Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol. 2 (2), July-Sept,2012

Stability indicating RP-HPLC method for simultaneous determination of amlodipine besylate and valsartan combination in bulk and commercial dosage forms.

Rahul R. Nahire[*1], Sagar S. Joshi[1], Varsha Meghnani[1], Nalini Shastri[1], K. V. Surendra Nath[2], J. Sathish[2]

[1] National Institute of Pharmaceutical Education and Research [NIPER], Balanagar, IDPL R&D Centre, Hyderabad 500037.

[2] United States Pharmacopeia-India Private Limited. Advanced Compendial Research Laboratory, ICICI Knowledge Park, Turkpally, Hyderabad 500078.

Corresponding author's Email: rahulandrd@yahoo.co.in , Rahul.Nahire@my.ndsu.edu

---

ABSTRACT:

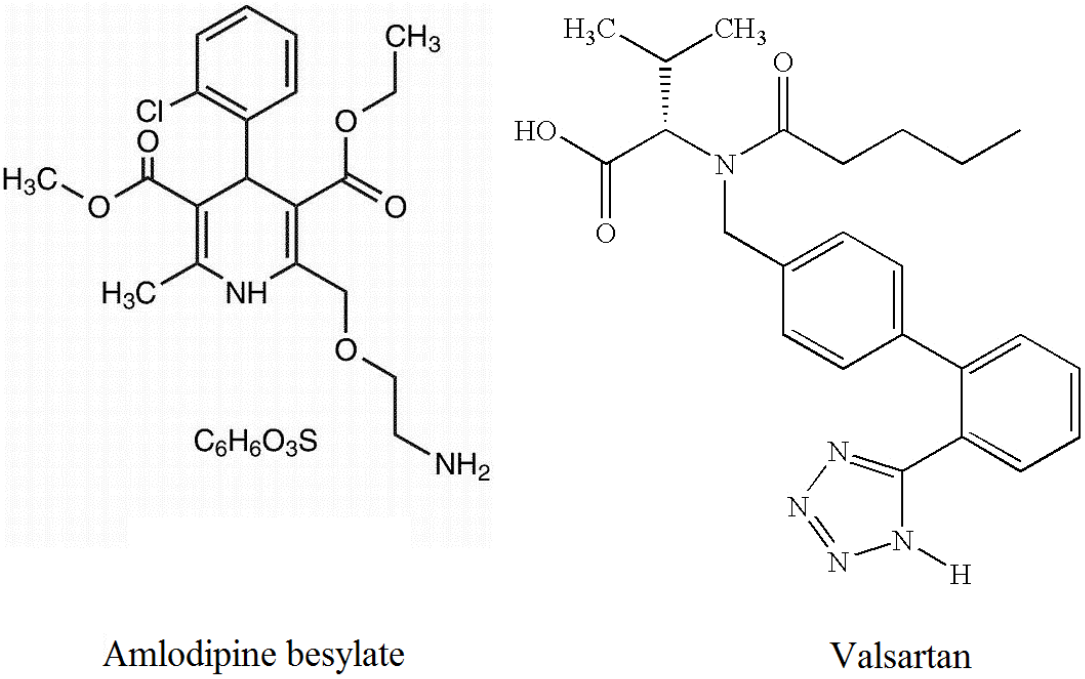
The present study describes development and validation of simple, rapid, sensitive and stability indicating high-performance liquid chromatographic assay method for simultaneous determination of Amlodipine besylate and Valsartan in bulk as well as in commercial formulation. Analytical separation was achieved with RP Waters Symmetry C18 Column [150×4.6 mm] using a combination of methanol and potassium dihydrogen phosphate buffer [0.01 M] pH 2.5, in ratio 60:40 v/v with flow rate of 1 mL min^{-1}. The retention times for Amlodipine and Valsartan were found to be 4.6 and 7.6 min respectively. Both the drugs were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions individually as well as in combination, subsequently samples were analyzed by the proposed method. Detection was done by PDA detector at 238 nm. Method was found to be very sensitive as LOD found to be 20 ng/mL and 44 ng/mL for Amlodipine besylate and Valsartan respectively. The method was found to be specific and stability indicating as no interfering peaks of degradation compounds and excipients were noticed. The developed method was validated with respect to linearity, accuracy, precision, specificity, robustness as per International Conference on Harmonization (ICH) guidelines. The proposed method hence useful for the application in quality control laboratories for quantitative analysis of both the drug individually or in combination, as it is simple and rapid method with excellent accuracy and precision.

