INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Development And Validation of RP-HPLC Method For Simultaneous Estimation of Amlodipine and Valsartan In Its Bulk And Tablet Dosage Form by Using The Quality By Design Approach

Shinde Mahesh*, Jondhale Vidhyatai, Pingale A. P., Dhikale G. K., Derle D. V., Wagh M. P.

NDMVP Samaj's College of Pharmacy, Gangapur road, Nashik-422002

ARTICLE INFO

Received: 22 April 2023
Revised: 24 April 2023
Accepted: 25 April 2023
Published: 26 April 2023

Keywords:
Quality by Design, Amlodipine, Valsartan, Box-Benhken, Design Space, Process Analytical Technology

DOI:
10.5281/zenodo.7866101

ABSTRACT

This study describes the implementation of a Quality by Design approach to develop and validate the RP-HPLC for amlodipine and valsartan. The reaction surface method performs the optimization by adopting the three-level Box Behnken design. The three factors selected are methanol and water concentrations (mobile phase), flow rate, and wavelength. The developed chromatographic method was validated against the ICH Q2(R1) guidelines for linearity, precision, range, precision, LOD, and LOQ. The maximum absorbance of amlodipine and valsartan (λ max) was found to be 245 nm. The optimized method consists of mobile phase Methanol: Water (pH 3.0) (80:20), and flow rate 0.9 ml/min, which was optimized by using design expert software. Linearity of the developed method was established over the concentration range of 1 10 µg/ml for Amlodipine and 30 – 200 µg/ml with correlation coefficient (r2) of 0.997 and 0.9993 respectively. The percent RSD for accuracy and precision of the method was found to be less than 2%. The limit of detection (LOD)was 0.08 µg/ml and 0.89 µg/ml for Amlodipine and Valsartan respectively. The limit of quantitation (LOQ) was 0.02 µg/ml and 2.7 µg/ml for Amlodipine and Valsartan respectively. They are relatively low to permit the determination of low concentrations of the drug.

INTRODUCTION

One of the most essential variables in the quality of pharmaceutical products is the design process. Throughout the product life cycle, the pharmaceutical industry has used the modern concept of Quality by Design (QbD) to apply science-based manufacturing principles to ensure the quality of the formulation and increase efficiencies, provide regulatory relief and flexibility, and offer important business benefits. It encourages the pharmaceutical industry and the

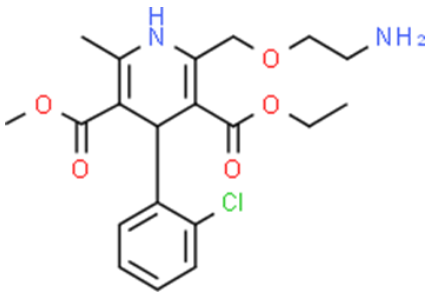
***Corresponding Author:** Mahesh Shinde
Address: *NDMVP Samaj's College of Pharmacy, Gangapur road, Nashik-422002*
Email : shinde111m@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

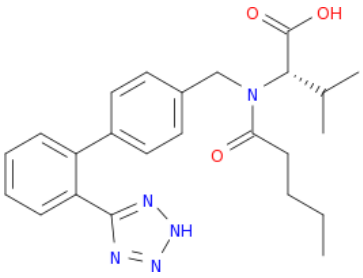
FDA to take a more scientific, risk-based, holistic, and proactive approach to drug development. It's also a significant problem for the pharmaceutical business, whose processes are time-bound despite inherent process and material unpredictability. It is crucial to establish the desired product performance profile Target Product Profile (TPP), Target Product Quality Profile (TPQP), and identify critical quality attributes while designing and developing a product (CQA). On this foundation, we designed the product formulation and process to satisfy the criteria for product attributes such as key material attributes (CMA), critical process parameters (CPP) on the CQAs, and identifying and controlling sources of variability.

High-overall performance liquid chromatography (HPLC) is the maximum flexible and extensively used analytical approach. It makes use of a liquid cellular segment to split the additives in combination. These additives (or analytes) are first dissolved in a solvent, after which pressured to float thru a chromatographic column below excessive stress. In the column, the combination is resolved into its additives. Amlodipine is used to treat high blood pressure (hypertension), some types of angina and other conditions caused by coronary artery disease. Amlodipine is a calcium channel blocker that works by changing the movement of calcium in the heart and blood vessel cells. This widens the blood vessels which increases the blood and oxygen supply to the heart and also lowers blood pressure. Amlodipine is considered a peripheral arterial vasodilator that exerts its action directly on vascular smooth muscle to lead to a reduction in peripheral vascular resistance, causing a decrease in blood pressure. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the influx of calcium ions into both vascular smooth muscle and cardiac muscle. Valsartan is used to treat high blood pressure (hypertension) in adults and children who are at least 1 year old. Lowering blood pressure may lower your risk of a stroke or heart attack.

