

## VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND ATORVASTATIN CALCIUM IN BULK AND PHARMACEUTICAL FORMULATION

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### ABSTRACT

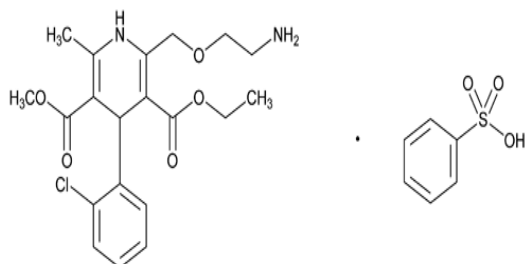
A simple, selective, sensitive and precise simultaneous high performance liquid chromatographic analysis of amlodipine and atorvastatin was described according to ICH guidelines. Good chromatographic separation was achieved by column ODS (4.6 cm × 250 mm, 5 μm) and a mobile phase consisting of acetonitrile 0.05M ammonium acetate buffer pH 4 (50:50, v/v) at a flow rate 1.5ml min<sup>-1</sup> at 30°C. The ultraviolet detector was set at wavelength 240 nm. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay method was found to be linear from 2.5- 40 μg/ml and 10-160 μg/mL with linear regression equations [ $X = (Y + 444.533532) / 24230184.65$  ( $R^2 = 0.99999$ ) and  $X = (Y + 2902.208644) / 29959770.57$  ( $R^2 = 0.99999$ )], accuracy [101.43 % and 101.12%] for amlodipine besylate and atorvastatin calcium, respectively, Precision developed through repeatability and Intermediate precision, repeatability express approved through 9 preparation from 3 different concentrations, while intermediate precision approved through day to day and intraday assays, the RSD was found to be less than 2%. , LOD & LOQ were determined from the blank response measurements, they were found (0.0047μg/ml-0.0035μg/ml) & (0.014 μg/ml-0.0102 μg/ml ), respectively. Comparison to previous studies there is no significant difference. The method was found to be robust after different deliberate changes ( flow rate & ratio of the organic phase to the aqueous phase). The tablets were stressed under different conditions in forced degradation studies, all validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of atorvastatin calcium and Amlodipine besylate in combined tablets dosage form.

**Keywords:** Amlodipine besylate, Atorvastatin Calcium, RP – HPLC.

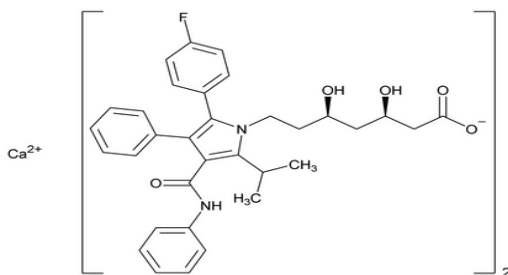
### INTRODUCTION

Amlodipine besylate; Chemical Name: 3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, (±)-, monobenzenesulfonate used for treatment of hypertension and prophylaxis of angina. Atorvastatin Calcium; 1H -Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β,α -dihydroxy-5-(1-

methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-, calcium salt (2:1) used for treatment of primary hypercholesterolaemia, heterozygous familial hypercholesterolaemia and homozygous familial hypercholesterolaemia or combined (mixed) hyperlipidaemia in patients who have not responded adequately to diet and other appropriate measures<sup>1-3</sup>.



**Chemical Structure of Amlodipine Besylate**



**Chemical Structure of Atorvastatin Calcium**

Various analytical methods have been attempted and reported for the assay of Amlodipine besylate alone and quite few in combinations with other anti-hypertensive agents in pharmaceutical formulations. These include UV spectroscopy<sup>4-7</sup> high performance liquid chromatography<sup>8-11</sup>. Many analytical methods were reported for the analysis of atorvastatin Calcium alone and in combination with other drugs by stability indicating methods and had been determined in plasma<sup>12, 13</sup>. In fact there is no available official method for the simultaneous determination for this combination. Moreover, based on the fact that, currently, HPLC-analytical tool and the procedures associated with it, specifically, RP-HPLC procedure have proved to be simple, accurate and of high degree of precision. Accordingly, the present study is an attempt to develop and validate an RP-HPLC-procedure for simultaneous estimation of amlodipine and Atorvastatin Calcium in bulk and in pharmaceutical preparations.

