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NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND VALSARTAN IN PHARMACEUTICAL FORMULATION

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Keywords:

Amlodipine Besylate, Valsartan, RP-HPLC, Stability indicating

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ABSTRACT: A simple, specific, accurate and precise and stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Amlodipine Besylate and Valsartan in pharmaceutical formulation. The method was developed using Enable C 18G column (250 × 4.6 mm, 0.5 µm) with a mobile phase consisting of sodium acetate buffer (pH 5.0) and methanol (35:65% v/v) with a flow rate of 1 mL/min. The UV detection was carried out at 234 nm. The retention time for Amlodipine Besylate and Valsartan were found to be 3.146 and 6.543 min respectively. The proposed method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was observed over a concentration range 0.5-250 µg/ml for amlodipine besylate (r^2 = 0.9996) and 1-90 µg/ml for Valsartan (r^2 = 0.9984). The % RSD for Intraday and Interday precision was found to be 0.37 and 0.57 for Amlodipine Besylate and 0.48 and 0.75 for Valsartan. The LOD and LOQ were found to be 0.01 µg/ml, and 0.04 µg/ml for Amlodipine Besylate and LOD and LOQ were found to be 0.04 and 0.14 µg/ml for Valsartan respectively. Amlodipine Besylate and Valsartan were subjected to stress conditions of degradation including acidic, alkaline, oxidative, thermal and photolysis.

INTRODUCTION: Amlodipine besylate **Fig. 1**, chemically designated as 2-[(2-aminoethoxy)-methyl]-4-(2- chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester, is a calcium channel blocker used to treat hypertension and angina. The drug is found to metabolize in the liver, and the produced metabolites are excreted via urine along with some unchanged drug.

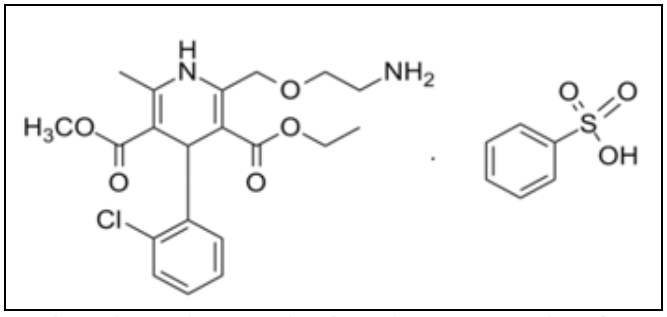

FIG. 1: STRUCTURE OF AMLODIPINE BESYLATE

Literature survey reveals various analytical method are reported either alone or combined with other drugs includes UV spectrophotometric [1-4], Visible spectrophotometric [5-7], spectrofluorometric [8], Titrimetry [5], LC-MS [9], HPLC [10-11] in pure drug, pharmaceutical formulations and biological fluids.

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Valsartan **Fig. 2** Chemically, is (2S)-3-methyl-2-[N- ({4- [2- (2H- 1, 2, 3, 4-tetrazol-5-yl)phenyl] phenyl} methyl) pentanamido] butanoic acid. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure. It is an angiotensin receptor blockers that selectively inhibits the binding of angiotensin- II to AT_1, which is found in many tissues such as vascular smooth muscles and the adrenal glands. This effectively inhibits the AT_1-mediated vaso-constrictive and aldosterone-secreting effects of angiotensin-II and results in a decrease in vascular resistance and blood pressure. Valsartan is selective for AT_1 and has virtually no affinity for AT_2. Inhibition of aldosterone secretion may inhibit sodium and water reabsorption in the kidneys while decreasing potassium excretion.

Valsartan is used in the treatment of hypertension, to lower blood pressure. Lowering blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions. It is used in the treatment of heart failure, and it significantly reduces hospitalization for heart failure. It reduces cardiovascular mortality in clinically stable patients with left ventricular failure or left ventricular dysfunction following myocardial infarction. It has an oral bioavailability of 25% and is available in tablet dosage forms. Several analytical methods have been reported for the determination of Valsartan either alone, or in combination with other drugs in pure drug, pharmaceutical dosage forms and biological samples using spectrophotometry [12-17], HPLC [18-38], LC-MS [39] and HPTLC [40-42] methods have been reported for the determination of Valsartan in pharmaceutical dosage forms. The combination of Amlodipine besylate and Valsartan is effective in the treatment of hypertension.

