipine Besylate and Celecoxib in Pure and Combined Dosage Form, Research J. Pharm. and Tech 2020; 13(9):4280-4284. Doi: 10.5958/0974-360X.2020.00756.8.
24. Sandhya TB, Chiranjeevi P*, Vasudha D, Pushpa D and Renusri B, Estimation of Amlodipine Besylate and Celecoxib in Combined Dosage Form by RP-HPLC, (IJBPAS), IJBPAS, August, 2022, 11(8): 3842-3853.
25. Somsubhra Ghosh1*, et al.,, Method Development and Validation of Aliskiren Hemifumarate and Valsartan in bulk drug by RP-HPLC method, Asian J. Research Chem. 6(1): January 2013, pages: 19-23.
26. Rizwan S H1*, Girija Sastry V2, Development and Validation of Stability Indicating Rp-Hplc Method for the Simultaneous Estimation of Aliskiren Hemifumarate and Valsartan in Bulk and Pharmaceutical Dosage Form, Asian Journal of Pharmaceutical and Clinical Research, vol. 8, no. 6, Nov. 2015, pp. 223-7.