## MATERIALS AND METHODS

### Materials

All analytical runs were performed in a HPLC-Shimadzu (Japan) chromatograph equipped with an LC – 20AB solvent delivery system, a universal loop injector (SIL20A) of injection capacity of 100  $\mu$ l, and an SPD – 20 AV UV-Visible detector set at 240 nm. The instrument was equipped with a GL SCIENCES C18

column of the dimensions (250mm x 4.6mm i.d., 5  $\mu$ m particle size). An isocratic elution was adopted using a mixture of ammonium acetate Buffer pH 4.0: Acetonitrile (50:50), as a mobile phase. Flow rate of mobile phase was adjusted to 1.0 ml min<sup>-1</sup> and injection volume was 20  $\mu$ l at 30°C temperature. Normal run time was chosen as 15 minutes. The equipment was controlled by a PC work station with LC Software. Analytically pure samples of amlodipine besylate and Atorvastatin Calcium were procured from Azal Pharmaceutical Company, Khartoum, Sudan as a gift and used as working standards. Acetonitrile of HPLC grade from CARLO ERBA (France), all other reagents are of analytical grade.

## Methods

### Preparation of buffer solution

Ammonium acetate (7.7 g) was transferred with stirring to water (2000 mL). The pH of the resulting solution was adjusted to pH 4.0 through the drop wise addition of *ortho*-phosphoric acid.

### Preparation of standard solution

Amlodipine Besylate (10 mg) and Atorvastatin calcium (20 mg) working standard were accurately weighed and introduced in 50 ml volumetric flasks. The contents were dissolved in the mobile phase (30 ml) and sonicated. The solutions were made up to 50 ml by the mobile phase. 5 ml of this solution was made up to 50 ml by the mobile phase in a 50 ml volumetric flask.

### Preparation of sample solutions

The sample drug (20 tablets) were accurately weighed and crushed to a coarse powder. Tablets powder containing an equivalent of 5mg amlodipine and 20 mg of Atorvastatin Calcium was transferred to a 50ml volumetric flask. The mobile phase (30 ml) was added and the mixture was shaken for complete solution and then sonicated for around 10 minutes with occasional shaking. The mobile phase was added to the mark to make up to 50ml solution. A portion of this solution (5 ml) was made up by the mobile phase to 50 ml in another volumetric flask. The final solution was filtered through 0.45  $\mu$ m GHP filter.

### Preparation of the Test Solutions (50%, 100% and 150% Solutions)

Amlodipine Besylate WS (2.5 mg), Atorvastatin Calcium WS (10.5 mg) and the placebo (162

mg) were thoroughly mixed and transferred into a 50 ml volumetric flask and then dissolved in the mobile phase (30 ml), sonicated to ensure complete dissolution. After cooling the volume was made up to the mark by the addition of the appropriate amount of the mobile phase. 5 ml portion of this solution was diluted to 50 ml with mobile phase to afford a 50% solution.

In a similar manner, for the preparation of a 100% and 150% different amounts (weights) of the drug combination were considered. Amlodipine Besylate WS (2.5 mg) and Atorvastatin Calcium WS should be 5 mg and 7.5 mg for the former drug and 20.9 mg, 31.4 mg for the latter drug, respectively. The appropriate volumes be taken and diluted to afford these two percentages.

### Specificity preparations

#### Standard preparation

Amlodipine besylate WS (10 mg) and Atorvastatin calcium WS (20 mg) were accurately weighed, mixed and transferred into a 50 ml volumetric flask and dissolved in the mobile phase (30 mL). The solution was sonicated for few minutes, then cooled and the volume was completed to the mark by the mobile phase. A volume (5 ml) of this solution was diluted to 50 ml by the mobile phase.

#### Test solution preparation

Amlodipine besylate WS (7.0 mg), Atorvastatin Calcium WS (20.9 mg) and placebo (152.5mg) were accurately weighed and transferred into a 50 ml volumetric flask and a 30 ml of the mobile phase was added. The contents were thoroughly mixed and sonicated for few minutes. The solution was allowed to cool and the volume was completed to the mark by the mobile phase. 5 ml of this solution was diluted to 50 ml with the mobile phase.