Valsartan belongs to a class of drugs called angiotensin II receptor blockers.



Structure of Amlodipine



Structure of Valsartan

Materials and methods

Chemicals and reagents-

Pure drug samples of Amlodipine (99%) and Valsartan (99%) were purchased from Macleods Pharmaceutical Ltd, Daman, India. All chemicals such as methanol (HPLC grade), water, ACN (HPLC grade), and O-phosphoric acid (AR grade) were used in experimentation and purchased from the rom Modern science apparatus PVT. Ltd, Nashik.

Instruments-

HPLC (WATERS 1525 with binary pumps, UV visible detector; WATERS 2489 with software), UV spectrophotometer (Make: Shimadzu, Model: UV2450 UV probe v 2.3.3), Weighing balance (Make: Shimadzu, Model: AUX220), Ultra-Sonicator (Citizen PVT Ltd), pH meter, FTIR-ATR (Bruker Eco-ATR). Design Expert® 12.0

Software (Design Expert trial version 12.0; State-Ease Inc., Minneapolis, MN, (USA)

Chromatographic conditions

HPLC (WATERS 1525 with UV visible detector WATERS 2489). The column Chemsil C18 (250×4.6×5) was used. The optimized mobile phase consists of methanol: water (80:20 v/v) pH 3 with a flow rate of 0.9 ml/min, the run time was 6.2 min, column temperature room temperature, Injection volume 20 µL and detection wavelength 245 nm.

Standard and stock solution preparation

A standard solution of 1000µg/ml of Amlodipine and valsartan was prepared by accurately weighing 10 mg of Amlodipine and Valsartan was transferred in to clean 10 ml volumetric flask, Add 5 ml of methanol in both volumetric flasks. Sonicate them to dissolve the drug completely and make up the volume up to 10ml by adding methanol. The solution is used as a standard solution of Amlodipine and Valsartan (1000µg/ml). 0.3 ml of each standard solutions was diluted with methanol up to 10 ml in volumetric flask to get mixed stock solution of concentration 30µg/ml of amlodipine and valsartan each.

Sample preparation

Weight an equivalent to 10 mg of the marketed formulation and transferred to a clean 10ml volumetric flask. Added methanol in a volumetric flask, sonicate it to dissolve the tablet in solvent. Further make up the volume up to 10 ml by using methanol. 0.3 ml of the solution was taken into a clean conical flask and the volume was made to 10ml using methanol. The solution was sonicated and filtered through a Whatman filter (0.45µ). This solution was injected into the HPLC.

Mobile phase preparation

The pure drug of Amlodipine and Valsartan was spiked into the HPLC system and run-in diverse solvent systems. Selection of Proper Column for RP-HPLC method, the various columns are available, but our main aim is to resolve the drugs. On the basis of RP-HPLC mode and number of carbon present in the molecule, the C18 column of following configuration was selected for further study. Waters Chemsil C18, 250×4.6mm, 5µ column is used. Selection and Optimization of Mobile Phase with the consideration of sample solubility, stability and suitability, the different mobile phases and its compositions were tried for obtaining a sharp peak. For selection of mobile phase, different mobile phase compositions containing Methanol and Water in different ratio were tried. Finally, mobile phase composition of Methanol: Water (80:20) pH 3.0 was found to give best resolution for drug.

Application of Design of experiments for method optimization

3^3 randomized response surface designs with a Box Behnken design were used with 17 trial runs to evaluate the effect of three factors on the three key response variables. In this design 3 factors were analysed, each at 3 levels, and experimental trials were carried out at all possible combinations. The flow rate, wavelength, mobile phase composition was selected as independent variables and retention time (RT), Theoretical Plate number (TPN) and Asymmetric Factor were selected as dependent variables based on risk analysis. The data resulted was processed into Design Expert 12.0 software and analysed statistically with the help of analysis of variance (ANOVA). The data were also exposed to 3-D response surface methodology to determine the impact of flow rate, pH and mobile phase composition on dependent variables. The Translation of coded levels in actual values and probable trial runs using 3 Box - Behnken designs are as shown in Table 1.

