International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

New Stability Indicating Method for Quantification of Impurities in Amlodipine and Atorvastatin calcium Tablets by Validated HPLC

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Manuscript No: IJPRS/V2/I4/00276, Received On: 30/12/2013, Accepted On: 31/12/2013

ABSTRACT

A stability indicating LC method was developed for the simultaneous determination of Amlodipine and Atorvastatin calcium in pharmaceutical dosage form. Efficient chromatographic separation was achieved on X-Select CSH, C18 4.6 x 150 mm, 3.5 µ or Equivalent stationary phase with simple combination of a mobile phase containing Buffer : Acetonitrile : Tetrahydrofuran (575 : 400 : 25, v/v/v)with pH 3.00 ± 0.05 using Ortho-Phosphoric Acid + 2 g of 1-Octane Sulfonic Acid Sodium Salt delivered in an Isocratic mode and quantification was carried out using UV detection at 246 nm at a flow rate of 1.0 mL min^{-1} with injection volume of 20 µl and ambient column temperature. This method is capable to detect both the drug components of Amlodipine and Atorvastatin calcium in presence of their degradation products (Amlodipine Imp-A and Atorvastatin Impurity-D, F, G and H) with detection level of 0.05 %. Amlodipine / Atorvastatin calcium in their combination drug product were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions, and the samples were analyzed. Peak homogeneity data of Amlodipine and Atorvastatin calcium is obtained using PDA detector, in the stressed sample chromatograms, demonstrating the specificity. The method shows excellent linearity over a range of 0.05-2.0% for Amlodipine, Amlodipine Impurity-A and 0.05-1.5 % for Atorvastatin calcium and Atorvastatin calcium Impurity-D,F,G and H. The correlation coefficient for Amlodipine and Atorvastatin calcium is 1. The relative standard deviation (RSD) was always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination and the stability study of Amlodipine and Atorvastatin calcium in pharmaceutical preparations. The developed HPLC method was validated with respect to linearity, range, accuracy, precision and robustness.

KEYWORDS

Column liquid chromatography, Method validation, Stability indicating study, Amlodipine and Atorvastatin calcium

INTRODUCTION

Amlodipine & Atorvastatin calcium tablets combine the calcium channel blocker amlodipine besylate with the HMG CoA-reductase inhibitor atorvastatin calcium.

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the movement of calcium ions into vascular smooth muscle cells

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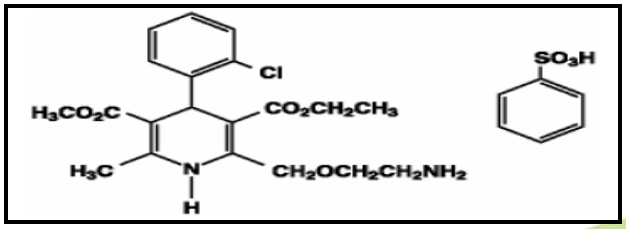
and cardiac muscle cells. Amlodipine besylate is a white to pale yellow crystalline powder, slightly soluble in water and sparingly soluble in ethanol. Amlodipine besylate has chemical name of 3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-cholorophenyl)-methyl-1-dihydropyridine-3,5-dicarboxylate benzenesulphonate[1, 2, 3].

Amlodipine besylate has empirical formula of $C_{20}H_{25}ClN_2O_5 \bullet C_6H_6O_3S$, and its molecular weight is 567.1, its structural formula is shown in Scheme 1.

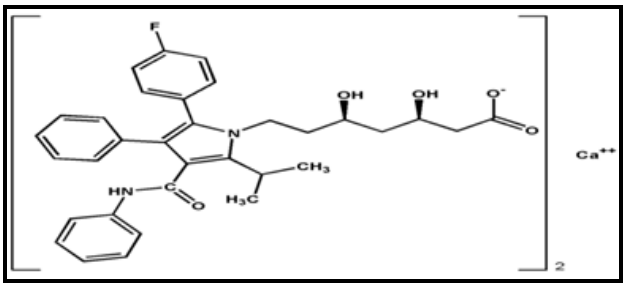


Scheme 1: Amlodipine Besylate Chemical Structure

Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis.

Atorvastatin calcium is a White to off white colored powder and soluble in Dimethyl sulphoxide. Atorvastatin calcium chemical name is 3[R-(R*,R*)]-2-(4-Fluorophenyl)-β,δ,-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1)[4-6]

Atorvastatin calcium has empirical formula of $C_{66}H_{68}CaF_2N_4O_{10}$ and its molecular weight is 1155.37and its structural formula is shown in Scheme 2.



