Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

RP-HPLC Method Development and Validation for the Simultaneous Determination of Amlodipine Besilate and Valsartan in Pharmaceutical Dosage Form

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Received on: 23-09-2013; Revised and Accepted on: 12-10-2013

ABSTRACT

A rapid, accurate, specific, linear, and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous determination of Amlodipine (Besilate) (AML) and Valsartan (VAL) in pharmaceutical dosage form. The chromatographic separation was performed on Thermo-scientific Hypersil BDS C18 Column (250mm×4.6mm, 5µm particle size) using a mobile phase A: Acetonitrile, Water, Methanol and Trifluoroaceticacid (300:700:110:1v/v) and mobile phase B: Acetonitrile, Water, Methanol and Trifluoroaceticacid (600:400:110:1 v/v), at a flow rate of 1.5 ml/min at 25°C column temperature with the detection wavelength at 240nm. The retention times of AML related compound A, AML, VAL related compound B and VAL were 5.29 min, 6.8 min, 7.34 min and 8.55 min respectively. The linearity was performed in the concentration range of 8-12.5µg/ml (AML) and 128-192 µg/ml (VAL) with a squared correlation coefficient of 0.9999 and 0.9999 for AML and VAL respectively. The percentage purity of AML and VAL was found to be > 99.8%. The Proposed method has been validated for specificity, linearity, precision, accuracy, ruggedness and robustness and were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the combined pharmaceutical dosage forms.

Key words: *Amlodipine Besilate, Valsartan, RP-HPLC, Validation.*

INTRODUCTION

Amlodipine Besilate (AML) is Calcium channel blocker. Chemically: 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4- dihydropyridine-3,5-dicarboxylate benzenesulphonate., its molecular weight is 567.1g/mol with an empirical formula $C_{20}H_{25}ClN_2O_5,C_6H_6O_3S$. **(Fig. 1)** [1].

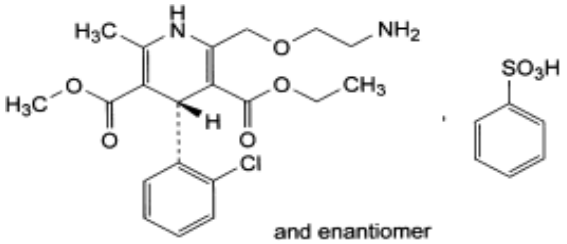

Fig. 1: Chemical Structure for Amlosipine Besilate

Valsartan (VAL) is chemically described as N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-L-valine. Its empirical formula is $C_{24}H_{29}N_5O_3$, its molecular weight is 435.5. **(Fig. 2)** [1].

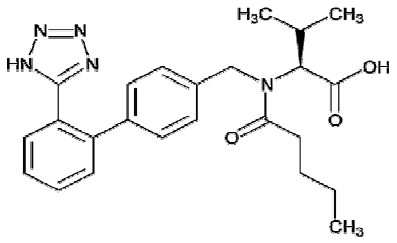

Fig. 2: Chemical Structure for Valsartan

Literature survey reveals that few HPLC methods [2-6], have been reported for the estimation of Amlodipine Besilate and Valsartan. The aim of the present study is to develop a simple, precise, linear and accurate reversed-phase HPLC method for the estimation of Amlodipine Besilate and Valsartan pharmaceutical dosage form [7-8].

MATERIALS AND METHODS

Instrumental and Analytical Conditions:
Reagents and Chemicals:

USP AML related compound A and USP VAL related compound B were used. AML and VAL were purchased from CADILA Pharmaceuticals and from JUBILANTS LifeSciences, respectively. All chemicals used of HPLC grade: Acetonitrile and Methanol were purchased from J.T. Baker, and Trifluoroacetic acid which was purchased from Alfa Aesar. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

Equipment:

A Dionex UltiMate 3000 HPLC system with Chromelen software "version 1.1", Photodiod Array Detector and Autosampler was used. It was manufactured by Dionex Corporation Company, USA.