Levels of variables	Concentration of factors		
	Flow rate (ml/min)	Wavelength	Mobile phase composition (methanol: water)
Low level (-1)	0.8	243	70:30
Medium level (0)	0.9	245	80:20
High level (+1)	1	247	90:10

Table 1: Box Behnken full factorial design

Method validation:

The method was validated for specificity, linearity, precision, accuracy, limit of detection, limit of quantification and stability, as per ICH guidelines Q2 (R2)

1. **System suitability** System suitability is a Pharmacopeial desideratum and is used to authenticate, whether if the resolution (here not apply) and reproducibility of chromatographic system are satisfactory for analysis to be done. The tests were performed by collecting data from five replicate injection of standard drug solution. (30 ppm) Acceptance Criteria: % Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %, Theoretical plates of analyte peak in Standard chromatograms should not be less than 2000 and Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 1.5.
2. **Linearity** Linearity was performed five levels over the range of 1-10 ppm of amlodipine and 30- 200 ppm. From stock solution aliquots of 0.01, 0.02, 0.03, 0.04, 0.05 ml of Amlodipine and 0.32, 0.64, 0.96, 1.28, 1.6 ml of Valsartan diluted to 10ml with methanol. Each linearity test solution of test concentration prepared in triplicate manner. Linearity was calculated by plotted graph mean area vs concentration. The correlation coefficient was calculated and recorded.
3. **Accuracy** Accuracy was performed over three ranges in triplicate manner i.e., three concentration and three replicate of each concentration. Accuracy of method was established over range of 80, 100 and 120% of working standards.

$$\% \text{ Recovery} = \frac{AT}{AS} \times 100$$

Where

AT- peak area of standard amlodipine/ valsartan

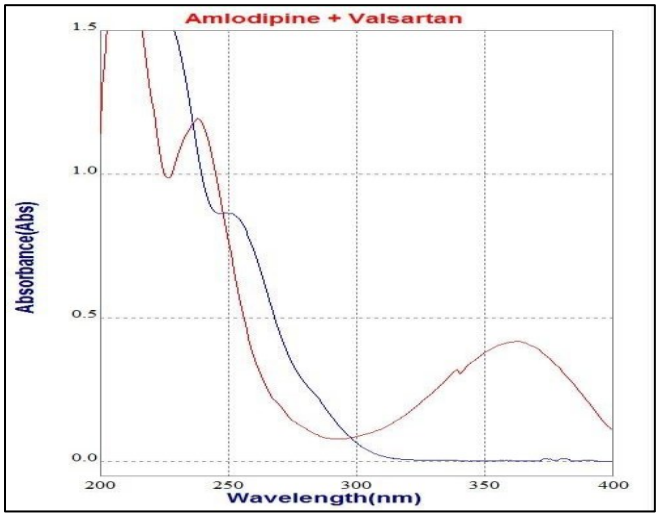
AS – peak area of tablet amlodipine/valsartan

4. **Precision** Precision was performed by analysing homogenous sample of amlodipine and valsartan in six times interday or intraday and %RSD calculated which should not be more than 2%.
5. **LOD and LOQ** LOD and LOQ was calculated from the Standard deviation of intercept and slope of intercept.

RESULTS AND DISCUSSION

Selection and optimization of wavelength

The sensitivity of the HPLC method is depending the on-proper selection of detected wavelength. An ideal wavelength is responsible drugs that are to be detected. The λmax of amlodipine and valsartan was observed at 245 nm.



Graph 1: Isosbestic wavelength for Amlodipine and Valsartan

Method Development:

The RP-HPLC method was developed for simultaneous estimation of amlodipine and valsartan by applying QbD. Range values of parameters were selected by applying Box Behnken Design of full factorial design. Chemsil C18 (250 × 4.6mm, 5µ) column was used and methanol: water (80:20) used as mobile phase, flow rate was maintained 0.9 µL/ml, method was performed at ambient temperature.