Scheme 2: Atorvastatin calcium chemical Structure

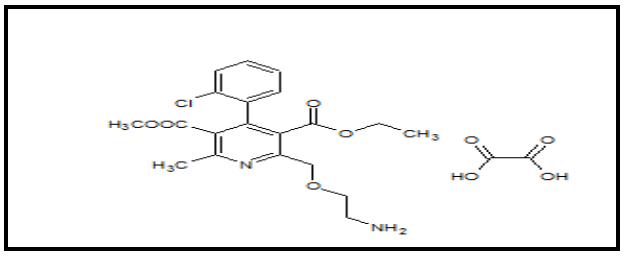
Amlodipine/Atorvastatin calcium Tablets Brand name is called as Caduet[7]. Caduet is a combination drug, containing Atorvastatin calcium (Lipitor) and amlodipine besylate (Norvasc) and is used for treating high blood pressure. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Amlodipine belongs to a class of medications called calcium channel blockers. These medications block the transport of calcium into the smooth muscle cells lining the coronary arteries and other arteries of the body. Since calcium is important in muscle contraction, blocking calcium transport relaxes artery muscles and dilates coronary arteries and other arteries of the body. Relaxing muscles of arteries lowers blood pressure. The FDA approved Caduet in the year 2001.

Caduet is the first commercial product that has been developed and marketed to treat two different conditions, viz., high blood pressure and high cholesterol, in one dosage form.

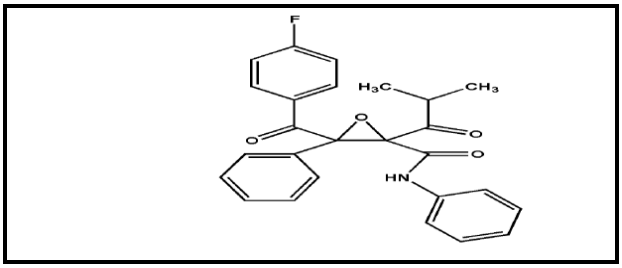
Caduet contains both Amlodipine besylate for the treatment of high blood pressure and Atorvastatin calcium for the treatment of hypercholesterolemia. Caduet tablets are intended for oral administration and are available in several different combinations including 2.5mg/10mg, 2.5mg/20mg, 2.5/40mg, 5mg/10mg, 5/20mg, 5mg/40mg, 5mg/80mg, 10mg/10mg, 10mg/20mg, 10mg/40mg and 10mg/80mg of Amlodipine and Atorvastatin respectively.

Amlodipine is official in USP[8] and Atorvastatin calcium is also official in USP[9] and their combination drug product doesn't have monograph in USP pharmacopoeia.

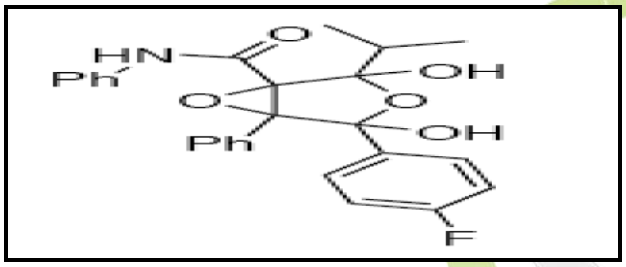
Even though the products has not been captured in USP, an in-house method has been developed and validated as per ICH guideline and monitoring of these impurities with good separation of peaks and quantification of impurities in Amlodipine / Atorvastatin calcium Tablets as shown in Scheme 3-7.



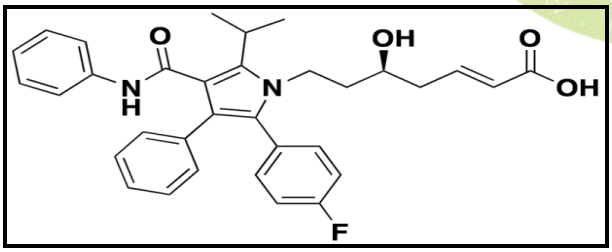
Scheme 3: Amlodipine Impurity-A Chemical Structure



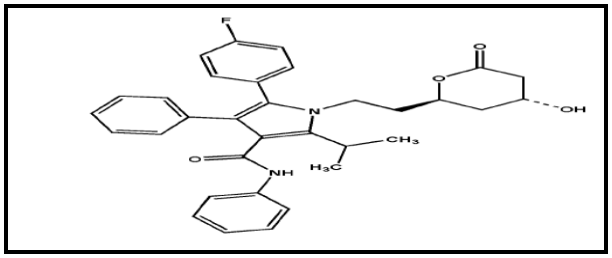
Scheme 4: Atorvastatin calcium Impurity-D Chemical Structure