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Chromatographic Conditions:

The column Thermo-scientific Hypersil BDS C18 Column (250mm×4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase A: Acetonitrile, Water, Methanol and trifluoroacetic acid (300:700:110:1v/v) and mobile phase B: Acetonitrile, Water, Methanol and trifluoroacetic acid (600:400:110:1 v/v)) with a gradient program as described in **Table 1**. The flow was adjusted to 1.5ml/min. The instrument was operated at 25°C temperature. The UV detection was achieved at 240nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL.

Table No. 1: Gradient program for mobile phase

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	90	10
8	0	100
12	0	100
12.1	90	10
16	90	10

Preparation of Analytical Solutions:

Preparation of mobile phase A:

Mix 300ml of Acetonitrile, 700ml of Water, 110ml of Methanol and 1ml of Trifluoroacetic acid and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of mobile phase B:

Mix 600ml of Acetonitrile, 400ml of Water, 110ml of Methanol and 1ml of Trifluoroaceticacid and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of diluent:

Mix 500ml of Acetonitrile, 500ml of Water, 110ml of Methanol and 1ml of Trifluoroaceticacid and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of system suitability solution:

Prepared by dissolving 1.0 mg of each of Amlodipine Besilate, Valsartan, Amlodipine related compound A and Valsartan related compound B in 100 ml of diluent. Filtered through 0.45µ filter.

Preparation of standard stock solution for Amlodipine (Besilate):

The Amlodipine (Besilate) standard stock solution was prepared by dissolving Amlodipine Besilate equivalent to 50.0mg Amlodipine as (Besilate) standard in 50 ml diluent, dissolved using sonicator, cooled to room temperature and filtered using 0.45µ filter to obtain a solution having a concentration of 1.0mg/ml.

Preparation of Amlodipine (Besilate) and Valsartan standard solution:

The standard was prepared by transferring an equivalent to 80.0mg of Valsartan standard to 50 ml volumetric flask, dissolved in about 30 ml of diluent. 5.0 ml of the Amlodipine (Besilate) standard stock solution were added to the same 50 ml flask and completed to volume with diluent and dissolved using sonicator, cooled to room temperature .5.0 ml of the resulting solution were transferred to 50 ml volumetric flask and the volume was completed with diluent to obtain a concentration of 0.16mg/ml of Valsartan and 0.01mg/ml Amlodipine (Besilate). Filter through 0.45µ filter.

Preparation of sample solution (Valsartan and Amlodipine 160/10mg tablet: Marketed formulation):

10 tablets were weighed and the average weight (349 mg) was calculated and finely powdered. 698 mg of powdered tablets were transferred to 200 ml volumetric flask; 150 ml of diluent were added and mixed to dissolve the active ingredient by the aid of sonicator for 10 minutes. Cooled and the volume was completed with diluent. 5.0 ml of the resulting solution was diluted to 50.0 ml with diluent, mixed well and filtered using 0.45 µ filter to obtain a solution having a concentration of 0.16mg/ml Valsartan and 0.01mg/ml Amlodipine (Besilate). Filter through 0.45µ filter.

Method Development and Validation of HPLC Method:

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, and system suitability.

Specificity:

The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak. Forced degradation studies are carried out by using 0.1M HCl, 0.3M HCl, 2M HCl, 2M NaOH, 0.1M NaOH, thermal, hydrogen peroxide degradation and U.V light.

Linearity:

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of AML and VAL, the solutions of five different concentration levels 128-192µg/ml (VAL) and 8.0-12.0 µg/ml (AML) are injected into the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, and correlation coefficient-R^2 were determined.

Precision:

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments).

In order to determine precision, six independent sample solution preparations from a single lot of formulation 10µg/ml for AML and 160µg/ml for VAL were injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Accuracy:

Accuracy was determined in terms of percentage recovery the accuracy study was performed for 80%, 100% and 120 % for AML and VAL. Standard and sample solutions are injected into HPLC system in triplicate and percentage recoveries of AML and VAL were calculated. The area of each level was used for calculation of % recovery.

Robustness:

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (± 6.6%), change in column temperature (± 5°C) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 1.5ml/min to 1.6ml/min and 1.4ml/min and the method is robust even by change in the mobile phase B ±5%.