#### Acid hydrolysis test (0.1N hydrochloric acid)

Amlodipine Besylate WS (6.9 mg), Atorvastatin Calcium

WS (20.8 mg) and of placebo (152 mg) were accurately weighed and transferred into a 50 ml volumetric flask. An aqueous hydrochloric acid (0.1N HCl, 5 mL) was added. The solution was allowed to stand for 2 hrs and about 30 mL of the mobile phase was added. The solution was then sonicated for few minutes, allowed to cool and the volume was made up to the mark with the mobile phase. A volume of 5 ml of this solution was diluted to 50 ml. The appropriate volume of this solution was injected in the

HPLC-system and the chromatogram was studied and recorded.

#### Base hydrolysis (0.1N sodium hydroxide)

Amlodipine Besylate WS (6.9 mg), Atorvastatin Calcium WS (20.9 mg) and placebo (152.5 mg) were weighed accurately and transferred into a 50 ml volumetric flask. An aqueous solution of sodium hydroxide (0.1 N, 5 mL) was added and the solution was allowed to stand for 2 hrs. 30 ml of the mobile phase was added and the contents of the flask were sonicated for few minutes, allowed to cool and the volume was made up to the mark by the mobile phase. 5 ml of this solution was diluted to 50 ml by the mobile phase. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded.

#### Hydrogen peroxide oxidation test

Amlodipine besylate WS (6.8 mg), Atorvastatin Calcium WS (20.8 mg) and placebo (152.5mg) were weighed accurately and transferred into a 50 ml volumetric flask. Hydrogen peroxide (5 ml, 30% solution) was added and the contents of the flask were allowed to stand for 2 hrs. 30 ml of the mobile phase was added and the contents were sonicated. It was then allowed to cool and the volume was made up to the mark by the mobile phase. 5 ml of this solution was diluted to 50 ml with the mobile phase. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded

#### Thermal stability test: Test preparation for Heat hydrolysis (at 80°C for 72 hours)

Amlodipine besylate WS (6.8 mg Atorvastatin Calcium WS (20.8mg) and the placebo (152 mg) were weighed accurately, transferred into a 50 mL volumetric flask. It was then placed into a dry oven set at 80 °C and allowed for 72 hr. Then 30 mL of the mobile phase was added, the contents of the flask were sonicated and then allowed to cool. The volume was then made up to the mark with the mobile phase. 5 mL of this solution was diluted to 50 mL by the mobile phase. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded.

### RESULTS AND DISCUSSION

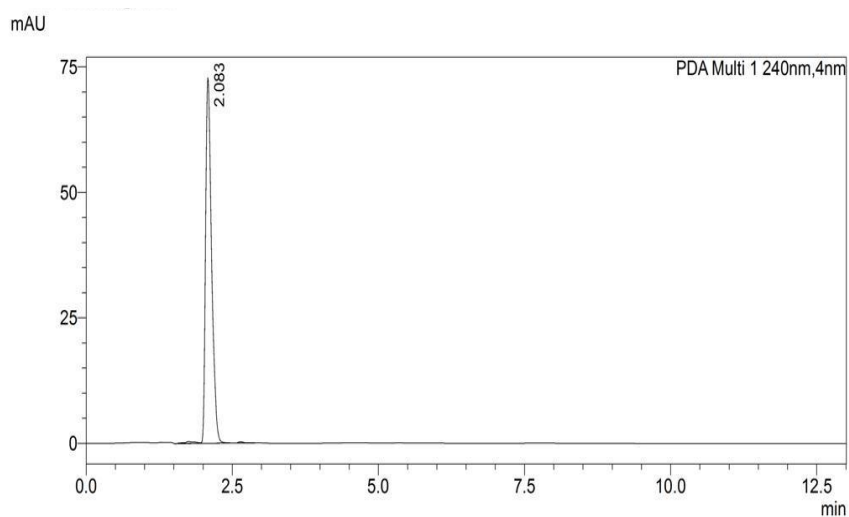
The protocol adopted for the establishment of the HPLC analytical procedure presented in this current work consisted of choosing optimum HPLC-conditions and suitable mobile phase

composition to achieve an excellent resolution of the individual working standards, amlodipine WS and Atorvastatin Calcium WS drugs and thereafter the resolution of a 1:1 ratio by weight mixture of the two drugs. The second phase comprises HPLC determinations of ranges of concentration levels of each component drug working standards to establish linearity plots. The third phase involves the derivation and determination of the validation parameters associated with the results obtained in terms of linearity, accuracy, precision, coefficient of variation, reproducibility and specificity of the sample applications. The fourth phase is a preliminary attempt for the application of method in monitoring drug stability and the final phase is the statistical data study for the derivation of a number of validation parameters.