Scheme 5: Atorvastatin calcium Impurity-F Chemical Structure



Scheme 6: Atorvastatin calcium Impurity-G Chemical Structure



Scheme 7: Atorvastatin calcium Impurity-H Chemical Structure

Stability testing forms an important part of the process of drug product testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommendation of storage conditions, retest periods and shelf life to be established. The two main aspects of drug products that play an important role in shelf life determinations are assay of active drug and degradants generated during the stability study. Stability-indicating methods have been reported for assays of various drugs in drug products containing only one active drug substance. Only few stability-indicating methods are reported for the Impurity assay of combination drug products containing two or more active drug substances. The objective of this work was to develop an analytical LC procedure, which would serve as stability-indicating Impurity assay method for combination drug products of Amlodipine and Atorvastatin.

The literature survey reveals that several methods were reported for the individual estimation of Amlodipine and Atorvastatin. There are many reported methods for the estimation of Amlodipine alone[10-20] or Atorvastatin calcium alone[21-27] or in combination with other agents[28-32]. In USP, there is no monograph for these combination products. (Amlodipine and Atorvastatin calcium in combined pharmaceutical dosage form).

If the reported individual methods are applied for the analysis of the tablets containing Amlodipine and Atorvastatin calcium it would require UPLC to have shorter runtime and it's not possible for all to afford the same and the method would not be rapid, less expensive, or economical, whereas the simultaneous determination of the ingredients of the tablets by HPLC would be rapid, stability indicative and also economical and can be afforded by all.

In the present study, attempts were made to develop a rapid, economical, precise and accurate method for the simultaneous estimation

of the ingredients of this combination in the presence of their degradants.

MATERIALS AND METHOD

Chemicals & Reagents

Samples of Amlodipine Impurity-A and the Atorvastatin calcium impurity-D, F, G and H were synthesized and characterized by Molcan Corporation Ontario, Canada. HPLC grade acetonitrile, Tetra hydro furan & Methanol was procured from Honeywell: Burdick & Jackson, Muskegon, MI 49442 and analytical grade Ortho phosphoric and HPLC grade 1-Octane Sulfonic Acid Sodium Salt acid was procured from Sigma Aldrich, St. Louis, MO, High purity De ionized water was generated in-house from Siemens water purification system.

Chromatographic Conditions

The chromatographic system used was Shimadzu LC 2010 HPLC system comprised of degasser, quaternary pump, auto injector, column compartment, UV detector and the system was controlled through Total chrome software. X-Select CSH, C18 4.6 x 150 mm, 3.5µ maintained at 25 °C using a column oven, eluted with mobile phase at the flow rate of 1.0 mL min^{-1} with Isocratic program.

Buffer: Weighed accurately 13.6 g of Monobasic Sodium Dihydrogen Phosphate and dissolved in 1000 mL of deionized water in a suitable beaker. Mixed well to dissolve

Mobile Phase: Transferred 575 mL of Buffer, 400 mL of Acetonitrile and 25 mL of Tetrahydrofuran into suitable container adjusted the pH to 3.00 ± 0.05 using Ortho-Phosphoric Acid and added 2 g of 1-Octane sulfonic acid sodium salt. Filtered through 0.45 µm nylon membrane filter and degassed.

Measurements were made with injection volume 20µL and ultraviolet (UV) detection at 246 nm. For standard and sample solution were prepared using the diluent of deionized water and Acetonitrile in the ratio of 1:1.

For analysis of forced degradation samples, the photodiode array detector (Model No. 2998) and Empower Software was used in scan mode with a scan range of 200–400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report using the above-mentioned software.

Standard Stock Solutions

Standard solutions were prepared by dissolving the drugs in the diluent and diluting them to the desired concentration.

Amlodipine

28.0 mg Amlodipine Standard (99.8%) was accurately weighed, transferred into a 100 mL volumetric flask, and dissolved with diluent.

Atorvastatin

166.0 mg Atorvastatin calcium standard (99.7%) was accurately weighed, transferred into a 100mL volumetric flask, and dissolved with diluent.

Low Level Standard Preparation

Transferred 1.0 mL of each above solution into a 200mL flask and diluted with diluent. The concentration of Low level standard Preparation contains 0.0014 mg/mL of Amlodipine Besylate and 0.0083 mg/mL of Atorvastatin.

Detectability Level Standard Preparation

Transferred 5.0 mL of the above Low level standard solution in 50mL flask and diluted with diluent. The concentration of Detectability level standard Preparation contains 0.00014 mg/mL of Amlodipine Besylate and 0.00083 mg/mL of Atorvastatin.