Various analytical methods were reported for simultaneous estimation of Amlodipine besylate and Valsartan in pure drug, pharmaceutical formulations and biological fluids by spectro-photometric [43-44], HPLC [45-56] and HPTLC[57]. From the reported RP-HPLC method for the simultaneous estimation of both drugs in pharmaceutical formulation, six methods are stability indicating one but the developed methods have has low linearity range, high flow rate, and mobile phase composition is complex.

Therefore, in the present study, an attempt was made to develop a simple, precise, accurate RP-HPLC method with forced degradation studies for the analysis of Amlodipine besylate and Valsartan in pharmaceutical formulation.

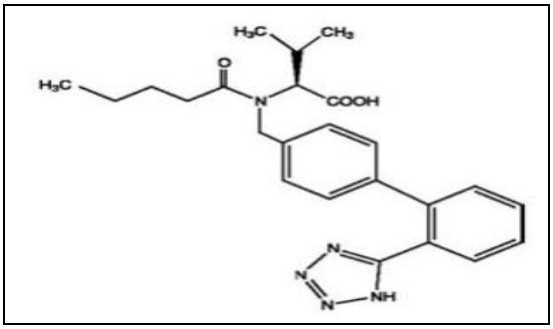

FIG. 2: STRUCTURE OF VALSARTAN

MATERIALS AND METHODS:

Materials and Chemicals: Amlodipine Besylate and Valsartan standard were obtained as a gifted sample from the pharma industry. Amlodipine Besylate and Valsartan tablets (EXFORGE FCT TABLETS) containing Amlodipine besylate 5 mg and Valsartan 80 mg were purchased from the medical store. HPLC grade water was from MERCK India Ltd. HPLC grade methanol was from standard reagent Pvt. Ltd., Hyderabad. Analytical grade acetic acid, sodium acetate, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were from SD Fine chemicals Mumbai, India. Nylon membrane filters 0.2 µm and 0.45 µm were from PALL life sciences Mumbai, India. Ultrasonicator used was from LAB India Ltd Mumbai. pH meter was of Elico LI 120 make. UV Spectrophotometer was of Elico SL 210 model consisted of spectral treats software.

Instrumentation: The chromatographic system used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector 7725i fitted with a 20 µL loop was used and data were recorded and evaluated by use of LC solutions software version 5.0.

Chromatographic Conditions: Chromatographic analysis was performed on Enable C18 G column (250 × 4.6 mm i.d, 5µ) .The mobile phase consisted of sodium acetate buffer (pH 5.0) and methanol (35:65, % v/v). The flow rate was 1 mL/min,

injection volume was 20 µL, and detection was carried out at 234 nm using a UV detector.

Preparations of Amlodipine Besylate and Valsartan Stock Solution: Stock solution of Amlodipine Besylate (1000 µg/ml) and Valsartan (1000 µg/ml) was prepared separately by transferring accurately weighed 50 mg of Amlodipine Besylate and 50 mg of Valsartan into a 50 ml volumetric flask and to it added a 20 ml methanol. The mixture was sonicated for 5 min to dissolve the drug and the solution was diluted up to the mark with methanol. Standard solution Valsartan (100 µg/ml) was prepared by diluting 10 ml of standard stock solution to 100 ml in a volumetric flask with the mobile phase. To prepare a binary mixture of Valsartan and Amlodipine Besylate appropriate volume of standard solution was transferred into a 100 ml volumetric flask and diluted with mobile phase to get a solution containing 100 µg/ml of Valsartan and 10 µg/ml of Amlodipine Besylate.

Analysis of Amlodipine Besylate and Valsartan in Combined Dosage Form: Accurately weighed about twenty tablets and an average weight of tablet was determined. The tablets were transferred into a mortar and triturated to a fine powder form. An a liquate of the powder equivalent to 160 mg of Valsartan and 10 mg of Amlodipine Besylate was transferred into a 100 ml volumetric flask. To it, 20 ml HPLC grade methanol was added and sonicated for 5 min to dissolve the drugs. The content of the flask was kept for 10 min at laboratory temperature and diluted up to mark with HPLC grade methanol this gives a concentration of Valsartan 1600 µg/ml and Amlodipine Besylate 100 µg/ml. The above solution was filtered through 0.2 µ membrane filter. The 5 ml of the filtrate was transferred into a 100 ml volumetric flask and diluted with mobile phase to get a concentration of 80 µg/ml and 5 µg/ml for Valsartan and Amlodipine Besylate respectively.