System suitability:

System suitability test was carried out on freshly prepared system suitability solution of Amlodipine Besilate, Valsartan, Amlodipine related compound A and Valsartan related compound B and it was calculated by injecting solution in six replicates and the values were recorded.

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of AML and VAL. The method developed was proceeding with wavelength selection. The optimized wavelength was 240nm.

In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

The mobile phase consisted of mobile phase A: Acetonitrile, Water, Methanol and trifluoroacetic acid (300:700:110:1v/v) and mobile phase B: Acetonitrile, Water, Methanol and trifluoroacetic acid (600:400:110:1 v/v) and the column used was Thermo scientific Hypersil BDS C18 Column (250mm×4.6mm, 5µm particle size). The flow rate was adjusted to 1.5ml/min. The instrument was operated at 25°C temperature. The UV detection was achieved at 240nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The linearity was determined as linearity regression of the claimed analyte concentration of the range 8.0-12µg/ml (AML) and 128-192µg/ml (VAL). The calibration curve obtained by plotting peak area versus concentration and presented in **Table 2** was linear and the squared correlation coefficient was found to be 0.9999 and 0.9999 for AML and VAL respectively. The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for system precision presented in **Table 3** was found to be 0.13 and 0.0664and the % Relative Standard Deviation for method precision presented in **Table 4** was found to be 0.081 and 0.08. The % Relative Standard Deviation for ruggedness presented in **Table 5** was found to be 0.175 and 0.174for AML and VAL respectively.

The accuracy study was performed in 80%, 100% and 120% .The percentage recovery was determined for AML and VAL and was found to be 100.2% and 100.2% presented in **Tables 6 & 7**.

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as presented in **Table 8**. Theoretical plates and tailing factor were observed and were found to be 52478 and 74628 (theoretical plates) and 1.07 and 1.03 (tailing factor) for AML and VAL respectively. The resolution was found to be 12.8 between Amlodipine related compound A and Amlodipine (Besilate), and 9.7 between Valsartan related compound B and Valsartan. And the Relative Standard Deviation in retention time were found to be zero for AML and 0.06 for VAL in six replicate injections of system suitability solution

The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table 9**.

Table No. 2: Linearity results for Amlodipine (Besilate) and Valsartn

Amlodipine (Besilate)		Valsartan	
Area	Concentration (ml/gµ)	Area	Concentration (ml/gµ)
4.836	8	57.51	128
5.455	9	64.597	144
6.058	10	71.722	160
6.659	11	78.814	176
7.28	12	85.988	192