Key words: *HPLC, Stability indicating assay method, Amlodipine besylate, Valsartan, Forced degradation studies.*

INTRODUCTION:

Amlodipine besylate [AMB], 3-ethyl 5-methyl [4RS]-2-[[2-aminoethoxy]methyl]-4-[2-chlorophenyl]-6-methyl-1,4-dihydropyridine -3,5-dicarboxylate benzenesulphonate[1] (Figure 1) is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina. AMB inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle [2]. Valsartan [VAL], N-[p-[o-1H-tetrazol-5-ylphenyl]benzyl] –N-valeryl-L-valine [3] (Figure 1) is an orally active, potent and specific competitive angiotensin II antagonist acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardiovascular system. VAL is widely used in the treatment of hypertension [4].

Two dosage forms Exforge tablet containing 5/160, 10/160, 5/320, 10/320 mg of AMB and VAL [Novartis, USA] and Valzaar-SM containing 2.5/80 mg of AMB and VAL are in market. Literature review reveals that methods have been reported for analysis of AMB alone or in combination with different other drugs by liquid chromatography [LC] [5-9]. For estimation of VAL also there are different methods published [10-14]**.** To the best of our knowledge this is the first Stability Indicating HPLC assay method for simultaneous determination of AMB and VAL with extensive stress studies and validation as per ICH guidelines. There are HPLC[15, 16], TLC[17], HPTLC[18] and UV[19] methods available in literature but they lack stability indicating nature. There are reports of stability indicating HPLC methods but are not extensive, elaborate and thus their usefulness for application in quality control departments will be limited. The present study reports stability indicating assay method for AMB and VAL by HPLC in bulk drug and in pharmaceutical dosage forms which is more efficient, accurate, sensitive and rapid compared to published methods [20, 21] . The proposed method is validated extensively as per ICH guidelines.



Amlodipine besylate Valsartan

Figure 1: Structure of amlodipine besylate and valsartan

MATERIALS AND METHODS

AMB and VAL were gratis samples from Hetero Drugs Ltd, Hyderabad, India and Mepro Chemicals, Gujarat, India respectively. Valzaar-SM® capsules containing AMB 2.5 mg and VAL 80 mg per capsule, were purchased for studies. Methanol, potassium dihydrogen phosphate, ortho phosphoric acid, sodium hydroxide, hydrochloric acid and hydrogen peroxide were obtained from Merck [Darmstadt, Germany]. All reagents used, were of analytical grade and methanol used was HPLC grade. HPLC grade water was obtained through a Milli-Q® system: Progard 2 [Millipore, Milford, MA, USA] and was used to prepare all solutions.

The HPLC system used was Waters 2695 separation module with 2996 PDA (Photo Diode Array) detector [Waters Corporation, USA] and chromatographic data handled with Empower software (version 6.10.01.00). Photostability studies were carried out in a photo stability chamber [Mack equipments, Thane, India]. Thermal stability study was performed in a dry air oven [Mack Pharmatech, Mumbai, India]. Phosphate buffer pH was adjusted on High precision 780 pH meter [Metrohm, Switzerland]. All pipettes and Glassware were A grade [Borosil, Illinois, USA] and all weighings were done with analytical balance [Sartorius, Germany].

Preparation of stock and standard solutions

Stock solutions of AMB [equivalent to 1 mg/mL of the free base] and VAL were prepared in methanol. The stock solutions were protected from light using aluminum foil and stored for three weeks at 40 °C and no evidence of degradation noticed. One mL of aliquots of stock solutions were transferred using A-grade bulb pipettes into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to yield final concentration of 100 µg/ml.