Method Validation: The method was validated for accuracy, precision, linearity, specificity, robustness, the limit of detection, the limit of quantitation and system suitability.

Linearity: Linearity was performed by preparing standard solutions of Valsartan and Amlodipine besylate at different concentration levels. Valsartan was prepared in the concentration range of 1-90 µg/mL and 0.5-250 µg/mL for Amlodipine besylate. Twenty micro liters of each concentration from both drug solutions were injected in duplicate into the HPLC system. The response was carried out at 234 nm, and the corresponding chromatograms were recorded from these mean peak areas were calculated. The calibration curve was plotted by taking concentration on x-axis and peak areas on the y-axis for both the drugs.

Accuracy: The accuracy of the method evaluated by standard addition method in which a known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery of Valsartan and Amlodipine besylate was calculated at three concentration levels of 80%, 100%, and 120%. The solutions were analyzed in triplicate at each level. The percent recovery and % RSD at each level was calculated.

Precision: Precision of the method was evaluated as system precision and method precision. To study the system precision, six replicate standard solutions of Valsartan and Amlodipine Besylate were analyzed. The percent relative standard deviation (% RSD) was calculated for both Valsartan and Amlodipine besylate. Method precision of the analytical method was carried out on six preparations from the tablet formulation, and percentage amount of Valsartan and Amlodipine Besylate in the tablet formulation was calculated. The intraday and interday precision studies were conducted for both Valsartan and Amlodipine Besylate. The mean % assay value, standard deviation and percent relative standard deviation was calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD was measured by serially diluting the standard solutions of Valsartan and Amlodipine Besylate and determining the concentration was the response of sample peaks are three times the noise peak. LOQ was measured by serially diluting the standard solutions of Valsartan and Amlodipine Besylate and determining the concentration was the response of sample peaks are ten times the noise peak.

Robustness: Robustness of the method was determined by making slight changes in the

composition of organic phase ± 5%, pH by ± 0.2, flow rate by ± 0.1 ml/min and detection wavelength by ± 2 nm.

Specificity: The specificity of the proposed method was determined against blank and placebo applications. Here mobile phase was used as a blank, and excipients like starch, lactose, magnesium stearate were used as placebo.

Forced Degradation Studies: Different stress conditions were used for the forced degradation studies of the formulation. These were also used to evaluate the specificity of the method. All the samples were diluted with mobile phase and filtered through 0.2 µ membrane filter.

Acidic Conditions: Weighed accurately about twenty tablets and triturated it to a fine powder form. An a liquate of the powder equivalent to 160 mg of Valsartan and 10 mg of Amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 10 ml of 5N HCl was added to it, refluxed for 3 h at 60 ºC, cooled to room temperature, neutralized with 5N NaOH and diluted up to the mark with the diluent. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 5 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Alkaline Conditions: Weighed accurately about twenty tablets and triturated it to a fine powder form. An a liquate of the powder equivalent to 160 mg of Valsartan and 10 mg of Amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 10 ml of 2N NaOH was added to it, refluxed for 6 hr at 60 ºC, cooled to room temperature, neutralized with 2N HCl and diluted up to the mark with the diluent. The above sample solution was filtered through a 0.2µ nylon membrane filter. Pipetted 5 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Oxidative Degradation: Weighed accurately about twenty tablets and triturated it to a fine powder form. An a liquate of the powder equivalent to 160 mg of Valsartan and 10 mg of Amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 5 ml of 3 % hydrogen peroxide was added, refluxed for 10 hr at 60 ºC, then cooled to room temperature and diluted up to the mark with diluents. The above sample solution was filtered through a 0.2µ nylon membrane filter. Pipetted 5 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Thermal Degradation: Weighed accurately about twenty tablets and triturated it to a fine powder form. The powder sample was subjected to thermal stress at 80 ºC for about 2 days. An a liquate of the powder equivalent to 160 mg of Valsartan and 10 mg of Amlodipine besylate was transferred into a 100 ml volumetric flask.