After entering the data in Design Expert software, fit summary applied to data after which "quadratic model" was suggested by the software. According to this model the following polynomial equation was obtained. Polynomial equation in coded terms,

Final Equation in Terms of Coded Factors:	
Retention Time =	+6.16 − 1.54 * A − 1.02 * B − 0.0324 * C

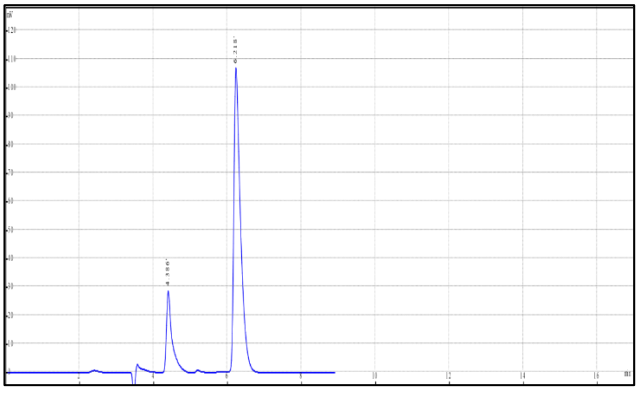
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

DOE optimization result:

The optimization was performed on the basis of response surface modelling by using the numerical and graphical optimization method. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimization finds a point that maximizes the desirability function. The characteristics of a goal may be altered by adjusting the weight or importance. For several responses and factors, all goals get combined into one desirability function. The goal of optimization is to find a good set of conditions that will meet all the goals.

Trial

Methanol: Water (80:20) pH 3



Name	RT (min)	Area (µV*sec)	USP Tailing	USP Plate Count
Amlodipine	4.3	667467	1.10	7144
Valsartan	6.2	1637042	1.07	7217

Observations: **The trial shows good peak shape, USP plate count is acceptable and retention time is desirable.**

Result: **Method selected**

Optimized Chromatographic Conditions:

The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Parameter/ conditions	Description/Values
Column name	ChemsilC18, 250 × 4.6mm, 5µ
Detector	245 nm
Flow rate	0.9 µL/ml
Injection volume	20 µL
Column oven Temperature	Ambient Temperature
Retention time	4.3 min and 6.2
Mobile phase	Methanol: Water (80:20) pH 3

Table 2: Optimized Chromatographic Condition

Level of Variables	Concentration of Factors		
	Flow rate (ml/min)	Wavelength	Mobile Phase Composition (Methanol: Water)
Low level (-1)	0.8	243	70:30
Medium level (0)	0.9	245	80:20
High level (+1)	1	247	90:10

Table 3: Translation of coded levels in actual values

Run	Factor 1 A: Methanol	Factor 2 B: Flow Rate	Factor 3 C: Wavelength	Response 1 Retention Time	Response 2 Retention Time	Response 3 Resolution
1	80	0.8	243	4.85	6.90	4.83
2	70	0.8	245	6.44	9.83	5.15
3	80	0.9	245	4.29	6.11	4.49
4	90	0.9	247	3.82	4.85	2.39
5	80	0.8	247	4.83	6.87	4.82
6	80	0.9	245	4.29	6.11	4.49
7	70	1.0	245	5.28	7.99	5.60
8	70	0.9	243	5.77	8.84	5.05
9	80	0.9	245	4.29	6.11	4.49
10	90	0.9	243	3.82	4.85	2.81
11	80	0.9	245	4.29	6.11	4.49
12	70	0.9	247	5.79	8.85	5.17
13	80	1.0	247	3.95	5.61	5.61
14	90	0.8	245	4.31	5.49	2.79
15	80	1.0	243	3.92	5.57	5.99
16	80	0.9	245	4.29	6.11	4.49
17	90	1.0	245	3.46	4.40	2.76

Table 4: Application of actual design of DOE with the subsequent response

Accuracy

Conc.	Conc.	Area	Mean	SD	% SD	% RSD
1	10	95867	96197	638.5264286	0.66376959	0.173992027
	10	96933				
	10	95791				
2	30	145875	144399.6667	1352.62424	0.93672255	
	30	143218				
	30	144106				
3	50	194646	195749.6667	1200.567505	0.61331778	
	50	195575				
	50	197028				

Table 5 Result and Statistical Data of Accuracy for Amlodipine

Conc.	Conc.	Area	Mean	SD	% SD	% RSD
1	10	2625272	2626372	12714.73716	0.48411791	0.165086452
	10	2614243				
	10	2639601				
2	30	5470416	5494281.333	42068.14094	0.7656714	
	30	5469573				
	30	5542855				
3	50	8606268	8636651.333	41070.87086	0.47554161	
	50	8683378				
	50	8620308				