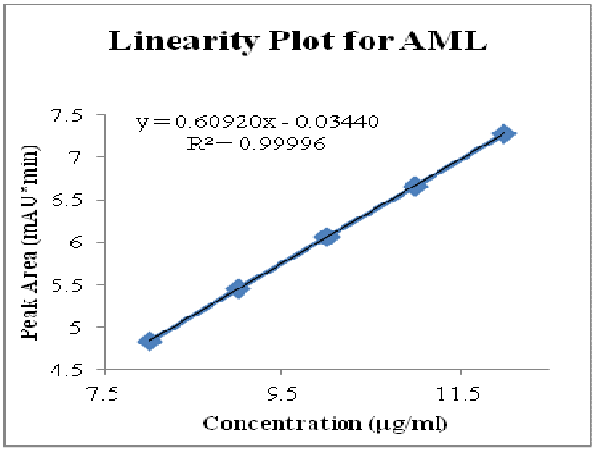

Fig. 3: Linearity plot for AML

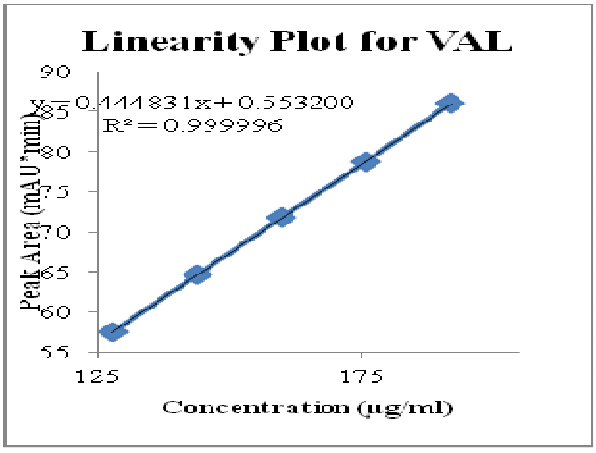

Fig. 4: Linearity plot for VAL

Table No. 3: System precision for AML and VAL

Injections	AML		VAL	
	RT	Area	RT	Area
1	6.82	6.054	8.58	71.714
2	6.82	6.053	8.58	71.767
3	6.82	6.042	8.58	71.761
4	6.82	6.055	8.59	71.782
5	6.82	6.067	8.59	71.858
6	6.82	6.05	8.59	71.8
Average	6.82	6.0535	8.585	71.78
Std. Dev.	0	0.0081	0.0055	0.0477
RSD%	0	0.13	0.064	0.0664

Table No. 4: Method precision for AML and VAL

Injections	AML		VAL	
	RT	Area	RT	Area
1	6.85	6.116	8.62	73.099
2	6.85	6.121	8.62	73.121
3	6.85	6.121	8.62	73.219
4	6.85	6.124	8.62	73.171
5	6.85	6.11	8.62	73.241
6	6.85	6.117	8.62	73.22
Average	6.85	6.118	8.62	73.179
Std. Dev.	0.000	0.005	0.000	0.058
RSD%	0	0.081	0	0.080

Table No. 5: Ruggedness values for AML and VAL

Injections	AML		VAL	
	RT	Area	RT	Area
1	6.87	6.063	8.64	70.985
2	6.87	6.069	8.64	71.09
3	6.87	6.072	8.64	71.112
4	6.87	6.08	8.65	71.167
5	6.87	6.084	8.65	71.332
6	6.88	6.092	8.65	71.258
Average	6.872	6.077	8.645	71.157
Std. Dev.	0.004	0.011	0.005	0.124
%RSD	0.059	0.175	0.063	0.174

Table No. 6: %Recovery for VAL

Concentration (at spesific level)	Active drug sol added (µg/ml)	Recovery amount (µg/ml)	Mean Recovery
80%	128	128.1	
100%	160	160.5	**100.20%**
120%	192	192.4	

Table No. 7: %Recovery for AML

Concentration (at spesific level)	Active drug sol added (µg/ml)	Recovery amount (µg/ml)	Mean Recovery
80%	8	8.02	
100%	10	10.006	**100.20%**
120%	12	12.03	

Table No. 8: Robustness values for VAL and AML Compound A

Parameter	Adjusted to	AML		VAL	
		RT	**Area**	**RT**	**Area**
Flow rate	1.4	7.17	6.555	8.97	78.56
	1.5	6.8	6.095	8.57	72.51
	1.6	6.57	5.744	8.32	68.797
Mobile B started	5%	7.13	6.132	8.85	73.345
	10%	6.8	6.095	8.57	72.51
	15%	6.57	6.125	8.39	73.379
Column Temp.	20 C°	6.96	6.139	8.75	73.51
	25 C°	6.8	6.095	8.57	72.51
	30 C°	6.75	6.129	8.5	73.462
Wavelength	235 nm	6.82	6.012	8.59	86.276
	240 nm	6.82	6.067	8.59	71.858
	245 nm	6.82	5.298	8.59	65.441

Table No. 9: System suitability values

Injection	AML related Compound A		AML		VAL related Compound A		VAL	
	RT	Area	RT	Area	RT	Area	RT	Area