To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely the diluted up to mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 5 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

Photolytic Degradation: Weighed accurately about twenty tablets and triturated it to a fine powder form. The powder sample was subjected to UV light in a photostability chamber for about 10 days. An a liquate of the powder equivalent to 100 mg of Valsartan and 10 mg of Amlodipine besylate was transferred into a 100 ml volumetric flask. To this added a 50 ml of diluent and sonicated for 10 min to dissolve the drug completely the diluted up to mark with diluents.

The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 6 ml of the above-filtered sample solution into a 100 ml volumetric flask and volume made up to the mark with diluent.

RESULTS AND DISCUSSION:

Optimization of Chromatographic Conditions: In the present work an analytical method based on RP-HPLC using UV detection was developed and validated for simultaneous estimation of Valsartan and Amlodipine besylate in pharmaceutical formulation. The selection of analytical conditions

was based on the chemical nature of valsartan and Amlodipine besylate. A systematic study of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant for development of the analytical method. Both Valsartan and Amlodipine besylate were soluble in polar solvents; therefore, RP-HPLC was chosen. The selection of stationary phase has been done based on back pressure, resolution, peak shape, theoretical plates and day to day reproducibility in retention time resolution between Valsartan and Amlodipine besylate peaks. After evaluating all these factors Enable C18 G column (250 × 4.6 mm i.d, 5µ) was chosen for the analysis. The selection of buffer was based on the chemical structure of selected drug molecules.

For optimization of the mobile phase, preliminary trials were conducted under isocratic conditions using mobile phases composed of a mixture of solvents like water, methanol, and acetonitrile with or without different buffers in different combinations. A mixture of sodium acetate buffer pH 5.0 and Methanol in the ratio of 30:70 v/v was found to be most suitable of all the combinations since the chromatographic peaks obtained had good system suitability parameters. The Flow rate of mobile phase was optimized based on the resolution between chromatographic peaks and minimal solvent consumption.

The flow rate of mobile phase was changed from 0.5-2 ml/min. It was found from trials that 1 ml/min flow rate was ideal for successful elution of both drugs. For the selection of analytical wavelength standard solutions of both drugs were scanned in the wavelength range of 200-350 nm. A detection wavelength of 234 nm was selected. The chromatogram of the sample was shown in **Fig. 3**.

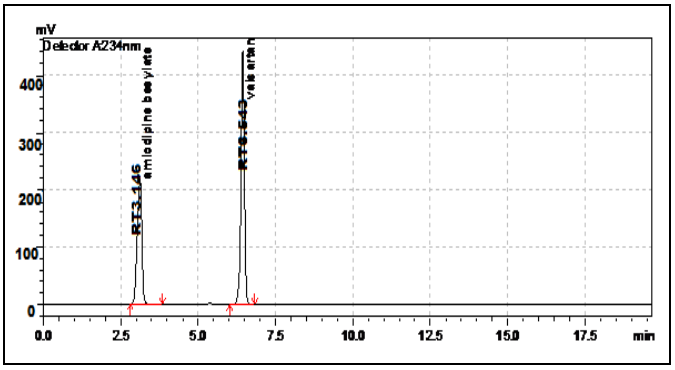

FIG. 3: CHROMATOGRAM OF AMLODIPINE BESYLATE AND VALSARTAN

Method Validation: Linearity was studied by preparing standard solutions at different concentration levels. The linearity ranges for Amlodipine Besylate and Valsartan were found to be 0.5-250 µg/mL and 1-90 µg/mL respectively. The linear regression equation for Amlodipine besylate was found to be 3456.5x + 9554.8 with correlation coefficient 0.9996. The linear regression equation for Valsartan was found to be 24099x + 16587 with correlation coefficient 0.9984. The calibration table for Amlodipine Besylate and Valsartan was shown in **Table 1** and **Table 2** respectively. The calibration curve of Amlodipine Besylate and Valsartan were shown in **Fig. 4** and **Fig. 5** respectively.