Table 6. Result and Statistical Data of Accuracy for Valsartan

Precision

Sr. No.	Area for Amlodipine	Area for Valsartan
1	145875	5470416
2	143218	5469573
3	144106	5542855
4	143605	5483103
5	145459	5459442
6	146364	5399900
Mean	144771.2	5470882
% RSD	0.90%	0.84%

Table 7: Intraday Precision for Amlodipine and Valsartan

Sr. No.	Area for Amlodipine	Area for Valsartan
1	145875	5470416
2	143218	5469573
3	144106	5542855
4	142840	5472721
5	143274	5454889
6	143542	5448667
Mean	143218.7	5458759
% RSD	0.76%	0.62%

Table 8: Interday Precision for Amlodipine and Valsartan

Sr. No.	Area for Amlodipine	Area for Valsartan
1	121954	3987675
2	122212	4023352
3	121068	4014905
Mean	121745	4008644
SD	600.041	18644.4
% SD	0.4928685	0.46510392

Table 9: Analytical data for change in pH of Amlodipine and Valsartan

Sr. No.	Area for Amlodipine	Area for Valsartan
1	121431	4034427
2	121068	4014905
3	122731	4065154
Mean	121743	4038162
SD	874.389	25331.9
% SD	0.71822341	0.62731165

Table 10: Analytical data for change in wavelength of Amlodipine and Valsartan

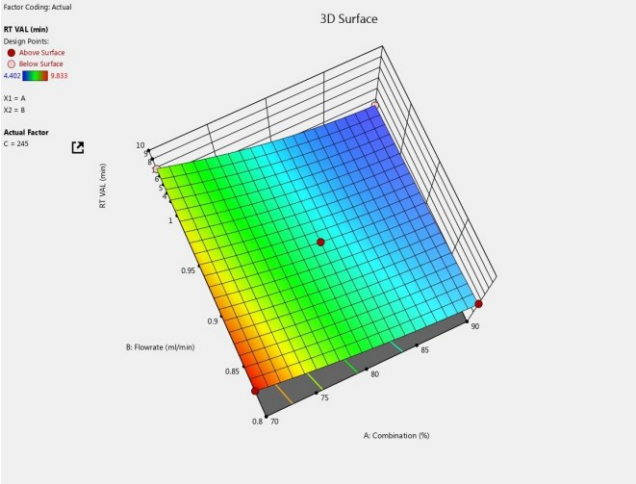


Figure 1: 3D Response Plot of Retention Time against Flow Rate and Combination for Amlodipine

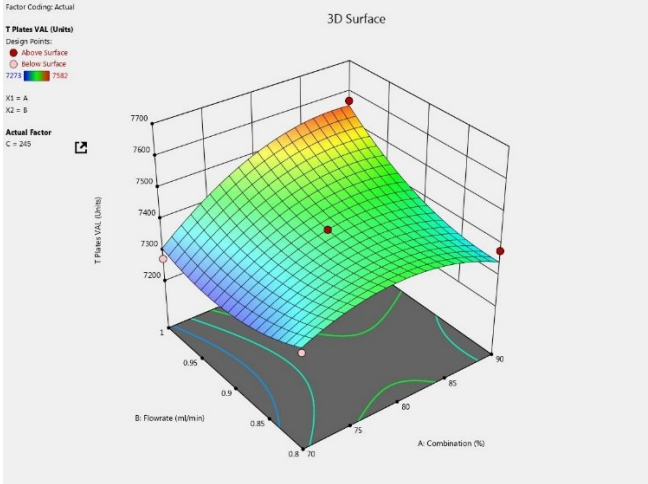


Figure 2: 3D Response Plot of Retention Time against Flow Rate and Combination for Valsartan

CONCLUSION

RP-HPLC binary isocratic system was used for the analysis. Chesil C18 (250mm x 4.6 ID, Particle size: 5 microns was used as a stationary phase. Solution of Amlodipine and Valsartan in appropriate dilution was scanned using UV visible spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm. The drug shows maximum absorbance at 245 nm (λ Max). The quality by Design approach has been successfully used for the Development of the RP-HPLC Method for estimation of Amlodipine and Valsartan. The developed method employed mobile phase Methanol: Water (80:20) (pH 3.0) pH adjusted by o-phosphoric acid, and flow rate 0.9 ml/min, which was optimized with the help of design expert software. Before method optimization, screening studies were carried out on different mobile phases of varying composition. Based on the results obtained from these studies, a suitable mobile phase with appropriate composition was selected and utilized for method development using the QbD methodology. A systematic approach was utilized to develop an efficient and robust method which includes beginning with the determination of target profile characteristics, risk assessment, design of experiment, and validation. The study was done by using 3^3 Box Behnken response surface designs. In this study interaction of 3 factors; Flow Rate, Wavelength, and Mobile Phase Composition vary at 3 levels. The effect of such critical process parameters on the critical quality attribute of the method is studied. Responses in terms of retention times and resolution were evaluated throughout all the runs in design. By taking such runs Method