Initial method development and optimization

After complete perusal of literature regarding HPLC assay methods of both the drugs it was explicitly clear that because of their nature and hence solubility, most commonly used solvents were phosphate buffer and acetonitrile with reversed phase system. Thus, we decided to start with buffer and Methanol instead of acetonitrile for economic purpose (methanol cheaper solvent compared to acetonitrile). It was clearly evident from Log P values of both the drugs (1.9 and 5.8 for AMB and VAL resp.) that they will have good retention on C18 column, and thus we decided to start with it. Since the injection concentration was set to 100 µg/mL, injection volume kept to 20 µL and column oven temperature was kept at room temperature (25°C). Although response of both the drugs at 210 nm was maximum but signal to noise ratio was very less. The detection wavelength was kept at 238 nm, as (which is λmax of AMB) both the drugs have sufficient response with low level of noise as compared to 210 nm.

The chromatographic separation was achieved on Waters Symmetry C18, 5 µm, 150×4.6 mm column using mobile phase consisting methanol-potassium phosphate buffer [0.01M pH: 2.5 adjusted with dilute ortho phosphoric acid] in proportion 60:40 v/v at flow rate of 1 mL/min in isocratic mode. The mobile phase was filtered through 0.45 µm Chrom Tech Nylon-66 filter prior to use.

Procedure for forced degradation studies

In order to establish stability indicating nature of developed assay method; pure active pharmaceutical ingredients AMB and VAL were exposed to various stress conditions. Stress conditions applied gradually from mild to severe in order to get considerable degradation. Least severe condition was selected to get considerable percentage of degradation so that it will be closer to realistic conditions that drug might get exposed to during its shelf life. As both the drugs are slightly soluble in water, for forced degradation studies methanol was used as co-solvent. All solutions were prepared by dissolving API or drug product in small volume of methanol and later diluted with aqueous solutions of degrading agents (hydrogen peroxide, hydrochloric acid, sodium hydroxide) to achieve concentration of 1 mg/ml. After considerable degradation these solutions were diluted with mobile phase to yield starting concentration of 100 µg/ml and then injected into HPLC system.

For thermal degradation both the drugs in solid and solution forms, as a separate and combination were placed in dry air heating oven at constant temperature 60 ºC for 10 days. Stock solution stability was determined by placing samples at 4 ºC for 4 weeks and after four weeks the solution was analyzed by injecting sample into HPLC system.

For photostability [UV and Fluorescent] studies, solid and solutions of each drug were prepared and exposed to light to determine the effects of UV and florescent light on the stability of the two drugs. Approximately 50 mg of each drug was spreaded on a glass petri dish in a layer that was less than 2 mm in thickness. Solution of each drug [1 mg/mL] was prepared in methanol and HPLC grade water [20:80 v/v]. Samples and capsules were then exposed to 1.2 million Lux hours of light. Following removal from the cabinet, all samples were prepared for analysis as previously described. The conditions at which we achieved a considerable degradation are mentioned in Table 1.

Validation procedure

The proposed method was extensively validated according to ICH guidelines Q2(R1), with respect to linearity, accuracy (recovery studies), precision, limit of detection and determination, specificity, robustness and system suitability. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present and typically these might include impurities, degradation products, excipients etc. The results of stress studies would indicate specificity in presence of degradants. Since Exforge tablet is not available in India, specificity studies were carried out in compounded formulation with common excipients like microcrystalline cellulose, starch, talc, Mg stearate. Valzaar-SM® capsules also were injected into system to see any possible matrix interferences.

Linearity was established by plotting area of each drug against concentration of 9 points (n=5) between 0.5 µg/mL to 100 µg/ml and evaluated by plotting regression equation. Accuracy was determined by recovery studies by spiking standard drug in placebo samples. Recovery was determined by interpolation of replicate (n=5) peak areas of three accuracy standards of different concentration (80-100-120% of target concentration) from a calibration curve that had been prepared for linearity.