TABLE 1: LINEARITY DATA FOR AMLODIPINE BESYLATE

Level	The concentration of Amlodipine Besylate (µg/mL)	Mean peak area
Level-1	0.5	1946
Level-2	50	176490
Level-3	100	353975
Level-4	150	526846
Level-5	200	694286
Level-6	250	881321
	Slope	3456.5
	Intercept	9554.8
	Correlation Coefficient	0.9996

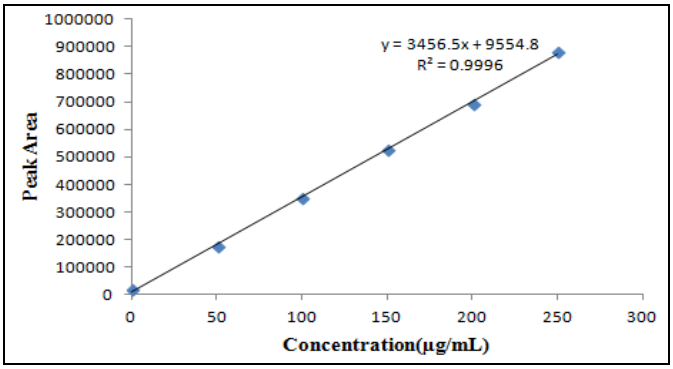

FIG. 4: LINEARITY PLOT OF AMLODIPINE BESYLATE

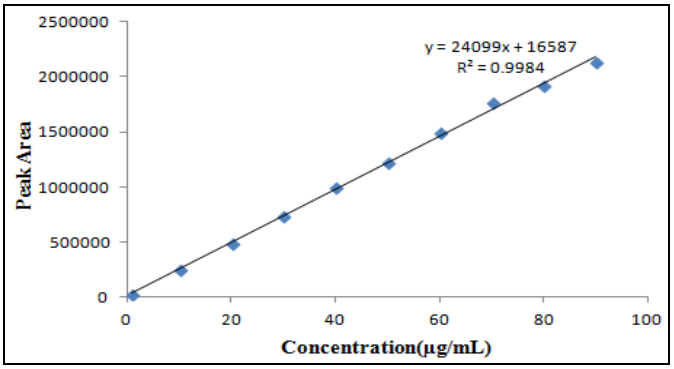

FIG. 5: LINEARITY PLOT OF VALSARTAN

TABLE 2: LINEARITY DATA FOR VALSARTAN

Level	The concentration of Valsartan (µg/mL)	Mean peak area
Level-1	01	26845
Level-2	10	248642
Level-3	20	488536
Level-4	30	737324
Level-5	40	994684
Level-6	50	1223218
Level-7	60	1491852
Level-8	70	1769494
Level-9	80	1925536
Level-10	90	2128419
Slope		24099
Intercept		16587
Correlation Coefficient		0.9984

Accuracy: The percent recovery of Valsartan and Amlodipine besylate was found to be 99.73-100.32% and 100.19-100.45%. This indicates the accuracy of the method. The results are shown in **Table 3** and **4**.

Precision:

System Precision: The % RSD for Valsartan was found to be 0.361 and for Amlodipine besylate was found to be 1.40 which are within the acceptance criteria of not more than 2.0 indicates the precision of the method. The result was shown in **Table 5**.

TABLE 3: ACCURACY RESULTS OF VALSARTAN

Accuracy level (%)	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery	Mean Recovery	% RSD
	4	4.04	101		
80	4	3.96	99	99.75	1.08
	4	3.97	99.25		
	5	4.94	98.8		
100	5	4.96	99.2	99.73	1.28
	5	5.06	101.2		
	6	6.09	101.5		
120	6	6.05	100.8	100.32	1.47
	6	5.92	98.66		

TABLE 4: ACCURACY RESULTS OF AMLODIPINE BESYLATE

Accuracy level (%)	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery	Mean Recovery	% RSD
	64	64.12	100.18		
80	64	63.98	99.96	100.19	0.23
	64	64.28	100.43		
	80	80.54	100.67		
100	80	80.22	100.27	100.45	0.20
	80	80.34	100.42		
	96	95.87	99.86		
120	96	96.42	100.43	100.23	0.32
	96	96.39	100.40		