Operable Design Region (MODR) also termed Analytical Design Space (ADS) was developed. A desirability function was applied to determine the optimum conditions. Optimum conditions were obtained; the one with higher desirability was selected. Replicates of the run having optimized conditions were taken to confirm the predicted response with the actual response. The RP-HPLC method developed for the estimation of Amlodipine and Valsartan was validated concerning ICH Q2 (R1) guideline. Linearity of the developed method was confirmed over the concentration range of 10 - 60 µg/mL for Amlodipine and Valsartan with correlation coefficients of 0.9997 and 0.9993 respectively. The percentage RSD for precision and accuracy of the method was found to be less than 2%. System suitability test ensures that the analytical system is working properly and can give accurate and precise results. System suitability tests include tailing factor, number of theoretical plates, area, etc. The results of all system suitability parameters were acceptable in their limits defined by official guidelines. The proposed high-performance liquid chromatographic method has also been evaluated for accuracy, and precision and proved to be convenient and effective for the quality control of Amlodipine and Valsartan. Moreover, the lower solvent consumption along with the short analytical run time of 8.91 min leads to a cost-effective and environmentally friendly chromatographic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good procedure for Amlodipine and Valsartan.

REFERENCES

1. ICH Expert Working Group, “Pharmaceutical Development Q8 (R2)”, ICH Harmonized tripartite guidelines,2009;(4):1-28.
2. ICH Expert Working Group, “Quality Risk Management Q9”, ICH Harmonized tripartite guidelines,2005;(4):1-23.
3. Patwardhan. D. M., Amrutkar S. S, Kotwal T. S, and Wagh M. P, “Application of quality by design to different aspects of pharmaceutical technologies”, International Journal of Pharmacy and Pharmaceutical Science,2017;8(9):3649-3662.
4. Bhatt D. A, Rane S. I, “QbD approach to analytical RP-HPLC method development and its validation”, International Journal of Pharmacy and Pharmaceutical Sciences,2011;3(1):179–187.
5. Thammana M, “A Review on High-Performance Liquid Chromatography, Journal of Pharmaceutical Analysis”, Research & Reviews: Journal of Pharmaceutical Analysis,2016;5(2):22–28.
6. ICH Expert Working Group, “Validation of Analytical Procedures: Text and Methodology Q2 (R1)”, ICH Harmonized tripartite guidelines,1994;(4):1-17.
7. ICH Expert Working Group, “Stability Testing of New Drug Substance and Product Q1 (R2)”, ICH Harmonized tripartite guidelines, 2003;(4):1- 24.
8. ICH Expert Working Group, Stability Testing: Photostability Testing of New Drug Substances and Products Q1(R2) (B), ICH Harmonized tripartite guidelines,1996;(4):1-12.
9. Gupta V. et al, “Development and validation of HPLC method - a review”, International Research Journal of Pharmaceutical and Applied Sciences,2012;2(4):17–25
10. Chilukuri. M, Hussain. K. R, Narayanreddy. P, Venkatramana. M, “Degradation pathway for Rilpivirine HCl by validated stability indicating UP-LC method”, International Journal of Clinical Pharmacology and Toxicology,2012;1(1):1-8.
11. Rajkumar. B, Dr. Subrahmanyam K. V, “A validated stability indicating RP-HPLC method for the determination of Rilpivirine”,

Journal of Global Trends in Pharmaceutical Sciences, 2014; 5(3):1822-26.