### HPLC-Resolution of the Drugs Combination

The HPLC-instrument employed in this work was a Shimadzu (Japan) Model prominence

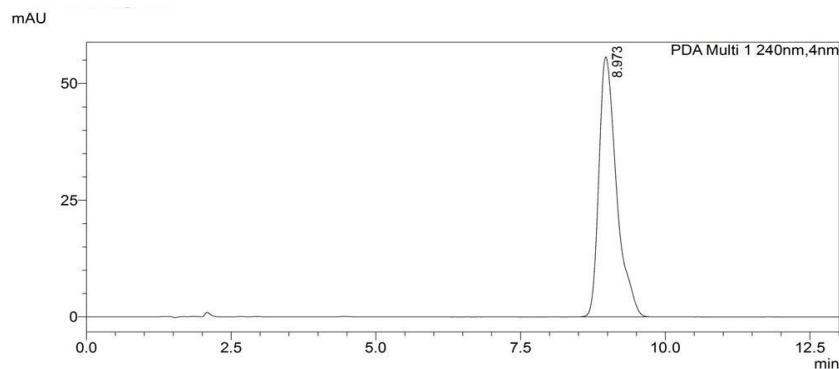
equipped with a UV-detector being set at  $\lambda$  240 nm and an RP-C18 Column. Other HPLC-conditions were presented in the Materials and Methods Section. The first organic solvent composition of the mobile phase was: ammonium acetate buffer pH 4.00: acetonitrile (50: 50), which has given good resolution and shapes of the peaks. HPLC-runs have been performed in which the buffer was kept constant and the composition of the organic solvents varied. An excellent resolution and best peak shapes were reached when the mobile phase composition of (Buffer pH 4.0: Acetonitrile 50:50) was attempted. This solvent mixture was used to resolve the individual working standards amlodipine WS and Atorvastatin Calcium WS drugs at similar concentrations affording a retention times of 2.351min for amlodipine and 9.13 min for Atorvastatin Calcium. Moreover, a 1:1 ratio combination of the two drugs mixture has shown an excellent resolution as shown below.



#### <Peak Table>

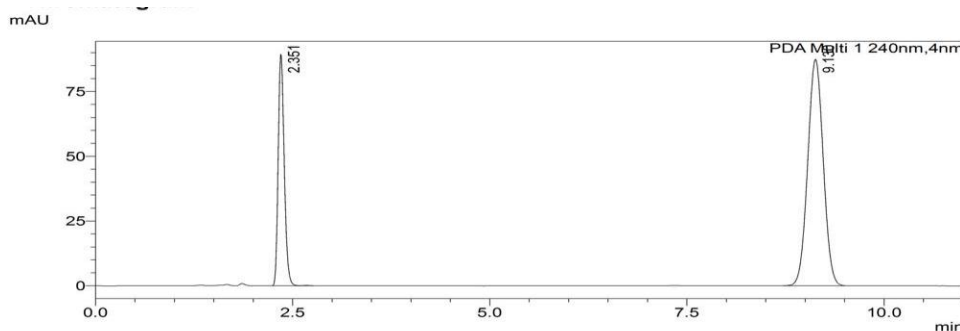
PDA Ch1 240nm		Amlostat						
Peak#	Name	Ret. Time	Area	Tailing Factor	Theoretical plate#	(k')	Resolution	Area%
1	Amlodipine	2.083	523347	1.494	1695	--	--	100.000
Total			523347					100.000

**Fig. 1: Chromatogram for Amlodipine**



&lt;Peak Table&gt;

Amlostat									
PDA Ch1 240nm									
Peak#	Name	Ret. Time	Area	Tailing Factor	Theoretical plate#	(k')	Resolution	Area%	
1	Atorvastatin	8.973	1183901	1.505	4411	--	--	100.000	
Total			1183901					100.000	