Sample Preparation

Weighed accurately about 20 tablets and determined the Average Tablet Weight (ATW) in mg. Crushed the tablets into fine powder. Weighed the powdered sample equivalent to 40.0 mg of Amlodipine and transfer it into 200 mL volumetric flask with the aid of diluent.

To this flask, 75 mL of diluent were added with intermittent shaking and with mechanical shaking for about 30 minutes. The solution was cooled to ambient temperature. Then the volume was made up with diluent and centrifuged for

about 15 minutes. Then the solution was used for injection.

Optimization of the Chromatographic Conditions

To develop the stability-indicating method different stationary phases like Symmetry C18 (150x4.6mm) 5u, Symmetry C8 (150x4.6mm) 5u,X-Bridge (150x4.6mm)5 u, X-Bridge (150x4.6mm) 5u and different mobile phases containing buffers like Tri fluoro acetic acid, Octylamine in water, Tetra butyl ammonium hydrogen sulfate with different pH (3–5) and organic modifier of Acetonitrile and Methanol were used.

Our objective of the chromatographic method development was to achieve a peak tailing factor < 2, Signal to Noise Ratio should be above 10, Theoretical plates should be above 1000 for Amlodipine and Atorvastatin calcium and % RSD for 5 consecutive injection should be less than NMT 10.0 % and very good separation between Amlodipine Impurity-A, Atorvastatin impurity -D,F,G& H along with drug peak amlodipine and Atorvastatin. As this method is used for quantifying impurities in drug product only degradation products are monitored. This method is capable of separating other process related impurities also but validation was done for only degradation products at this time.

The chromatographic separation was achieved using a X-Select CSH, C18 4.6 x 150 mm, 3.5 micron or equivalent. Changing the composition of mobile phase optimized the chromatographic method. Segregation of both peaks (Amlodipine and Atorvastatin) was observed on any C_{18} or CN column but it was difficult to separate both drug degradants on these columns (amlodipine Impurity-A and Atorvastatin Impurity-D, F, G &H). The X-Select CSH column showed better performance as compared to other columns.

Analytical Method Validation

The developed chromatographic method was validated for selectivity, linearity, range, precision, accuracy, sensitivity, robustness[33-35] and system suitability.

Selectivity / Specificity

Selectivity of the developed method was assessed by performing forced degradation studies. According to ICH stress testing of the drug substance can help the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used. Photo stability testing should be an integral part of stress testing. The standard conditions for photo stability testing are described in ICH Q1B. The specificity of the developed LC method for Amlodipine and Atorvastatin calcium was determined in the presence of its related compounds Amlodipine impurity-A, Atorvastatin calcium impurity-D, F, G& H and Placebo.

The stress conditions employed on Amlodipine and Atorvastatin calcium Tablets as degradation study includes acid hydrolysis (5mL of Conc. HCl), base hydrolysis (2mL of 2N NaOH), oxidation (2 mL of 3% H_2O_2), photolysis (ICH Q1B) and thermal (80°C) study.

As such sample provided the Total Impurities level as 0.14 % which includes Atorvastatin Impurity-H and Amlodipine Impurity –A, Atorvastatin calcium Impurity –D,F,G and H as BRL whereas in acid degradation the sample subjected to 5mL of Conc. HCl for 2 hours and the Total degraded impurities were found 1.91 % .

In Base degradation the sample subjected to 2mL of 2N NaOH for 2 hours which produces the total impurities of 0.27% and the samples under Oxidation with 2mL of 3% H_2O_2 provides the Total impurities result as 5.23%.

Under UV treatment of Sample on 3 days provides the Total impurities as 0.10 % and in Thermal condition at 80°C the total impurities were found 0.11%

For all the above degradation studies results were presented in in Tables 1(a) and 1(b) and related chromatograms were represented in Figure 1 through 6.