TABLE 5: SYSTEM PRECISION FOR VALSARTAN AND AMLODIPINE BESYLATE

Injection No.	Peak Area of Valsartan	Peak Area of Amlodipine Besylate
1	994684	526846
2	991556	516842
3	990463	528990
4	999438	531475
5	995631	528824
6	998464	539856
Mean	995039.3	528805.5
SD	3595.2	7432.8
% RSD	0.361	1.40

Method Precision: The % RSD for Intraday and Interday precision assay results of six preparations for Valsartan were found to be 0.48 and 0.75 respectively which are within the acceptance criteria of not more than 2.0 indicates the precision of the method. The % RSD for Intraday and Interday precision assay results of six preparations for Amlodipine Besylate was found to be 0.37, and 0.57 respectively which are within the acceptance criteria of not more than 2.0 indicates the precision of the method. The result was shown in **Table 6**.

Limit of Detection and Limit of Quantitation: The LOD and LOQ were found to be 0.01 µg/mL, and 0.04 µg/mL for Amlodipine Besylate and the LOD and LOQ for Valsartan were 0.04 µg/mL and 0.14 µg/mL respectively.

Robustness: To evaluate the robustness of the developed method, small, deliberate variations in

optimized method parameters were made. The effect of change in flow rate, change in pH, change in the composition of mobile phase and detection wavelength on retention time, tailing factor and theoretical plates were studied. The method was found to be unaffected by small changes in flow rate, change in pH, change in the composition of mobile phase and detection wavelength. The results were shown in **Table 7** and **Table 8**.

TABLE 6: METHOD PRECISION FOR VALSARTAN AND AMLODIPINE BESYLATE

Set	Valsartan (%Assay)		Amlodipine Besylate (% Assay)	
	Intraday (n=6)	Interday (n=6)	Intraday (n=6)	Interday (n=6)
1	100.21	100.63	100.84	100.98
2	100.18	100.44	100.44	100.86
3	101.24	100.16	100.69	100.41
4	99.92	101.87	100.12	100.72
5	99.97	100.18	100.75	99.54
6	100.46	101.64	99.89	99.82
Mean	100.33	100.82	100.45	100.38
SD	0.48	0.74	0.38	0.58
% RSD	0.48	0.75	0.37	0.57

TABLE 7: ROBUSTNESS RESULTS FOR AMLODIPINE BESYLATE

Conditions	% Assay	System Suitability parameters	
		Theoretical Plates	Tailing Factor
Flow Rate 0.9 mL/min	100.24	6089	1.19
Flow Rate 1.1 mL/min	100.12	6523	1.24
Mobile Phase- Buffer(35):Acetonitrile(65)	99.94	6545	1.24
Mobile Phase- Buffer(25):Acetonitrile(75)	99.87	6365	1.22
Mobile Phase pH 4.8	100.06	6821	1.32
Mobile Phase pH 5.2	100.11	6798	1.25
Wavelength 232 nm	100.31	6431	1.19
Wavelength 238 nm	100.46	6486	1.18

TABLE 8: ROBUSTNESS RESULTS FOR VALSARTAN

Conditions	% Assay	System Suitability parameters	
		Theoretical Plates	Tailing Factor
Flow Rate 0.9 mL/min	99.67	8054	1.12
Flow Rate 1.1 mL/min	99.84	7667	1.23
Mobile Phase- Buffer(35):Acetonitrile(65)	99.63	7642	1.24
Mobile Phase- Buffer(25):Acetonitrile(75)	100.12	7895	1.21
Mobile Phase pH 4.8	100.08	8213	1.26
Mobile Phase pH 5.2	100.42	8654	1.23
Wavelength 232 nm	99.83	7687	1.22
Wavelength 238 nm	99.79	7632	1.21

Specificity: Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. The specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purities of Amlodipine besylate and Valsartan were assessed by comparing the retention times of standard Amlodipine besylate and Valsartan and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected, and there were no peaks. There is no interference of degradation peaks on drug peaks; hence, the method is specific. The specificity results are shown in **Table 9**.

TABLE 9: SPECIFICITY RESULTS OF THE METHOD

Name of solution	Retention Time
Blank	No peaks
Placebo	No Peaks
Valsartan	6.543 min
Amlodipine Besylate	3.146 min

Analysis of Commercial Formulation: The proposed method was applied for the determination of Amlodipine Besylate and Valsartan in marketed formulations available (EXFORGE FCT TABLETS). The % recovery was found to be 99.60 ± 0.68 and 99.60 ± 0.89 for Amlodipine Besylate and Valsartan respectively in **Table 10**.