**Fig. 2: Chromatogram for Atorvastatin**

&lt;Peak Table&gt;

Amlostat									
PDA Ch1 240nm									
Peak#	Name	Ret. Time	Area	Tailing Factor	Theoretical plate#	(k')	Resolution	Area%	
1	Amlodipine	2.351	479251	1.225	3569	--	--	28.502	
2	Atorvastatin	9.130	1202228	1.021	9564	2.883	25.540	71.498	
Total			1681479					100.000	

**Fig. 3: Resolution of amlodipine WS and Atorvastatin Calcium WS 1:1 mixture**

It was observed that optimizing acetonitrile composition in the mobile phase was a determining factor in improving the resolution, maintaining good peak shape and minimizing the HPLC-run time. Accordingly, the following optimum mobile phase ratio: buffer pH 4.00 : Acetonitrile 50: 50, was reached after conducting a number of HPLC-trials involving varying volumes of acetonitrile versus fixed volume of buffer. The components of the combination drug have been resolved without any interference, **Figure 1**. Accordingly, the fore-mentioned composition of the mobile phase has been used throughout the work at a flow rate of 1.0 ml/min.

#### Determination of the Linearity Parameter

The linearity parameter was determined by

injecting a series of seven concentration levels within the range 2.5- 40 µg/ml and 10-160 µg/mL, for each amlodipine WS and Atorvastatin Calcium WS, respectively. The response of each of the two drugs was found to be linear within its investigated concentration range and the linear regression equation was  $y = 24230184.65x - 444.533532$  with a correlation coefficient 0.99999 for amlodipine and  $y = 299599770.57x - 2902.208644$  with a correlation coefficient of 0.99999 for Atorvastatin Calcium. The results obtained for both drugs have shown an excellent coefficient of variation and reproducibility, which was evident from the low relative standard deviation RSD ranging from 0.04 to 0.53 for amlodipine and 0.02 to 0.4 for Atorvastatin Calcium see **Table 1** and **Table 2**, below.

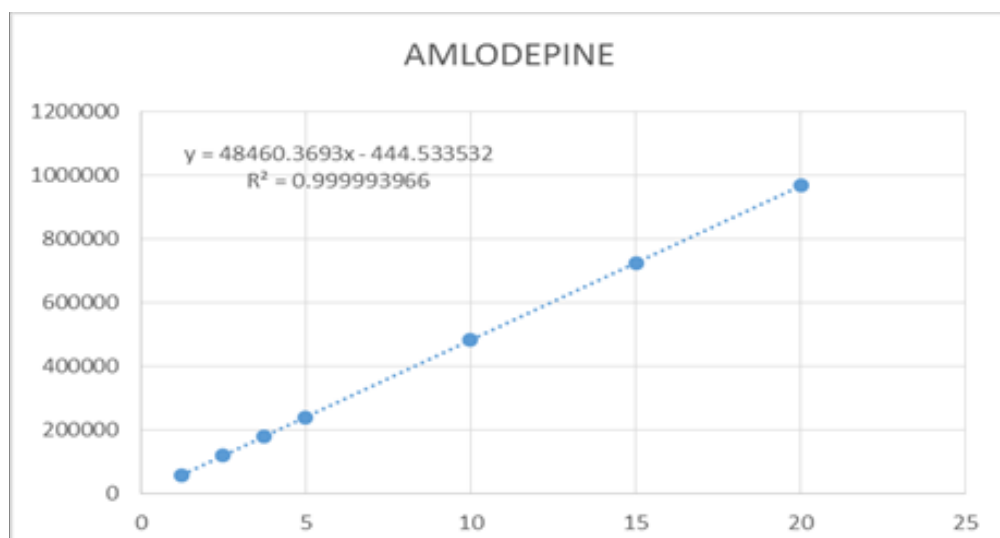
**Table 1: Regression analysis data for Amlodipine besylate**