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. Precision studies were done at three levels; intraday precision (repeatability), reproducibility and intermediate precision (ruggedness). Intra-day precision was investigated by injecting samples (n=5) of each drug for three different concentrations. Intermediate precision was assessed by two different analysts on two different days and reproducibility was assessed by injecting the same three samples in two different laboratories.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices. LOD and LOQ were determined on the basis of signal to noise ratio i.e. 3 for LOD and 10 for LOQ.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the method was investigated under a variety of conditions including changes in pH of the buffer, flow rate, temperature of column and mobile phase composition. The degree of reproducibility of the results obtained as a result of small deliberate variations is determined by percent recoveries and effect on system suitability parameters (USP resolution, tailing and plate count)

Assay procedure for Valzaar-SM® capsules

Validated assay method then applied to commercially available Valzaar-SM® capsules. Twenty capsules were emptied, weighed and mixed with mortar and pestle. Powder equivalent to weight of one capsule was accurately weighed into each of three 100 ml volumetric flasks and 20 ml of HPLC-grade methanol was added to each flask. The volumetric flasks were sonicated for 5 min to affect complete dissolution and the solutions were then made up to volume with methanol. Aliquots of the solution were filtered through a 0.45 μm filter and 1 ml of the filtered solution was transferred to a 10 ml volumetric flask and volume made up to with mobile phase, to yield target concentration.

RESULTS AND DISCUSSION

Forced degradation studies/Specificity

Degradation studies indicated the specificity of developed method in presence of degradation products. Degradation was carried out in combination and separately for each drug, so that we could able to assign degradant peak in combination samples. Purity of drug peaks was confirmed by purity angles. Both the drugs were found to be stable towards oxidation since with 3% H_2O_2 even after 5 days there was negligible degradation. So concentration was increased to 10%, after 3 days at RT around 14.5% degradation was found. At 2.6 min a prominent degradant peak came which constitutes around 4% degradation. (Fig. 2G)

Table 1. Forced degradation studies

Sr. No.	Stress study	Condition	Duration	% Degradation
1	Oxidation degradation	10% H_2O_2	6 days	14
2	Acid degradation	1N HCl	3 days	16
3	Base degradation	0.1N NaOH	6 days	07
4	Neutral degradation	Reflux	12 hrs	07
5	Thermal degradation	60 ^{0}C	10 days	0.2
6	UV light degradation	1.2 Million Lux Hrs	10 days	14
7	Fluorescent degradation	1.2 Million Lux Hrs	10 days	1.2