TABLE 10: ANALYSIS OF AMLODIPINE BESYLATE AND VALSARTAN IN COMMERCIAL FORMULATION

Formulation	Labeled claim (mg)		Amount found* (mg)		% Recovery* ± % RSD	
	Amlodipine besylate	Valsartan	Amlodipine besylate	Valsartan	Amlodipine besylate	Valsartan
EXFORGE TABLETS	5	80	4.98	79.95	99.60 ± 0.68	99.93 ± 0.89

*Average of three determinations

Results of Forced Degradation Studies: Under alkaline conditions Amlodipine besylate degraded to 28.64% and Valsartan degraded to 15.86%. In these stress conditions, the retention time of degradation peaks appears at 2.124 min, 9.621 min, and 10.903 min. In acidic conditions, Amlodipine Besylate degraded to 10.44 % and Valsartan degraded to 12.47%. Under these conditions, degradant peak appears at 5.921 min and 13.563 min. In oxidative conditions, Amlodipine Besylate degraded to 26.36% and Valsartan to 19.62%. Although both drugs degraded to a significant extent, but only one minor peak detected at retention times of 2.484 min. In thermal conditions, Amlodipine Besylate degraded to 0.56% and Valsartan degraded to 2.86%. The two degradant peaks appear at retention times of 2.314 min and 2.602 min on the chromatogram. In photolytic conditions, Amlodipine Besylate degraded to 24.88% and Valsartan degraded to 5.74% here also both the drugs degraded to a significant extent, but only one minor peak are detected at retention times of 2.401 min. The degradation chromatograms were shown in from **Fig. 6 - Fig. 10**. The system suitability parameters of chromatograms were summarized in **Table 11**.