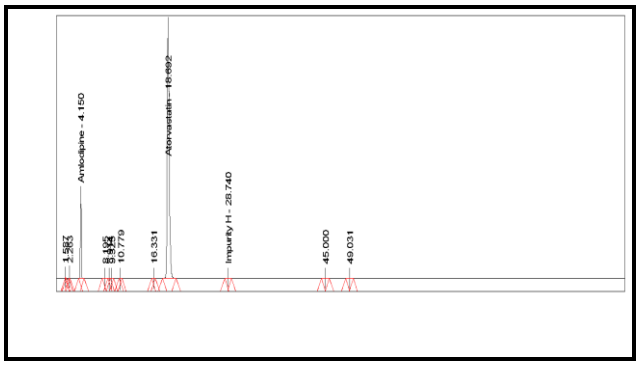


Figure 1: Chromatogram of 10/80 mg sample on as such

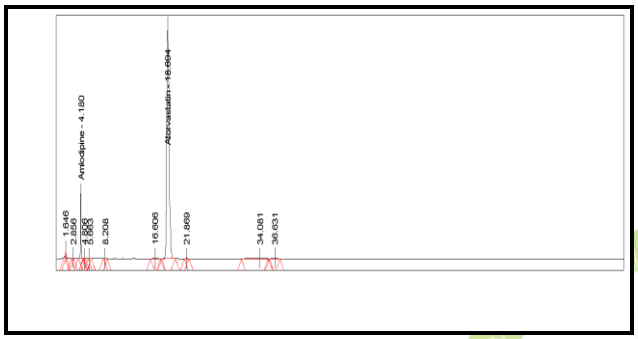


Figure 2: Chromatogram of 10/80 mg sample on acid degradation

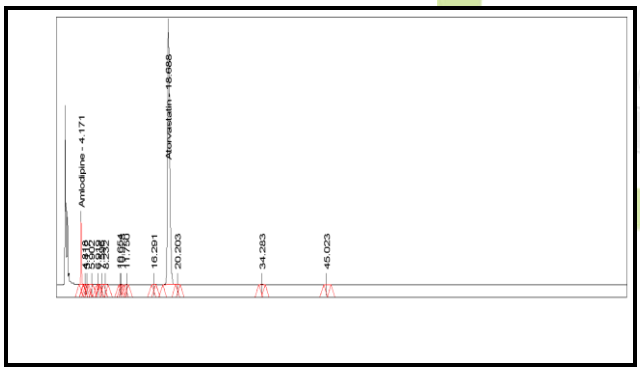


Figure 3: Chromatogram of 10/80 mg sample on Base degradation

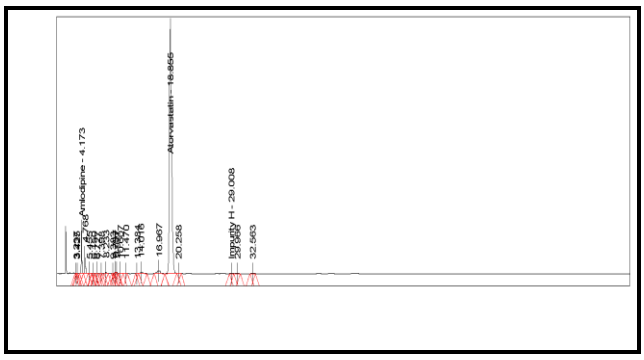


Figure 4: Chromatogram of 10/80 mg sample on oxidation degradation

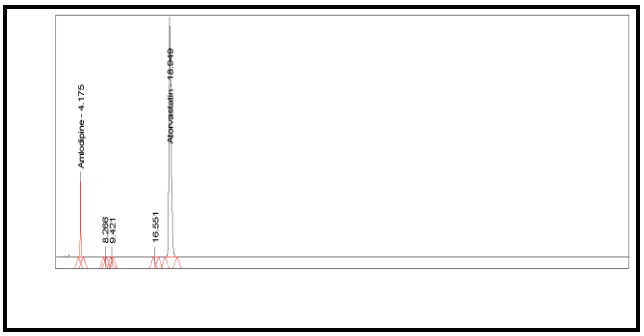


Figure 5: Chromatogram of 10/80 mg sample on UV treatment

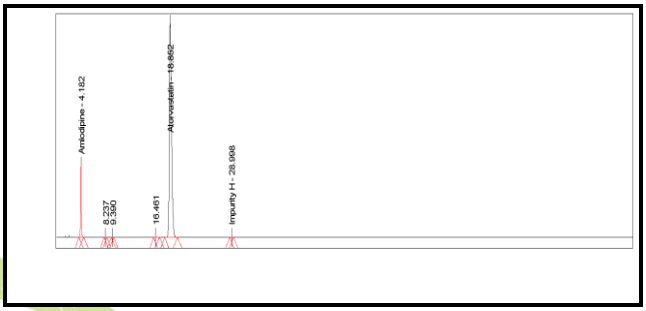


Figure 6: Chromatogram of 10/80 mg sample on Thermal treatment

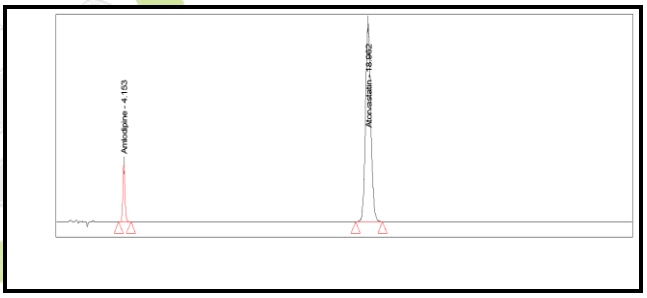


Figure 7.1: Related compound and Assay HPLC chromatogram of Amlodipine & Atorvastatin calcium 10/80 mg Tablets