1	5.29	2.23	6.8	5.151	7.34	7.453	8.55	5.503
2	5.29	2.23	6.8	5.151	7.34	7.461	8.55	5.511
3	5.29	2.23	6.79	5.15	7.34	7.459	8.55	5.513
4	5.29	2.23	6.79	5.147	7.34	7.464	8.55	5.517
5	5.28	2.23	6.79	5.15	7.34	7.467	8.55	5.522
6	5.29	2.22	6.8	5.145	7.34	7.455	8.55	5.488
Avg.	5.288	2.227	6.795	5.149	7.340	7.460	8.550	5.509
Std.Dev .	0.004	0.003	0.005	0.002	0.000	0.005	0.000	0.012
%RSD	0.077	0.112	0.081	0.048	0.000	0.071	0.000	0.219
USP Theoretical Plates	32921		52478		56524		74628	
Resolution	Between AML related Comp. A and AML: 12.8				Between VAL related Comp. B and VAL: 9.7			

Between VAL related Comp. B and VAL: 9.7

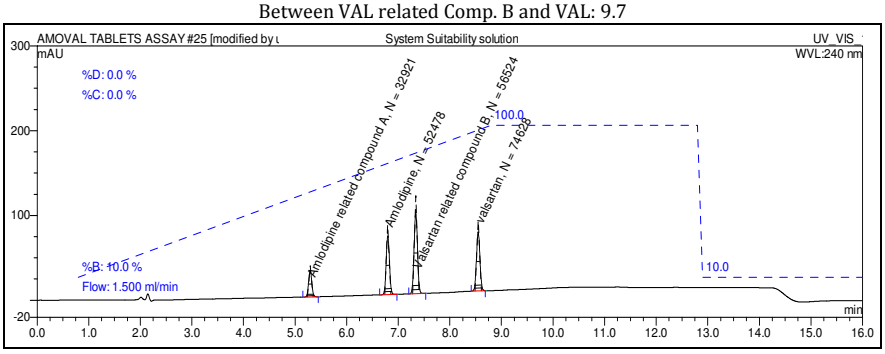

Fig. 5: Chromatogram for system suitability solution

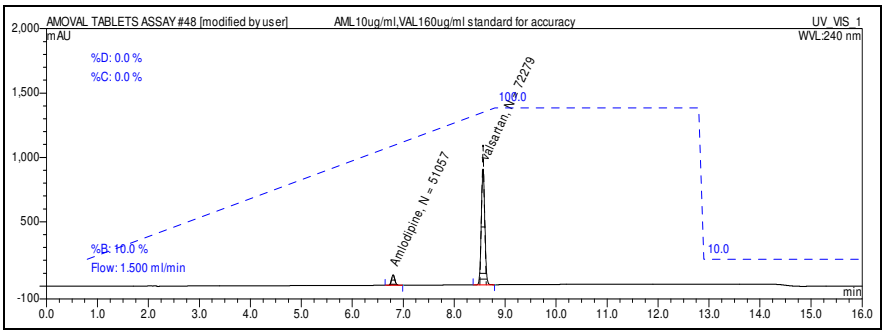

Fig. 6: Chromatogram for standard solution

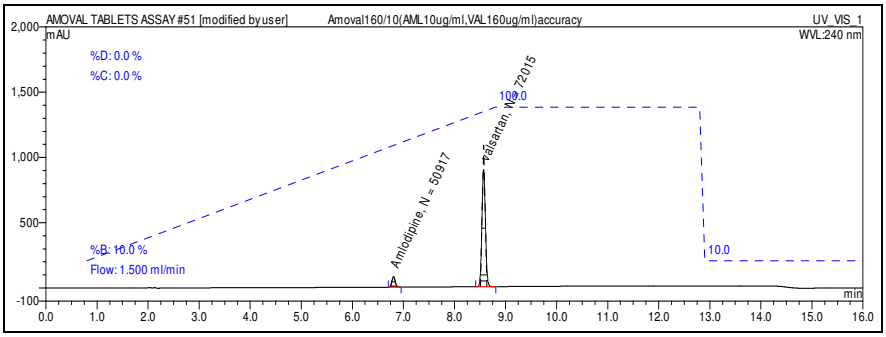

Fig. 7: Chromatogram for sample solution

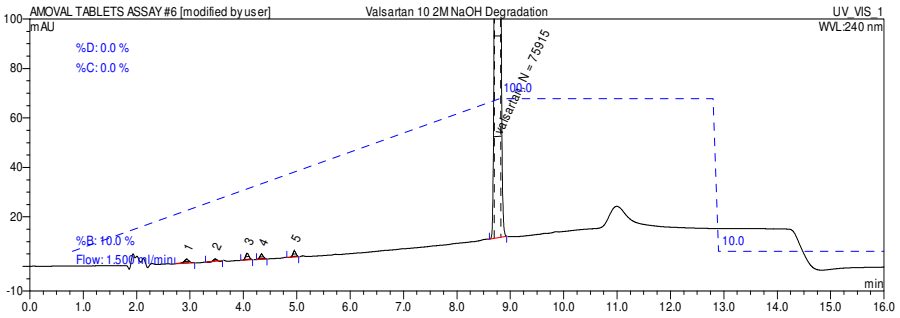

Fig. 8: Chromatogram for 2 M NaOH Degradation

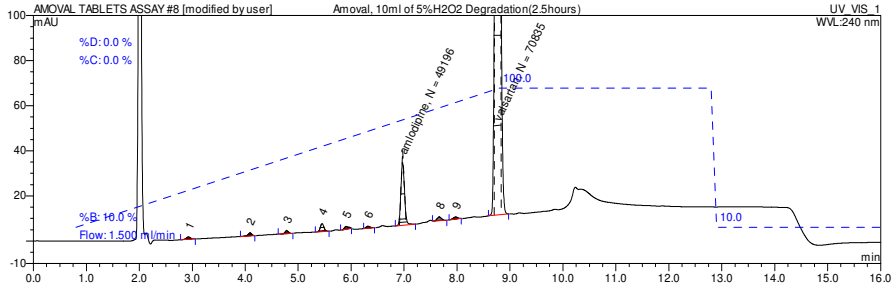

Fig. 9: Chromatogram for 5% H2O2 Degradation (2.5 hrs)