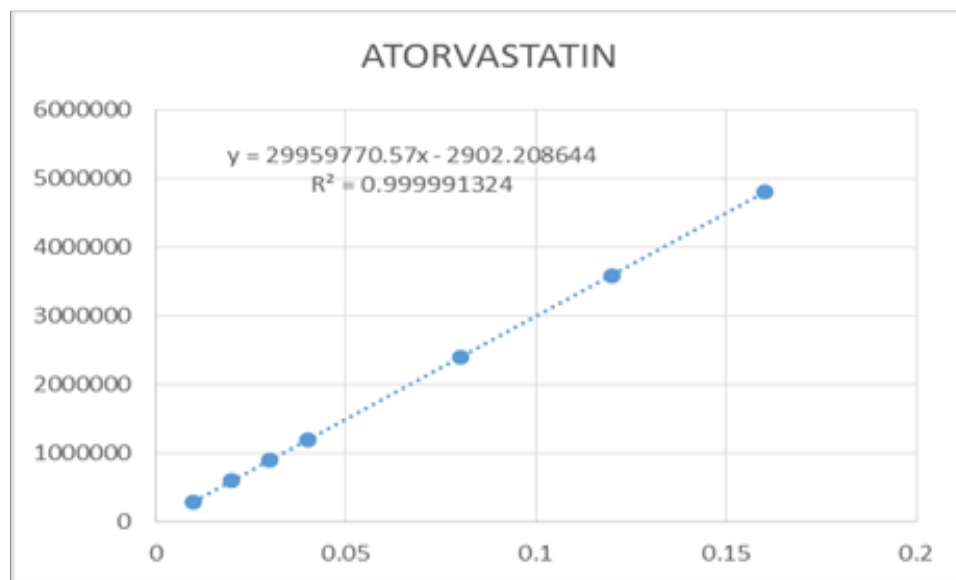
	1 <sup>st</sup> level	2 <sup>nd</sup> level	3 <sup>rd</sup> level	4 <sup>th</sup> level	5 <sup>th</sup> level	6 <sup>th</sup> level	7 <sup>th</sup> level
Amlodipine conc.	0.0025	0.005	0.0075	0.01	0.02	0.03	0.04
Actual content 'mg'	1.25	2.5	3.75	5	10	15	20
1st response	58883	120795	182732	241734	485184	725898	968323
2nd response	59323	120597	182627	241580	484024	725144	969875
Average of response	59103	120696	182679.5	241657	484604	725521	969099
STDV	311.13	140.007	74.24621	108.8944	820.244	533.1585	1097.43
%RSD	0.53	0.12	0.04	0.05	0.17	0.07	0.11

**Table 2: Regression analysis data for Atorvastatin Calcium**

	1 <sup>st</sup> level	2 <sup>nd</sup> level	3 <sup>rd</sup> level	4 <sup>th</sup> level	5 <sup>th</sup> level	6 <sup>th</sup> level	7 <sup>th</sup> level
Atorvastatin conc.	0.01	0.02	0.03	0.04	0.08	0.12	0.16
Actual content 'mg'	5	10	15	20	40	60	80
1st response	295921	594472	903137	1191555	2394363	3585180	4792569
2nd response	296195	597352	903481	1190210	2393753	3585511	4798656
Average of response	296058	595912	903309	1190883	2394058	3585346	4795613
STDV	193.75	2036.47	243.2447	951.0586	431.335	234.0523	4304.16
%RSD	<b>0.0654</b>	<b>0.34174</b>	<b>0.026928</b>	<b>0.079862</b>	<b>0.01802</b>	<b>0.006528</b>	<b>0.08975</b>

A linearity plot of concentration versus intensity (area under the peak) was established for each of the working standards Amlodipine & Atorvastatin, **Figure (4)** and **Figure (5)**, respectively.

**Fig. 4: Linearity plot of amlodipine besylate**



**Fig. 5: Linearity plot of Atorvastatin Calcium**

#### Determination of Precision and Accuracy Parameters

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing the same procedure on the different day (intraday) by another analyst

under the same experimental conditions. The intermediate precision, which is less than 2.0%, is an evidence for the excellent repeatability of the results indicating that the method is of high precision. It is noteworthy to mention that, the repeatability parameter could be determined from the precision and accuracy, since all three parameters are inter-related.