Spectrum Index plot – Amlodipine & Atorvastatin

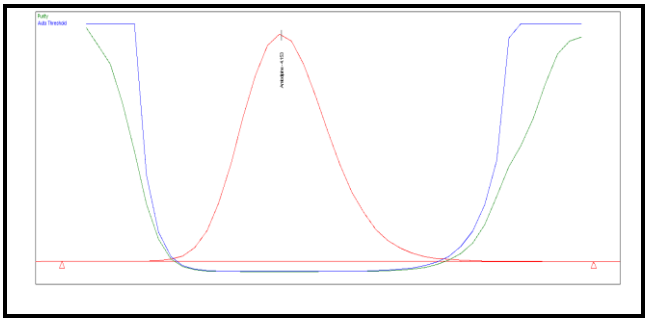


Figure 7.2: Purity Plot of Amlodipine from Amlodipine & Atorvastatin calcium10/80 mg Tablets

Tables 1(a) and 1(b) Results of analysis of forced degradation study samples using the proposed method, indicating percentage of degradation and peak purity of Amlodipine and Atorvastatin calcium Tablets

Table 1 (a)

Stress Condition	Time	Amlodipine	Atorvastatin calcium				Maximum individual unknown impurity	Total Impurities
		Imp-A	Imp-D	Imp-F	Imp-G	Imp-H		
		(%)	(%)	(%)	(%)	(%)	(%)	(%)
As such sample	0 hour	ND	ND	ND	ND	0.06	0.08	0.14
Conc. HCl (5 mL)	2 hours	0.05	ND	ND	0.32	ND	0.72	1.91
2N NaOH (2 mL)	2 hours	ND	ND	ND	BRL	ND	0.05	0.27
3 %H_2O_2 (2 mL)	2 hours	ND	ND	ND	ND	0.06	2.27	5.23
UV Light	3 days	ND	ND	ND	ND	ND	0.06	0.10
Thermal Condition at 80°C	7 days	ND	ND	ND	ND	0.04	0.05	0.11

ND: not detected; BRL: below reporting level (BRL = 0.05%), Imp: Impurity

Table 1 (b)

Stress Condition	Time	Peak	Peak area	Retention time (min)	Purity angle	Purity threshold	Match angle	Match threshold
Conc. HCl (5 mL)	2 hours	Amlodipine	375242	4.16	0.877	1.530	1.160	2.272
		Atorvastatin	3581719	18.96	0.087	0.328	0.208	1.236
2N NaOH (2 mL)	2 hours	Amlodipine	262664	4.16	1.061	1.507	0.849	1.879
		Atorvastatin	3788730	18.97	0.050	0.272	0.146	1.145
3 %H_2O_2 (2 mL)	2 hours	Amlodipine	216393	4.19	0.485	1.002	0.566	1.598
		Atorvastatin	3583132	19.09	0.054	0.281	0.132	1.124
UV light	3 days	Amlodipine	419683	4.19	0.090	0.276	0.461	1.147
		Atorvastatin	3756080	19.06	0.028	0.218	0.125	1.112
Thermal condition 80°C	7 days	Amlodipine	414545	4.19	0.093	0.295	0.467	1.418
		Atorvastatin	3701414	19.08	0.030	0.221	0.128	1.112

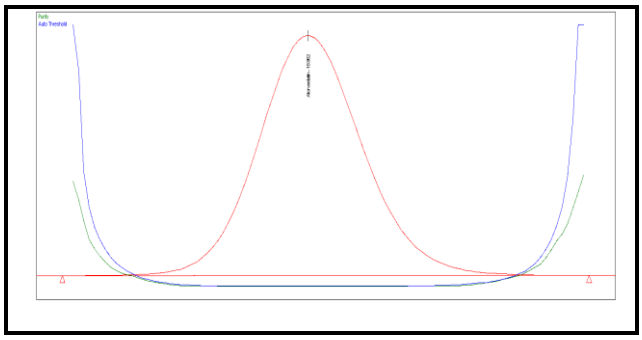


Figure 7.3: Purity Plot of Atorvastatin calcium from Amlodipine & Atorvastatin calcium10 / 80 mg Tablets

RESULTS AND DISCUSSION

From the development studies, it was determined that using mobile phase as 575 mL of Buffer,400 mL of Acetonitrile and 25 mL of Tetrahydrofuran into a suitable container adjusted the pH of the solution to 3.00 ±0.05 with the aid of Ortho phosphoric acid with Isocratic flow rate of 1.0mL/min and ambient temperature the analytes of this combination had adequate retentions, peak shape, less tailing, more resolution between drug and it's degradants and the chromatographic analysis time was about 90minutes. In optimized conditions Amlodipine, Atorvastatin and their degradants were well separated. Typical retention times of Amlodipine and Atorvastatin were about 4.36 min and 19.64 min and for amlodipine Impurity–A, Atorvastatin Impurity-D,F,G &H and benzene sulphonic acid were about 2.96 min, 68.15 min,49.67 min,37.68 min and 29.66 min respectively.

USP doesn't have the combination of Amlodipine/Atorvastatin tablets monograph but as per in-house validated method the retention time was about 90 minutes which is little higher run time even though for combination products the separations were achieved to greater extent and the methods proved to be stability indicating. No EP methods are available on this combination. Instead of regular HPLC if we use UPLC the retention time can be reduced by another 30 minutes with the same suitability parameters. Development on UPLC is not an option in the present study keeping in view this application for cost effective product.

During the initial forced degradation experiments, it was observed that acid hydrolysis was a fast reaction for Amlodipine and Atorvastatin Tablets and almost complete degradation occurred when 5 mL of Conc. HCl solution was used. Both drugs showed extensive degradation in acidic condition and indicating homogeneous peaks and thus establishing the specificity of the Impurity assay method.

Calibration and Linearity

Calibration curve obtained by the least square regression analysis between average peak area and concentration showed linear relationship with a regression coefficient of 0.999 over the calibration ranges tested.

The results of linearity and range obtained for these 5 potential impurities were found well within the acceptance criteria. Out of which Atorvastatin Imp-D and Impurity –H were provided for Linearity calibration plot (Figures 8 and 9) for this chromatographic method was obtained over the calibration ranges tested, i.e. 0.05 % to 10.0 % for Atorvastatin impurity-D and for Atorvastatin calcium impurity-H. The correlation coefficient obtained was greater than 0.999 for all the five impurities and the major compounds Amlodipine and Atorvastatin .The method exhibited good linearity with correlation coefficient values greater than 0.999.

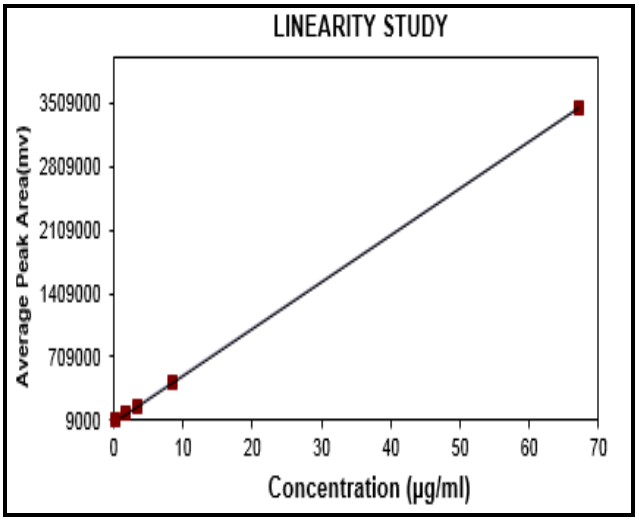


Figure 8: Linearity for Atorvastatin Impurity –D

Atorvastatin Impurity -D

Slope (m) = 51632.029

Intercept (c) = -9156.842

Correlation Co-efficient (r) = 0.9999

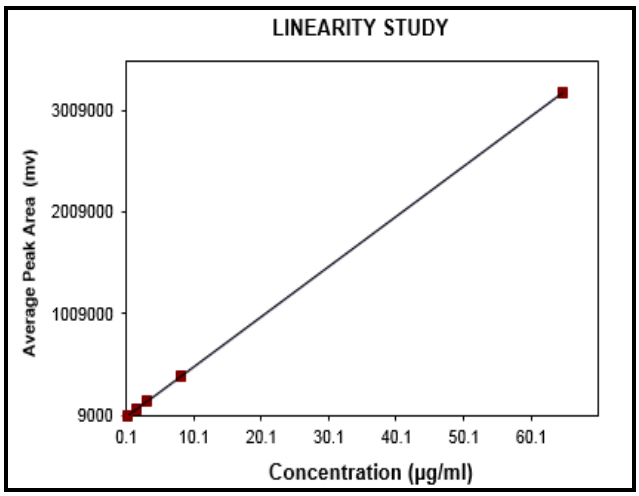


Figure 9: Linearity for Atorvastatin Impurity -H

Atorvastatin Impurity-H

Slope (m) = 49306.305

Intercept=-4574.156

Correlation Co-efficient (r) = 0.9999

The precision of the method was studied by determining the concentrations of each drug as 0.13 and 0.16. The results of the precision study indicate that the method is reliable (RSD% < 10), in Tables 2(a) and 2(b).