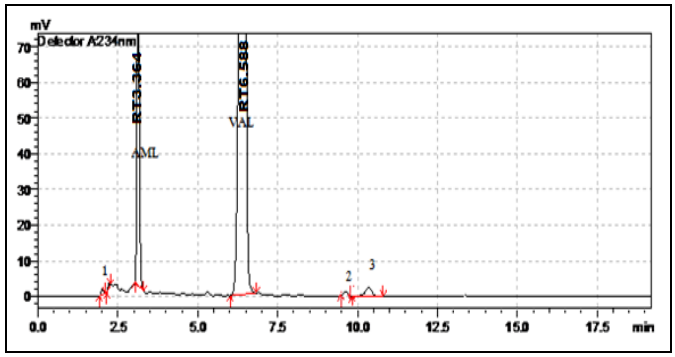

FIG. 6: BASE DEGRADATION CHROMATOGRAM

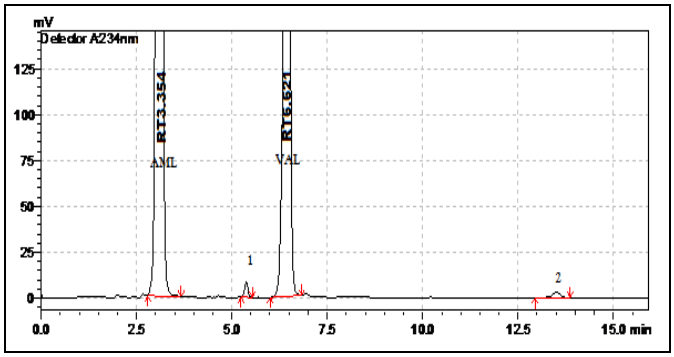

FIG. 7: ACID DEGRADATION CHROMATOGRAM

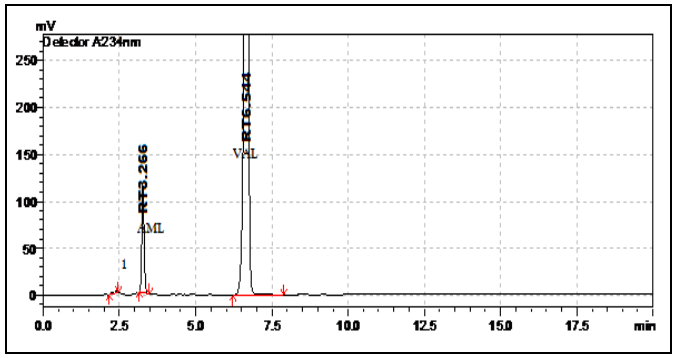

FIG. 8: OXIDATIVE DEGRADATION CHROMATOGRAM

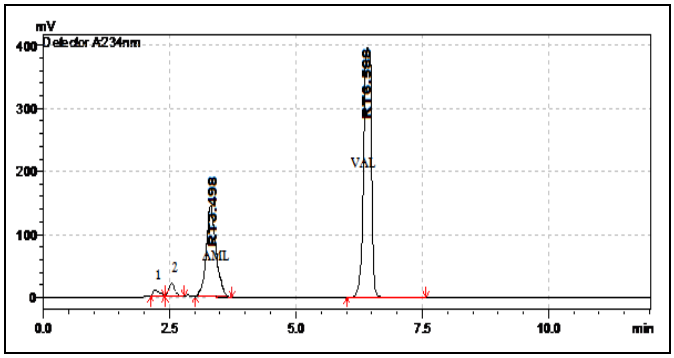

FIG. 9: THERMAL DEGRADATION CHROMATOGRAM

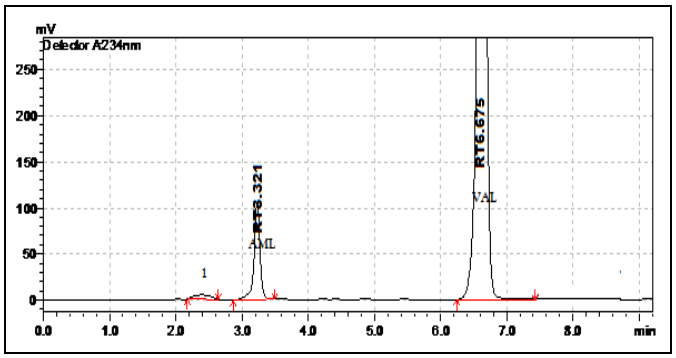

FIG. 10: PHOTOLYTIC DEGRADATION CHROMATOGRAM

TABLE 11: FORCED DEGRADATION STUDIES OF VALSARTAN AND AMLODIPINE BESYLATE

Stress Conditions	% Drug Recovered	% Drug decomposed	Retention Time (min)	Theoretical Plates	Tailing Factor
Valsartan					
Control Sample	99.93	--	6.543	8221	1.21
Alkaline Degradation (2N/60°C/6 h)	84.07	15.86	6.588	8362	1.23
Acid Degradation (5 N /60°C/3 h)	87.46	12.47	6.621	8275	1.23
Oxidative Degradation (3 % H_2O_2/60°C /10 h)	80.31	19.62	6.544	8232	1.31
Thermal Degradation (80 °C/2 days)	97.07	2.86	6.588	8467	1.14
Photolytic Degradation (1.2 million lux hours/10 days)	94.19	5.74	6.675	8645	1.43
Amlodipine Besylate					
Control Sample	99.60	--	3.146	6486	1.22
Alkaline Degradation (2N/60°C/6 h)	96.62	28.64	3.364	5853	1.23
Acid Degradation (5 N/60°C/3 h)	69.62	10.44	3.354	5674	1.26
Oxidative Degradation (3 % H_2O_2/60°C /10 h)	69.7	26.36	3.266	5281	1.12
Thermal Degradation (80 °C/2 days)	99.63	0.56	3.498	5736	1.31
Photolytic Degradation (1.2 million lux hours/10 days)	71.34	24.88	3.321	5452	0.92

CONCLUSION: The proposed method for the simultaneous estimation of Amlodipine Besylate and Valsartan validated as per the ICH guidelines, and it is simple, specific and reliable. The data generated from the forced degradation studies enabled the evaluation of Amlodipine Besylate and Valsartan stability under a variety of ICH recommended conditions. These data are valuable for the safety and potency assessment of a drug product. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Amlodipine Besylate and Valsartan in pharmaceutical formulations without any interference from the excipient.

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CONFLICT OF INTEREST: No

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