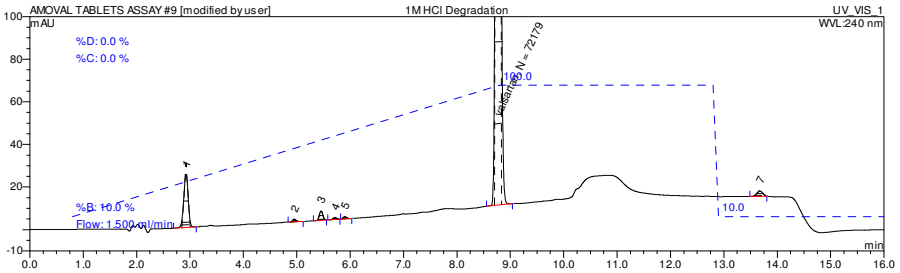

Fig. 10: Chromatogram for 1 M HCl Degradation

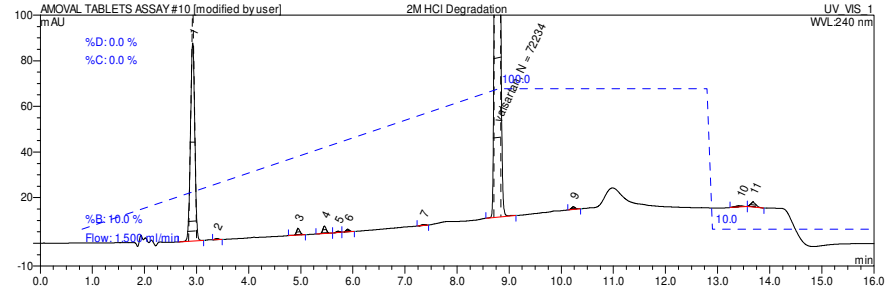

Fig. 11: Chromatogram for 2 M HCl Degradation

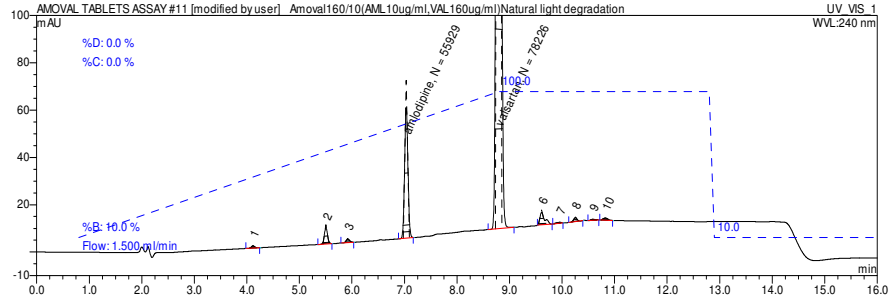

Fig. 12: Chromatogram for Light Degradation

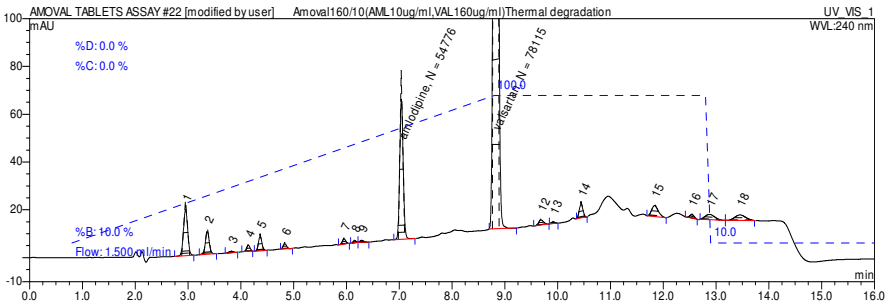

Fig. 13: Chromatogram for Thermal Degradation (24 hrs at 105°C)

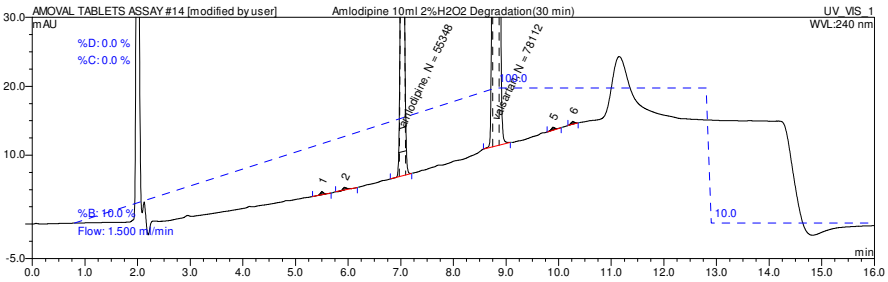

Fig. 14: Chromatogram for 2% H2O2 Degradation (30 min)

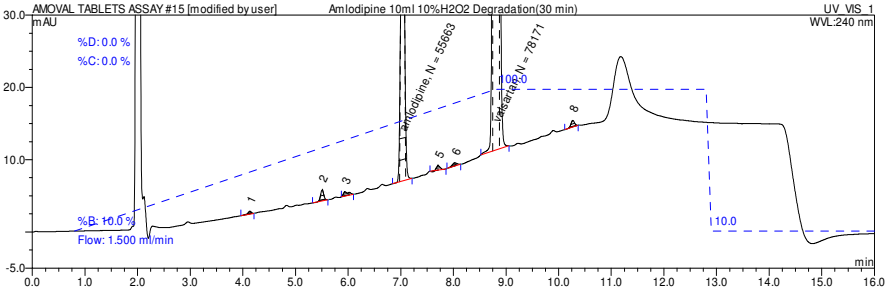

Fig. 15: Chromatogram for 10% H2O2 Degradation (30 min)

SUMMERY AND METHODS

The method was found to be precise accurate and linear for determination of Amlodipine Besilate and Valsartan. The method was developed and validated for system suitability linearity, specificity, accurace, robustness and ruggedness. All parameters tested were found to be within limits. The study indicates that the method has a significant advantages in term of shorter analysis time, good resolution between active drugs and there related substances and other system suitability parameters, high purity of active drug peaks, accuracy and precision.

ACKNOWLEDGMENTS

Thankful to Sama Pharmaceuticals Manufacturing Co. for providing facilities to execute the research work.

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Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil