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Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Determination of Atorvastatin and Amlodipine in Pharmaceutical Dosage Forms

Zahi Mohammad Turabi*, O'hood Atef Khatatbeh
Quality Control Department, Sama Pharmaceuticals Manufacturing Co., Nablus, Palestine.

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ABSTRACT

A rapid, accurate, specific, linear, and sensitive stability indicating reverse phase-HPLC method has been developed and validated for the simultaneous determination of Amlodipine (Besilate) (AML) and Atorvastatin (Calcium Trihydrate) (ATO) in pharmaceutical dosage forms. The chromatographic separation was performed on Luna 5µ C18 (e) 100A Column (250mm×4.6mm). A mobile phase: Ammonium dihydrogen phosphate buffer pH 4.4, Acetonitrile, and Tetrahydrofurane (61:37:3v/v) was used, at a flow rate of 2 ml/min , 35°C column temperature and a detection wavelength at 242nm. The retention times of AML related compound A, AML, ATO related compound A, ATO related compound B, ATO, and ATO related compound C were 2.48 min, 3.56 min, 21.44 min, 24.31min , 25.77and 28.69 min respectively. The linearity was performed in the concentration range of 16-24µg/ml (AML) and 32-48 µg/ml (ATO) with a squared correlation coefficient of 0.9999 and 9995 for AML and ATO respectively. The percentage purity of AML and ATO was found to be >99.5%. 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, Atorvastatin Calcium Trihydrate, Stability indicating, 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].

Atorvastatin Calcium Trihydrate (ATO) is chemically described as Calcium((3R,5R)-7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate trihydrate. Its empirical formula is $C_{66}H_{68}CaF_2N_4O_{10},3H_2O$, its molecular weight is 1209. **(Fig. 2)** [2].

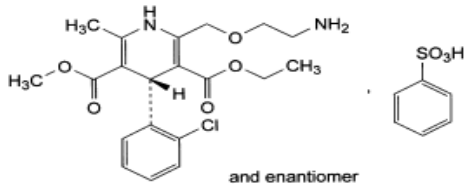

Fig. 1: Chemical Structure for Amlodipine Besilate

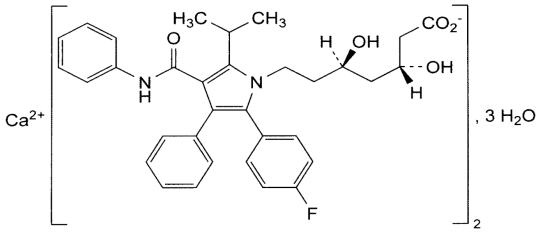

Fig. 2: Chemical Structure for Atorvastatin Calcium Trihydrate

***Corresponding author:**
Zahi Mohammad Turabi
Quality Control Department, Sama Pharmaceuticals Manufacturing Co., Nablus, Palestine. Phone no:+97292313767; Fax: 2313748
**E-Mail: zahibpharm@yahoo.com*

Literature survey reveals that few HPLC methods [3-11], have been reported for the estimation of AML and ATO. The aim of the present study is to develop a simple, precise, linear and accurate reversed-phase HPLC method for the estimation of AML and ATO pharmaceutical dosage form [12-13].

MATERIALS AND METHODS

Instrumental and Analytical Conditions:
Reagents and Chemicals:

USP AML related compound A, USP AML, USP ATO related compound A, USP ATO related compound B, USP ATO and USP ATO related compound C were used. All chemicals used of HPLC grade: Acetonitrile, Tetrahydrofurane and Methanol were purchased from J.T. Baker. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

Equipment:

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

Chromatographic Conditions:

The column Luna, 5µ C18 (e) 100A, (250mm×4.6mm) was used for analytical separation, using a mobile phase: Ammonium dihydrogen phosphate buffer, Acetonitrile, and Tetrahydrofurane (61:37:3v/v). The flow rate was adjusted to 2.0ml/min. The instrument was operated at 35°C temperature. The UV detection was achieved at 242nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL.

Preparation of Analytical Solutions:
Preparation ammonium dihydrogen phosphate pH 4.4:

3.83g of ammonium dihydrogen phosphate were dissolved in 1000 ml distilled water; pH was adjusted to 4.4 with ammonium hydroxide.

Preparation of mobile phase:

Mixture of 37 portions of Acetonitrile, 61 portions of ammonium dihydrogen phosphate buffer and 3 portions of

tetrahydrofurane, degassed in ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum filtration.

Preparation of diluent:

Mixture of 100 ml Acetonitrile, 150 ml Water, 100 ml Methanol and 150 ml Tetrahydrofurane, degassed in ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum filtration.

Preparation of system suitability solution:

Prepared by dissolving 1.0 mg of each of Amlodipine Besilate, Amlodipine related compound A, Atorvastatin related compound A, Atorvastatin related compound B, Atorvastatin and Atorvastatin related compound C in 100 ml of diluent to have a solution of 10 µg/ml of each. Filtered through 0.45µ filter.

Preparation of standard solution:

The Standard solution was prepared by dissolving Amlodipine Besilate standard equivalent to 10.0 mg Amlodipine (as Besilate) and equivalent to 20.0 mg of Atorvastatin (as calcium trihydrate) in 100 ml diluent, dissolved using sonicator, cooled to room temperature, and diluting 10 ml of the resulting solution to 50ml with diluent, filtered using 0.45µ filter to obtain a solution having a concentration of 20 µg/ml of AML and 40 µg/ml of ATO.

Preparation of sample solution (Atorvastatin and Amlodipine 20/10 mg tablet: Marketed formulation):

10 tablets were weighed and finely powdered. Equivalent to two 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 20 minutes. Cooled and the volume was completed with diluent. 10.0 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 40 µg/ml Atorvastatin and 20 µg/ml Amlodipine (Besilate). Filtered 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, 0.1M NaOH, thermal degradation, 1% hydrogen peroxide degradation and photo degradation.

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 ATO, the solutions of five different concentration levels 32 -48µg/ml (ATO) and 16 -24 µg/ml (AML) were injected into the HPLC system. The calibration curves for the standard solutions were constructed 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 20µg/ml for AML and 40µg/ml for AML 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 ATO. Standard and sample solutions are injected into HPLC system in triplicate and percentage recoveries of AML and ATO 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 (± 5%), change in column temperature (± 5°C) and change in wave length (± 2nm). The robustness was performed for the flow rate variations from 2.0ml/min to 1.9ml/min and 2.1ml/min and the method is robust even by change in the mobile phase component (± 5%).

System suitability:

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

RESULTS AND DISCUSSION

The present investigation reported is a new stability indicating RP-HPLC method development and validation of simultaneous estimation of AML and ATO. The method developed was proceeding with wavelength selection.

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

The mobile phase consisted of: Ammonium dihydrogen phosphate buffer pH 4.4, Acetonitrile, and Tetrahydrofurane (61:37:3v/v), at a flow rate of 2 ml/min at 35°C column temperature) and the column used was Luna 5µ C18 (e) 100A Column (250mm×4.6mm). The flow rate was adjusted to 2.0ml/min. The instrument was operated at 35°C column temperature. The UV detection was achieved at 242nm 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 16.0-24µg/ml (AML) and 32-48µg/ml (ATO). The calibration curve obtained by plotting peak area versus concentration and presented in **Table 1** was linear and the squared correlation coefficient was found to be 0.9999 and 0.9995 for AML and ATO 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 2** was found to be 0.173 and 0.0.215 and the % Relative Standard Deviation for method precision presented in **Table 3** was found to be 0.174 and 0.126 .The % Relative Standard Deviation for ruggedness presented in **Table 4** was found to be 0.159 and 0.289 for AML and ATO respectively.

The accuracy study was performed on 80%, 100% and 120% of the target concentrations. The percentage recovery was determined for ATO and AML and was found to be 99.6% and 99.95% presented in **Tables 5 & 6**.

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength (±2nm), column temperature (±5°C), changing the percentage of the minor components of the mobile phase (±5%) and flow rate (±5%). Theoretical plates and tailing factor were observed and were found to be 7548 and 10227 (theoretical plates) and 1.01 and 0.97 (tailing factor) for AML and ATO respectively. The resolution was found to be 8.1 between Amlodipine related compound A and Amlodipine (Besilate), and 1.5 between ATO related compound B and ATO. And the Relative Standard Deviation in retention time were found to be zero for AML and 0.069 for ATO in five replicate injections of system suitability solution. The percentage purity of AML and ATO was found to be >99.5%. Typical chromatogram for system suitability solution, standard solution and test solution presented in **(Fig. 5)**, **(Fig. 6)** and **(Fig. 7)** respectively and Stress conditions

chromatograms presented in **(Fig. 8)**, **(Fig. 9)**, **(Fig. 10)**, **(Fig. 11)** and **(Fig. 12).**

The system suitability parameters like theoretical plates (N), Resolution (R) and Tailing factor (T) were calculated and were found to be more than 2000 , more than or equal to 1.5 and not more than 2 respectively and ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table7**.

Table No. 1: Linearity results for Amlodipine (Besilate) and Atorvastatin (calcium trihydrate)

Amlodipine (Bisilate)		Atorvastatin	
Absorbance	**Concentraion (µg/m)**	**Absorbance**	**Concentraion (µg/m)**
6.701	16	12.331	32
7.548	18	13.852	36
8.375	20	15.309	40
9.212	22	16.786	44
10.071	24	18.444	48

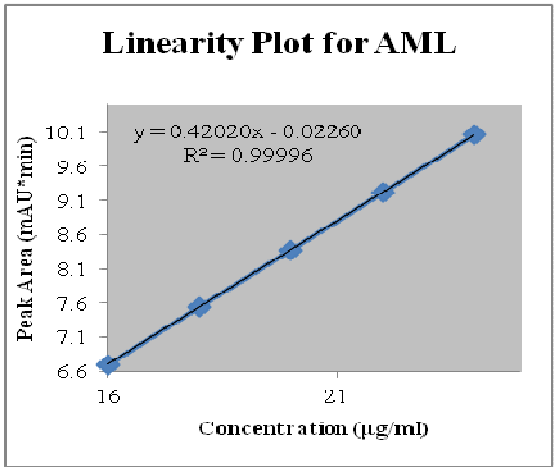

Fig. 3: Linearity plot for AML

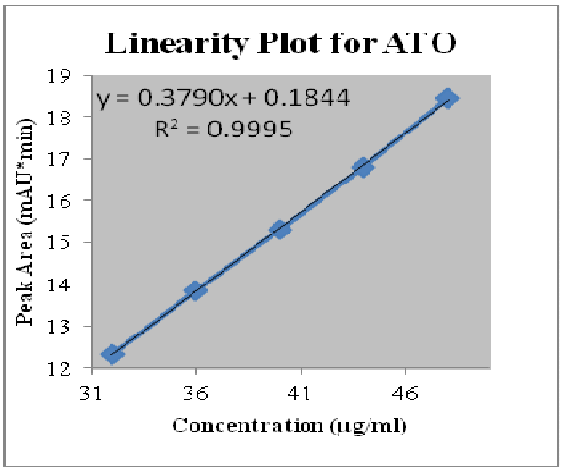

Fig. 4: Linearity plot for ATO

Table No.2: System precision for AML and ATO

Injection	AML		ATO	
	RT	**Area**	**RT**	**Area**
1	3.34	8.183	26.48	15.501
2	3.34	8.196	26.47	15.6
3	3.34	8.199	26.47	15.546
4	3.35	8.2	26.46	15.538
5	3.35	8.226	26.45	15.573
6	3.35	8.207	26.47	15.554
Average	3.345	8.202	26.467	15.552
Std. Dev.	0.005	0.014	0.010	0.033
%RSD	0.164	0.173	0.039	0.215

Table No.3: Method precision for AML and ATO

Injection	AML		ATO	
	RT	**Area**	**RT**	**Area**
1	3.65	8.442	25.92	15.362
2	3.65	8.441	25.94	15.321
3	3.66	8.472	25.93	15.321
4	3.66	8.466	25.92	15.361
5	3.66	8.443	25.93	15.331
6	3.65	8.438	25.92	15.352
Average	3.655	8.450	25.927	15.341
Std. Dev.	0.005	0.015	0.008	0.019
%RSD	0.000	0.174	0.031	0.126

Table No.4: Ruggedness values for AML and ATO

Injection	AML		ATO	
	RT	**Area**	**RT**	**Area**
1	3.66	8.444	25.19	14.864
2	3.66	8.468	25.15	14.953
3	3.66	8.472	25.13	14.932
4	3.67	8.468	25.12	14.907

5	3.67	8.453	25.1	14.903
6	3.67	8.481	25.07	14.836
Average	3.665	8.464	25.127	14.899
Std. Dev.	0.005	0.013	0.041	0.043
%RSD	0.149	0.159	0.164	0.289

Table No.5: %Recovery for ATO

Concentraion (at Specific Level)	Active Druge adding in mg	Recovered Amount in mg	Mean Recovery
80%	16	15.98	99.60%
100%	20	19.74	
120%	24	24.04	

Table No.6: %Recovery for AML

Concentraion (at Specific Level)	Active Druge adding in mg	Recovered Amount in mg	Mean Recovery
80%	8	8.069	99.95%
100%	10	9.89	
120%	12	12.03	

Table No.7: System suitability values

Injection	AML Related Comp. A		AML		ATO Related Comp. A		ATO Related Comp. B		ATO		ATO Related Comp. C	
	RT	Area	RT	Area	RT	Area	RT	Area	RT	Area	RT	Area
1	2.48	1.126	3.56	3.506	21.44	4.785	24.31	2.736	25.77	3.302	28.69	3.56
2	2.48	1.128	3.56	3.502	21.46	4.824	24.33	2.699	25.79	3.302	28.72	3.599
3	2.48	1.125	3.56	3.499	21.45	4.807	24.33	2.719	25.78	3.273	28.71	3.596
4	2.48	1.125	3.56	3.514	21.43	4.809	24.3	2.715	25.75	3.328	28.67	3.573
5	2.48	1.131	3.56	3.508	21.43	4.803	24.3	2.705	25.75	3.308	28.67	3.573
Average	2.480	1.127	3.560	3.506	21.442	4.806	24.314	2.715	25.768	3.303	28.692	3.580
Std. Dev.	0.000	0.003	0.000	0.006	0.013	0.014	0.015	0.014	0.018	0.020	0.023	0.017
%RSD	0.000	0.226	0.000	0.164	0.061	0.291	0.062	0.525	0.069	0.596	0.079	0.466
USA Theoretical Plates	7548		8717		9964		10019		10227		9736	
Resolution	**Between AML Related Comp. A and AML**				**Between ATO Related Comp. B and ATO = 1.5**							

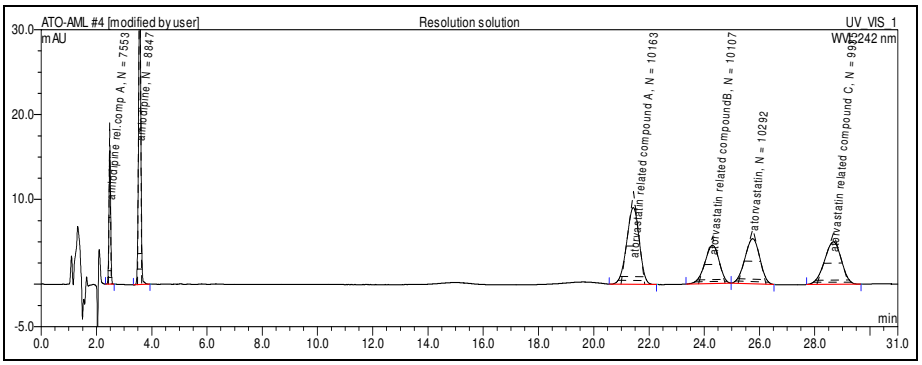

Fig. 5: Chromatogram for System Suitability Solution

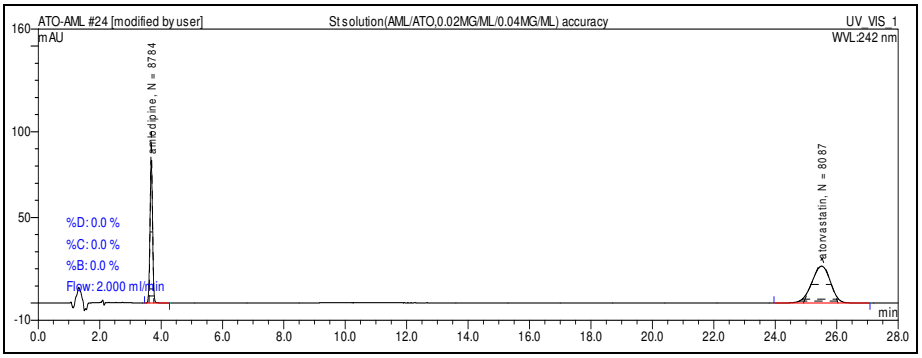

Fig. 6: Chromatogram for Standard Solution

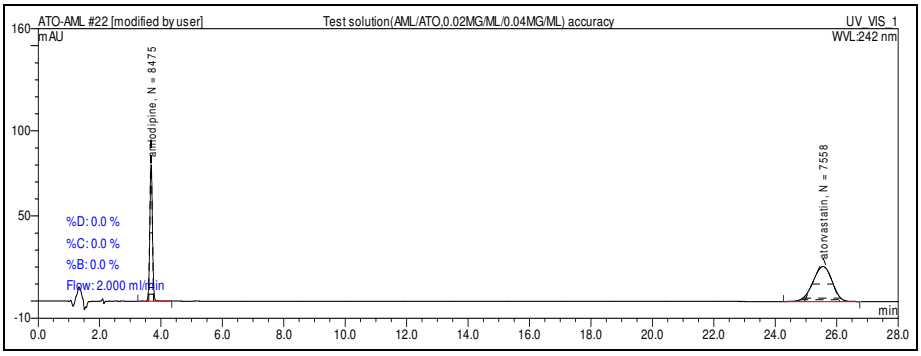

Fig. 7: Chromatogram for Test Solution

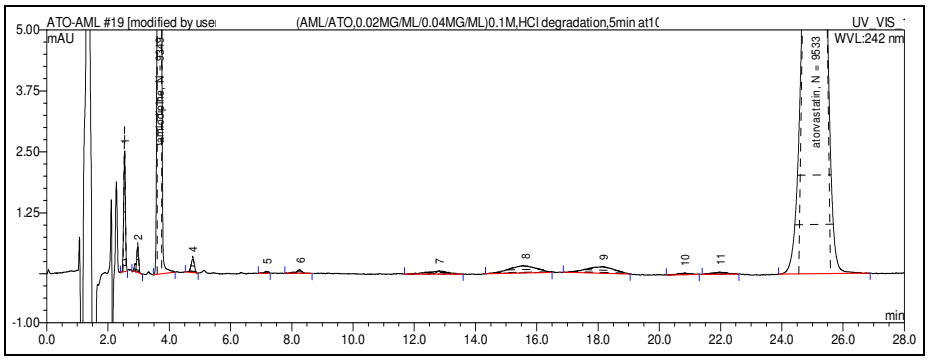

Fig. 8: Chromatogram for 0.1M HCl degradation

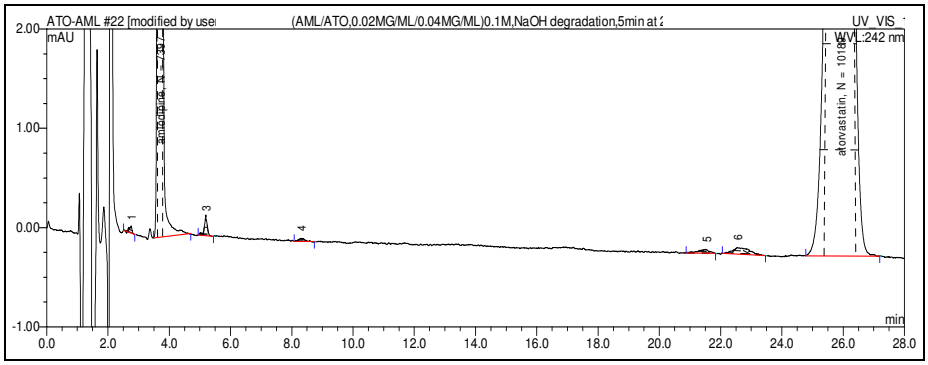

Fig. 9: Chromatogram for 0.1M NaOH degradation

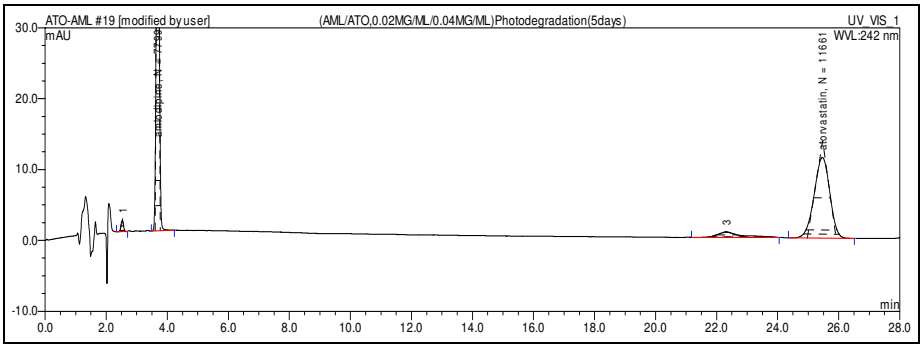

Fig. 10: Chromatogram for photo degradation

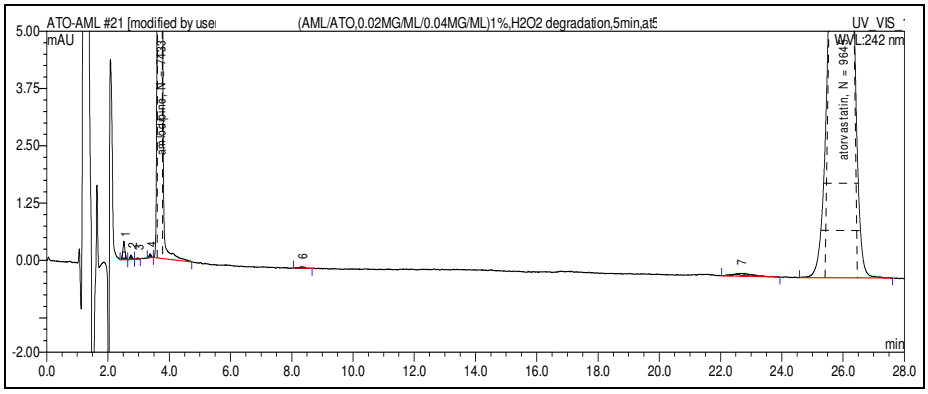

Fig. 11: Chromatogram for 1% H2O2 degradation

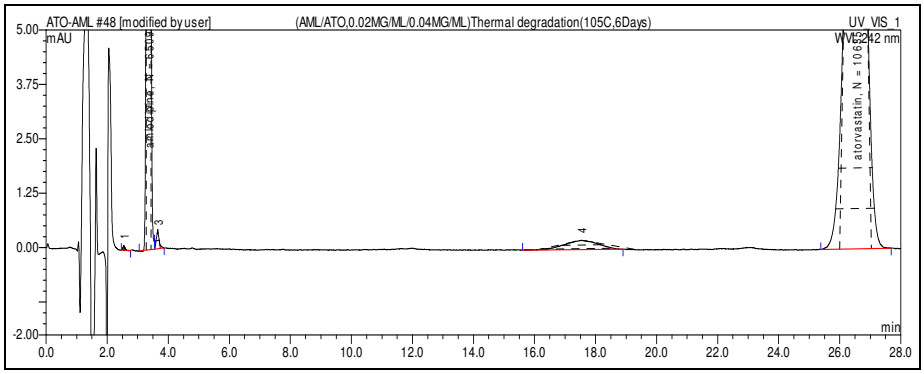

Fig. 12: Chromatogram for thermal degradation

SUMMERY

The method was found to be precise accurate and linear for determination of Amlodipine Besilate and Atorvastatin. The method was developed and validated for system suitability linearity, specificity, accuracy, 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.

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