**Table 3: Intraday Precision for Amlodipine Besylate and Atorvastatin Calcium**

Precision 1									
Amlodipine besylate					Atorvastatin Calcium				
P		WC	average	Claim	P		WC	average	Claim
99.16		0.17	180	5	101.75		4.79	180	20
M.W. Amlodipine Besylate			567.05						
M.W. Amlodipine			408.88						
	STD1	STD2	Test1	Test2		STD1	STD2	Test1	Test2
weight	10.5	10.6	191.1	183.3	weight	20.5	21.2	191.1	183.3
Inj#01	479251	483396	333539	314719	Inj#01	1202228	1236199	1263236	1239089
Inj#02	479325	483303	333525	313802	Inj#02	1202770	1235109	1263069	1240549
Inj#03	479533				Inj#03	1204920			
Inj#04	481053				Inj#04	1205187			
Inj#05	479460				Inj#05	1204709			
average	479724.4	483349.5	333532	314260.5	average	1203963	1235654	1263153	1239819
<b>RSD</b>	<b>0.16</b>	<b>0.01</b>	<b>0.003</b>	<b>0.21</b>	<b>RSD</b>	<b>0.11</b>	<b>0.06</b>	<b>0.01</b>	<b>0.08</b>
Agree	100.2	assay	100.16	98.59	Agree	100.77	assay	98.04	100.31
		average	99.38				average	99.18	
		<b>RSD</b>	<b>1.12</b>				<b>RSD</b>	<b>1.62</b>	

**Table 4: Interday Precision for Amlodipine Besylate and Atorvastatin Calcium**

Precision 2									
Amlodipine besylate					Atorvastatin Calcium				
P	WC	average	Claim		P	WC	average	Claim	
99.16	0.17	180	5		101.75	4.79	180	20	
M.W. Amlodepine Besylate		567.05							
M.W. Amlodipine		408.88							
	STD1	STD2	Test1	test2		STD1	STD2	Test1	test2
weight	10.4	10.1	177.5	178.2	weight	50.1	50	177.5	178.2
Inj#01	485663	470191	312046	313938	Inj#01	1182187	1179147	1205975	1206800
Inj#02	485603	467226	313749	315980	Inj#02	1183423	1171276	1215981	1209529
Inj#03	485740				Inj#03	1459892			
Inj#04	485849				Inj#04	1461488			
Inj#05	486071				Inj#05	1463102			
average	485785.2	468708.5	333532	314260.5	average	1183024.8	1175211.5	1479146	1472448
<b>RSD</b>	<b>0.04</b>	<b>0.45</b>	<b>0.36</b>	<b>0.46</b>	<b>RSD</b>	<b>0.001</b>	<b>0.005</b>	<b>0.32</b>	<b>0.57</b>
Agree	100.7	assay	98.95	98.89	Agree	100.66	assay	100.47	99.73
		average	98.92				average	100.10	
		<b>RSD</b>	<b>0.04</b>				<b>RSD</b>	<b>0.52</b>	

The accuracy of the method was determined by recovery of spiked pre-analyzed sample formulation of the drug in triplicate sets of concentration levels: 50%, 100%, and 150%. The robustness of procedure was investigated to evaluate the influence of small but deliberate variations in the chromatographic conditions, such as changes in the flow rate [ $\pm 0.1$  ml/min], and changes in the mobile phase composition by changing ( $\pm 10\%$ ) of acetonitrile.

**Table 5: Percentage Recoveries of Spiked Amlodipine Besylate**

	50% Assay			100% Assay			150% Assay		
Actual Assay	42.30	48.64	48.64	96.58	97.28	96.58	145.22	145.92	149.44
Assay Found	41.87	48.70	49.32	98.47	98.89	98.09	145.46	147.97	153.68
Difference	-0.43	0.06	0.68	1.90	1.61	1.51	0.25	2.05	4.24
% Recovery	98.99	100.12	101.40	101.97	101.65	101.56	100.17	101.40	102.83
Average % Recovery	100.17			101.73			101.47		
%RSD	1.21			0.21			1.31		
Average % for all Recovery	101.12								
<b>RSD% over all</b>	<b>0.16</b>								



**Table 6: Percentage recoveries of spiked Atorvastatin Calcium**

	50% Assay			100% Assay			150% Assay		
Actual Assay	48.48	48.48	48.71	97.42	97.89	96.01	144.96	144.72	144.96
Assay Found	49.31	49.10	49.52	98.69	98.66	97.02	146.73	145.83	149.47
Difference	0.83	0.62	0.81	1.27	0.78	1.01	1.77	1.11	4.51
% Recovery	101.72	101.28	101.66	101.31	100.79	101.05	101.22	100.77	103.11
Average % Recovery	101.55			101.05			101.70		
<b>RSD %</b>	<b>0.23</b>			<b>0.25</b>			<b>1.22</b>		
Average all Recovery				101.43					
<b>RSD for all recovery</b>				<b>0.57</b>					

### Specificity of the Method

It is noteworthy to mention that preliminary tests were performed where by the specificity of the method was firstly determined against placebo. It was the found that there were no interferences between the drug and the excipients of the claimed placebo. Secondly the specificity of the method toward the drug was approved via the non-existence of interferences between the peaks of the drug and the degradation products resulting from exposure to forced stress conditions of acidic, alkaline, photolytic and oxidative conditions.

### CONCLUSION

A new analytical method has been developed to be routinely applied to simultaneous determination of amlodipine besylate and Atorvastatin Calcium in pharmaceutical dosage form. In this study, stability of amlodipine besylate, Atorvastatin Calcium in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also for stability sample analysis.

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### REFERENCES

1. Pharmacopeia, U.S. Pharmacopeia National Formulary. 2014. USP 37/NF32.
2. Group, B., British National Formulary (BNF) 67. 2014, London: BMJ Group and the Royal Pharmaceutical Society of Great Britain.
3. FDA, Caduet (amlodipine besylate/ atorvastatin calcium) tablets. [://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm208616.htm](http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm208616.htm), 04/15/2015
4. Chaudhari, B.G. and A.B. Patel, Simultaneous spectrophotometric estimation of atorvastatin calcium and amlodipine besylate in tablet dosage forms. *Int J Chem Tech Res*, 2010. 2(1): p. 633-639.
5. Ibrahim, N., et al., Simultaneous determination of amlodipine besylate and atorvastatin calcium by using spectrophotometric method with multivariate calibration and HPLC method implementing "design of experiment". *Int J Pharmacy Pharm Sci*, 2014. 6(1): p. 419-25.
6. Kumbhar, S.T., et al., Development and validation of derivative spectrophotometric method for estimation of atorvastatin calcium and amlodipine besylate in tablet dosage form. *Int J Pharm Pharm Sci*, 2011. 3(4): p. 195-7.
7. Ramesh, D. and S. Ramakrishna, New spectrophotometric methods for simultaneous determination of amlodipine besylate and atorvastatin

- calcium in tablet dosage forms. *Int J Pharm Pharm Sci*, 2010. 2(4): p. 215-219.
8. Hafez HM1\*, E.A., Abdelaziz LM2, and M. MS1, Development of a Stability-Indicating HPLC Method for Simultaneous Determination of Amlodipine Besylate and Atorvastatin Calcium in Tablets. *Austin Journal of Analytical and Pharmaceutical Chemistry*. 2014.
  9. Jena, A., M. Madhu, and S. Latha, Analytical method development and validation of simultaneous determination of atorvastatin calcium and amlodipine besilate in tablet dosage form by RP-HPLC. *International Journal of Pharmaceutical Sciences and Research*, 2010. 1(11): p. 100.
  10. Kurakula, M., et al., Development and validation of a RP-HPLC method for assay of atorvastatin and its application in dissolution studies on thermosensitive hydrogel-based nanocrystals. *Tropical Journal of Pharmaceutical Research*, 2014. 13(10): p. 1681-1687.
  11. Pathak, A. and S. Rajput, Development of a stability-indicating HPLC method for simultaneous determination of olanzapine and fluoxetine in combined dosage forms. *Journal of chromatographic science*, 2009. 47(7): p. 605-611.
  12. Virani, P., et al., Atorvastatin: A Review on Analytical Methods and its Determination in Pharmaceuticals and Biological Matrix.
  13. Virani, P., et al., Atorvastatin: A review on analytical method and its determination in pharmaceuticals and biological matrix. *Asian Journal of Pharmaceutical Analysis*, 2015. 5(3): p. 151-160.