 ISSN 2231 – 4423

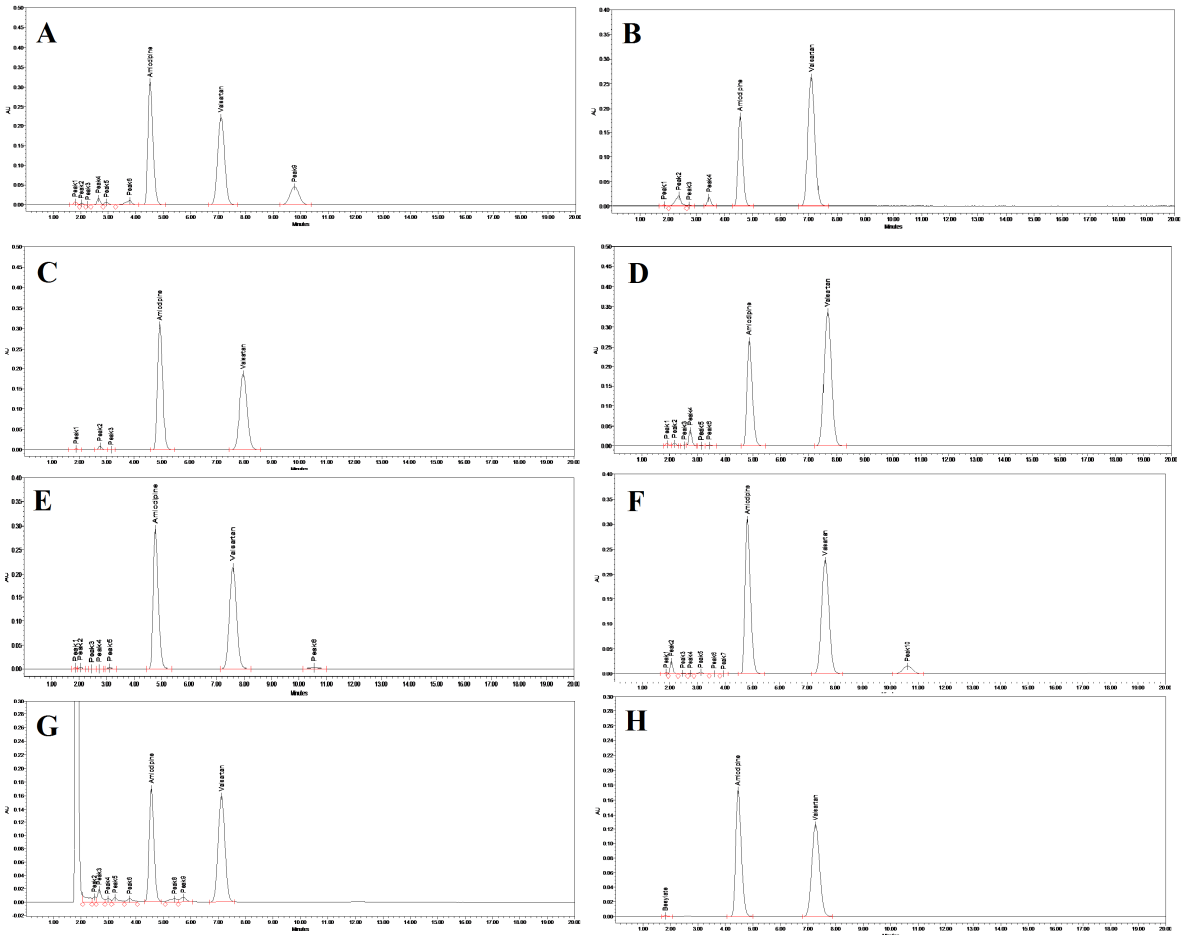


Figure 2: Chromatograms of degradation studies (a) acid degradation (b) alkali degradation (c) fluorescent light degradation (d) uv light degradation (e) thermal degradation (f) neutral condition degration (in water) (g) oxidation degradation (h) stock solution stability

For degradation in acidic condition, initially 0.1 N HCl at RT was used, but both drugs were found to be stable for 5 days with negligible degradation. So concentration of HCl was increased to 1N. After 48 hrs around 16 % degradation was found which was mostly contributed by VAL; which complied with previously published report[22]. A prominent degradation peak came at 9.7 min. (Fig. 2A) which constitutes around 11% of area.

For alkali degradation initially mild condition; 0.1 N NaOH at RT was used but after 3 days only 3 to 4% degradation was found. We then used 1 N NaOH but within 30 min total VAL got degraded so decided to keep concentration at 0.1 N for longer time. After 6 days considerable (7%) of degradation was found. A prominent degradant peak eluted at 2.4 min with area around 4%. (Fig. 2B)

For hydrolysis in neutral pH, both the drugs were refluxed with water in combination and separately for 12 hours. At intervals of 2 hrs samples were withdrawn and analyzed. In combination, around 7% degradation was found after 12 hrs of reflux. In individual degradation, AMB was found to be more prone to neutral degradation and degraded around 33% whereas VAL found to be stable and degradation was less than 1%. AMB gave prominent degradation peak at around 2.5 min and VAL at 10.5 min. (Fig. 2F)

For thermal stability studies, solid samples and solutions of each drug as well as combination were kept in oven at constant temperature 60°C for 10 days. After 10 days all samples were injected into the system, it was found that both the drugs were stable towards heat, producing negligible degradation in all samples. (Fig. 2E)

According to ICH guidelines; for UV and fluorescent degradation studies samples (each solid, solutions and combination) must be exposed total 1.2 million Lux hours of light in photo stability chamber. Since, AMB is a photosensitive drug[23] (a property of all dihydropyridines), it underwent considerable degradation in all samples, around 24% in solid, 10% in solution. In combination total 35% of degradation was found which was mostly contributed by AMB. A prominent degradation peak came at about 2.7 (Peak 4, Fig. 2D) of AMB. VAL found to be stable towards UV light and only 1% degradation was detected.
Similar to UV degradation samples were also kept under florescent light for 1.2 million Lux hours in photo stability chamber. After stipulated time, all samples were removed and injected into HPLC system. Around 4% degradation in VAL solution and 1% in AMB solution was detected. So it can be inferred that, AMB is sensitive to UV light whereas VAL to fluorescent light. (Fig. 2C) Stock stability was assessed by placing a combination stock solution in methanol was kept at 4°C for 4 weeks. It was found very stable and no degradation observed.

Validation

The results of stress studies indicated high degree of specificity in presence of degradants of this method. Since Exforge tablet is not available in India, specificity studies carried out in compounded formulation with common excipients like MCC, starch, talc, Mg stearate and Valzaar-SM® capsule was injected into system to see any possible matrix interference. No interfaring peaks were observed and both peaks were found to be pure indicating high degree of specificity method has.

Table 2. Accuracy data [n=5]

Interpolated concentration [mean± SD]	% Recovery	%RSD
Amlodipine besylate concentration [µg/ml]		
Added Determined		
2 1.9979±0.0056	99.89	0.2837
2.5 2.5319±0.0287	101.28	1.1335
3 2.9532±0.0103	98.44	0.3487
Valsartan concentration [µg/ml]		
Added Determined		
64 63.9808±0.1795	99.97	0.2805
80 79.5328±0.5057	99.41	0.6359
96 95.0170±0.1644	98.97	0.1730

Table 3. Precision data [n=5]

Actual Concentration [µg/ml] Measured concentration [µg/ml], %RSD

	Repeatability	Ruggedness	Reproducibility
Amlodipine besylate			
5	4.99±0.18	4.99±0.36	4.97±0.17
10	9.97±0.09	9.94±0.2624	9.93±0.41
20	19.93±0.18	19.83±0.05	19.776±0.08
Valsartan			
5	4.99±0.61	4.98±0.67	4.95±0.50
10	9.96±0.11	9.99±0.17	9.92±0.29
20	19.98±0.17	19.98±0.16	19.91±0.12

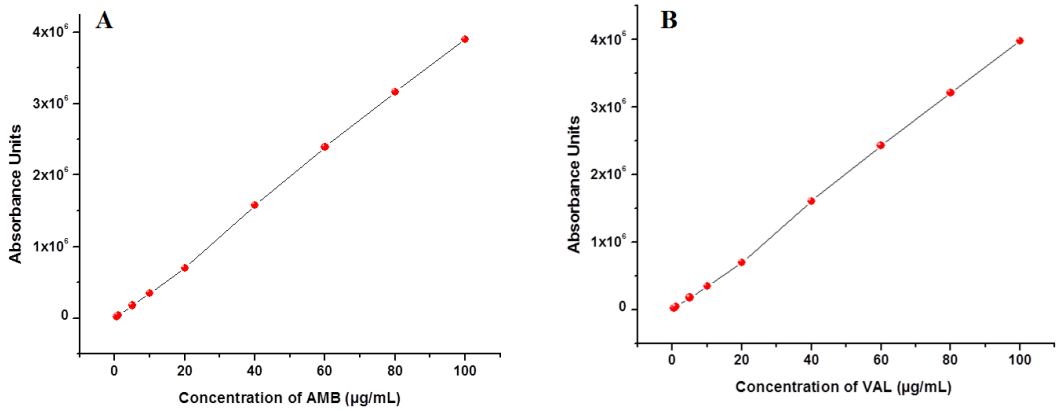


Figure 3. Linearity graphs for amlodipine besylate (a) and valsartan (b)

Linearity was established by plotting area of each drug against concentration for 9 points (n=5). The linearity found to be from 0.5 µg/ml to 100 µg/ml for both the drugs. Typically, regression equations were Y= 39369X – 11613 (R^2= 0.996) for AMB and Y= 40146X-15689 (R^2= 0.997) for VAL. Accuracy was determined by interpolation of replicate (*n*=5) peak areas of three accuracy standards of different concentration at three different levels across range of method (Table. 2). In each case, the Standard deviation was found to be less than 1.5 % for each drug. Precision studies were done at three levels. Percent RSD were found to be less than 1 for all precision studies indicating that method is precise and can reproduce the results (Table. 3). Detection limits and quantification limits were determined on the basis of signal to noise ratio, 3 for LOD and 10 for LOQ. It was found that LOD for AMB was 20 ng/ml and for VAL 44 ng/ml using formula 3.3xSD/Slope however with signal to noise ratio we found that LOD for AMB was 15 ng/ml and VAL was 70 ng/ml. Whereas LOQ was found to be 50 ng/ml and 300 ng/ml for AMB and VAL resp (Table. 4). System suitability of method was also checked by injecting sample at target concentration, method complies with standards of USP for all three parameters USP resolution (>2), tailing (<2) and theoretical plates (>2000).

Table 4. Limit of detection and limit of quantification

Method	Parameter	Amlodipine besylate [ng/mL]	Valsartan [µg/ml]
Calculation 3.3xSD/Slope	LOD	20	44
	LOQ	62	133
Signal to Noise ratio	LOD	15	70
	LOQ	50	200

Method was found to be robust for small deliberate changes in parameters like pH, mobile phase flow rate, mobile phase composition, temperature of column. There were no significant changes in system suitability parameters of method; USP resolution, tailing and plate count (Table. 5). The validated method was then applied to determination of AMB and VAL in commercial available dosage form (Valzaar-SM). The result of the assays (n = 3) undertaken yielded 99.98 % (%RSD = 0.0) and 100.12 % (%RSD = 0.04) of label claim for AMB and VAL, respectively. The results of the assay indicate that the method is selective for the analysis of both AMB and VAL.

Table 5. Robustness studies

Parameter		USP Resolution	USP Tailing		USP Plate count	
			AMB	VAL	AMB	VAL
pH	2.4	7.39	1.63	1.04	1750	3950
	2.5	6.90	1.62	1.05	1660	3850
	2.6	7.06	1.68	1.05	1540	3870
Flow	0.9	7.08	1.67	1.05	1730	4090
mL/min	1.0	6.90	1.62	1.05	1660	3850
	1.1	6.96	1.63	1.05	1660	3680
MP*	62:38	6.75	1.56	1.06	1790	3560
	60:40	6.90	1.62	1.05	1660	3850
	58:42	7.39	1.65	1.04	1630	3950
Temp	22^0C	7.06	1.68	1.05	1540	3870
	25^0C	6.90	1.62	1.05	1660	3850
	28^0C	7.08	1.67	1.05	1730	4090

*MP: Mobile Phase Organic Phase: Aqueous Phase

CONCLUSION

A simple, rapid, accurate and precise stability-indicating HPLC method has been developed and validated for the routine analysis of AMB and VAL in API and commercial dosage form. The results of stress testing undertaken according to the International Conference on Harmonization guidelines reveal that the method is specific and

stability-indicating. The proposed method has the ability to separate these drugs from their degradation products, related substances; excipients found in tablet dosage forms and can be applied to the analysis of samples obtained during accelerated stability experiments.

ACKNOWLEDGEMENTS

The authors wish to thank Sathish Shetty along with the management and analysts of United States Pharmacopeia, India and Dr. Prakash Diwan, along with NIPER Hyderabad, for wholeheartedly supporting this work.

References

[1] United States Pharmacopeia NF, in, Rockville, MD,, 2009, pp. 1532.

[2] S. Dhein, A. Salameh, Amlodipine releases nitric oxide from canine coronary microvessels: An unexpected mechanism of action of a calcium channel-blocking agent, Circulation, 100 (1999) 1011.

[3] United States Pharmacopeia NF 27, in, The United States Pharmacopeial Convention, Rockville, MD, 2009, pp. 3842.

[4] A. Markham, K.L. Goa, Valsartan. A review of its pharmacology and therapeutic use in essential hypertension, Drugs, 54 (1997) 299-311.

[5] S. Shah, A. Asnani, D. Kawade, S. Dangre, S. Arora, S. Yende, Simultaneous Quantitative Analysis of Olmesartan Medoxomil and Amlodipine Besylate in Plasma by High-performance Liquid Chromatography Technique, Journal of young pharmacists : JYP, 4 (2012) 88-94.

[6] S. Nalwade, V. Ranga Reddy, D. Durga Rao, I. Koteswara Rao, Rapid simultaneous determination of telmisartan, amlodipine besylate and hydrochlorothiazide in a combined poly pill dosage form by stability-indicating ultra performance liquid chromatography, Scientia pharmaceutica, 79 (2011) 69-84.

[7] Q. Zou, Y. Zhan, Z. Ge, P. Wei, P. Ouyang, Liquid chromatography-mass spectrometry method for the determination of amlodipine in human plasma and its application in a bioequivalence study, Arzneimittel-Forschung, 59 (2009) 383-391.

[8] A.K. Sarkar, D. Ghosh, A. Das, P.S. Selvan, K.V. Gowda, U. Mandal, A. Bose, S. Agarwal, U. Bhaumik, T.K. Pal, Simultaneous determination of metoprolol succinate and amlodipine besylate in human plasma by liquid chromatography-tandem mass spectrometry method and its application in bioequivalence study, Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 873 (2008) 77-85.

[9] B. Suchanova, R. Kostiainen, R.A. Ketola, Characterization of the in vitro metabolic profile of amlodipine in rat using liquid chromatography-mass spectrometry, European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences, 33 (2008) 91-99.

[10] A.S. Doshi, S.S. Bhagwan, T.N. Mehta, V.K. Gupta, G. Subaaiah, Determination of nebivolol and valsartan in a fixed-dose combination by liquid chromatography, Journal of AOAC International, 91 (2008) 292-298.

[11] H. Li, Y. Wang, Y. Jiang, Y. Tang, J. Wang, L. Zhao, J. Gu, A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma, Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 852 (2007) 436-442.

[12] N. Koseki, H. Kawashita, H. Hara, M. Niina, M. Tanaka, R. Kawai, Y. Nagae, N. Masuda, Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry, Journal of pharmaceutical and biomedical analysis, 43 (2007) 1769-1774.

[13] B. Kocyigit-Kaymakcoglu, S. Unsalan, S. Rollas, Determination and validation of ketoprofen, pantoprazole and valsartan together in human plasma by high performance liquid chromatography, Die Pharmazie, 61 (2006) 586-589.

[14] J. Macek, J. Klima, P. Ptacek, Rapid determination of valsartan in human plasma by protein precipitation and high-performance liquid chromatography, Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 832 (2006) 169-172.
[15] M. Çelebier, M.S. Kaynak, S. Altinoz, S. Sahin, Validated HPLC method development: the simultaneous analysis of amlodipine and valsartan in samples for liver perfusion studies, Hacettepe University J Faculty of Pharmacy, 28 (2008) 15-30.
[16] D. Kul, B. Dogan-Topal, T. Kutucu, B. Uslu, S.A. Ozkan, High-performance liquid chromatographic and first derivative of the ratio spectrophotometric determination of amlodipine and valsartan in their binary mixtures, Journal of AOAC International, 93 (2010) 882-890.
[17] S.R. Dhaneshwar, N.G. Patre, M.V. Mahadik, Validated TLC Method for Simultaneous Quantitation of Amlodipine Besylate and Valsartan in Bulk Drug and Formulation, Chromatographia, 69 (2009) 157-161.
[18] S.J. Varghese, T.K. Ravi, QUANTITATIVE SIMULTANEOUS DETERMINATION OF AMLODIPINE, VALSARTAN, AND HYDROCHLOROTHIAZIDE IN "EXFORGE HCT" TABLETS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY, Journal of Liquid Chromatography & Related Technologies, 34 (2011) 981-994.
[19] N.G. Mohamed, Simultaneous Determination of Amlodipine and Valsartan, Analytical chemistry insights, 6 (2011) 53.
[20] S. Chitlange, K. Bagri, D. Sakarkar, Stability indicating RP-HPLC method for simultaneous estimation of valsartan and amlodipine in capsule formulation, Asian J. Research Chem, 1 (2008) 15-18.
[21] S.B. Patel, B.G. Chaudhari, M.K. Buch, A.B. Patel, Stability indicating RP-HPLC method for simultaneous determination of Valsartan and amlodipine from their combination drug product, Inter J ChemTech Res, 1 (2009) 1257-1267.
[22] C. Krishnaiah, A.R. Reddy, R. Kumar, K. Mukkanti, Stability-indicating UPLC method for determination of Valsartan and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms, J Pharm Biomed Anal, 53 (2010) 483-489.
[23] G. Ragno, E. Cione, A. Garofalo, G. Genchi, G. Ioele, A. Risoli, A. Spagnoletta, Design and monitoring of photostability systems for amlodipine dosage forms, Int J Pharm, 265 (2003) 125-132.