12. Ghosh. S, Bomma. S, Laxmiprasad. V, Vidyasagar. S, Banji. D, Roy. S, "Method development and validation of Rilpivirine in bulk and tablet dosage form by RP-HPLC method", Research Journal of Pharmacy and Technology,2013;6(3):240-45.
13. Dr. Yashoda. A Rani. J, Venkataih. G, Shivakumar. A "RP-HPLC Method Development and Validation of Rilpivirine", International Journal of Pharmacy and Analytical Research, 2017; 6(1):18-38.
14. Reddishdiah. V C, Rama Devi. P, Mukkanti. K., "Effective Estimation Of Rilpivirine By HPLC Method In Tablet Dosage Forms And Its In-vitro Dissolution Assessment", International Journal of Pharmacy and Pharmaceutical Science,2012;4(3):595-599.
15. Vogt. F. G, Kord A. S, "Development of quality by design analytical methods", Wiley Online Library,2010;100(3):797–812.
16. Ford. N, Lee. J, Meyer.r I. A, Alexandra, "Safety efficacy and pharmacokinetics of rilpivirine: a systematic review with an emphasis on resource-limited settings", Journal HIV/AIDS Research and Palliative Care,2011;(3):35-44.
17. Garvey. L, Winston. A "Rilpivirine: A novel non-nucleoside reverse transcriptase inhibitor", Expert Opinion Investigation Drugs,2009;18(7); 1035-1041.
18. Sharma. M and Saravolatz. L. D, "Rilpivirine: A new non-nucleoside reverse transcriptase inhibitor", Journal of Antimicrobial Chemotherapy, 2013; 68:250–256.
19. Lemke L, Williams D. A, Roche V. F, Zito S. W, "Foye's Principles of Medicinal Chemistry", 6th edition, Published by Lippincott Williams & Wilkins, 2008; 900-901.
20. Sethi P. D, "High-Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulations",1st edition, CBS Publishers and Distributors, New Delhi, 2001; 311,23, 53-54, 116-120.
21. Munson J. W, "Pharmaceutical Analysis, Modern methods-Part B", International Medical book Distributors, Mumbai,2001:51-54.
22. Kasture A.V, Mahadik K. R, Wadodkar S. G, More H. N, "Pharmaceutical AnalysisInstrumental Methods",2012;(2):6-7, 28-30, 49, 64.
23. Stahl E, "Thin Layer Chromatography-A Laboratory Handbook", 2nd edition, Springer, India, 2006:52-66.
24. Connors K. A, "A textbook of Pharmaceutical Analysis", 3rd edition, John Wiley and sons, 1999:196-198.
25. Beckett A. H, Stenlake J. B, "Practical Pharmaceutical Chemistry-Part- 2", CBS Publishers and Distributors, New Delhi, 2002, 275-288.
26. Kalsi P. S, "Spectroscopy of Organic Compounds", 6th edition, New Age International Publishers, New Delhi,2007:7-10.
27. Bolton S, Charles B, "Pharmaceutical Statistics and Clinical Application", 3rdedition, Marcel Dekker Inc, New York, 2005: 24-25, 416,428.
28. Indian Pharmacopoeia, Vol. II, Govt. of India, Ministry of Health and Family Welfare. New Delhi Published by The Controller of Publications, 2007:1142-1144.
29. British Pharmacopoeia, Vol. I, Published by The Stationery Office on behalf of Medicines & Healthcare Products Regulatory Agency (MHRA), 2009, 904-906, 913- 915.
30. Chatwal G. R, Sharma A, "Instrumental Methods of Chemical Analysis", 5th edition,

Himalaya Publishing House, Delhi, 2004:1.1-1.5.

31. Willard H. H, Jr. Merritt, L. L, Dean, J.A., Jr. Settle F.A, "Instrumental Methods of Analysis", 7th edition, CBS Publishers and Distributors, Delhi, 2001:1-4.
32. Skoog D. A, Holler, F. J, Crouch S. R, "Principle of Instrumental Analysis", 6th edition, Thomson Publications, India, 2007:1-3, 145-147, 180.
33. Sharma B. K, "Instrumental Methods of Chemical Analysis", 25thedition, Goel Publication Co. Meerut, 1983:3-6.
34. Mendham J, Denney R. C, Barnes, J. D, Thomas M, "Vogel's Textbook of Quantitative Analysis", Pearson Education, Singapore, 2003:8-9.

HOW TO CITE: Shinde Mahesh*, Jondhale Vidhyatai, Pingale A. P., Dhikale G. K., Derle D. V., Wagh M. P., Development and Validation of RP-HPLC Method For Simultaneous Estimation of Amlodipine and Valsartan In Its Bulk And Tablet Dosage Form by Using The Quality By Design Approach, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 4, 186-196. https://doi.org/10.5281/zenodo.7866101