Table 2: (a) % RSD of six (6) replicate injections of each impurity should be less than 10.0, theoretical plates should be NLT 1000 and tailing factor should be NMT 2.0 for system precision.

System precision			
Amlodipine		**Atorvastatin**	
% RSD	0.18	% RSD	1.63
Theoretical plates (N)	5387	Theoretical plates (N)	14408
Tailing factor (T)	1.16	Tailing factor (T)	1.07

Table 2(b): % RSD of six (6) sample preparations for each impurity should be less than 10.0 for method precision

Method Precision	
Amlodipine/Atorvastatin tablets 10/80 mg	
Impurity Name	**% RSD**
Amlodipine Impurity-A	0.28%
Atorvastatin calcium Impurity-D	1.20%
Atorvastatin calcium Impurity-F	0.36%
Atorvastatin calcium Impurity-G	0.42%
Atorvastatin calcium Impurity-H	0.16%

Accuracy (Recovery Test)

The percentage recovery was established for all the analytes throughout the range concentration as explained under linearity studies and obtained results are tabulated in Table-3

Robustness

Robustness study was conducted by making small but deliberate changes in the optimized method parameters.

To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between Amlodipine and Atorvastatin calcium were evaluated.

The flow rate of the mobile phase was 1.0 mL min^{-1}. To study the effect of flow rate on the retention time of Amlodipine and Atorvastatin calcium it was changed to 0.9 ml/min and 1.1 ml/min. The effects of pH variation were studied at pH 2.90 and pH 3.10 instead of pH 3.00, while other mobile phase components were held constant.

At all conditions the relative retention time found for Amlodipine Impurity-A , Atorvastatin calcium Impurity-H, G, F and D were found 0.68, 1.54,1.97,2.58 and 3.53 respectively. See Table – 4.

Table 3: Accuracy data of Impurities

Concentration µg/mL	% Spiking level	Average peak area	% Recovery	Mean recovery %
Amlodipine Imp-A				
0.1283	0.05	1821	92.0	95.0
1.2838	0.5	19042	96.1	
10.2704	5.0	153395	96.8	
Atorvastatin calcium Imp-D				
0.1999	0.05	64555	116.3	104.0
3.1992	0.5	119433	107.5	
63.984	10.0	1960314	88.3	
Atorvastatin calcium Imp-F				
0.1988	0.05	12639	94.4	97.5
3.1812	0.5	259568	97.0	
63.624	10.0	5408630	101.0	
Atorvastatin calcium Imp-G				
0.2102	0.05	9322	90.1	95.9
3.3633	0.5	159721	94.8	
67.2672	10.0	3464675	102.8	
Atorvastatin calcium Imp-H				
0.2025	0.05	9248	94.4	98.3
3.24	0.5	154792	98.7	
64.8	1.0	3190851	101.7	

Table 4: Impurities RRT in Robustness study

Parameters	Amlodipine	Atorvastatin			
	Impurity-A	Impurity-H	Impurity-G	Impurity-F	Impurity-D
Flow Rate of 1.0 mL/minute	0.68	1.54	1.97	2.58	3.53
Variation in Flow Rate – 0.9 mL/minute	0.69	1.56	1.99	2.59	3.55
Variation in Flow Rate - flow rate of 1.1 mL/minute	0.67	1.53	1.96	2.57	3.52
Variation in pH of Mobile Phase – 2.90	0.68	1.55	1.98	2.58	3.53
Variation in pH of Mobile Phase – 3.10	0.69	1.55	1.96	2.59	3.54

Table 5: S/N ratio of amlodipine, Atorvastatin calcium and composite impurities at LOQ (0.05%) level

Component name	Concentration µg/mL	% RSD	Signal to noise ratio (S/N)
Amlodipine impurity-A	0.1283	4.50	46.10
Amlodipine	0.1387	1.76	144.04
Atorvastatin Impurity-D	0.1999	2.38	46.83
Atorvastatin	0.2161	0.63	114.35
Atorvastatin Impurity-F	0.1988	1.46	63.41
Atorvastatin Impurity-G	0.2102	5.75	61.72
Atorvastatin Impurity-H	0.2025	3.32	74.88

Determination of Limit of Quantification

Prepare Amlodipine and Atorvastatin calcium LOQ solution as per the method containing the concentration of about 0.331 µg/mL of Amlodipine and 0.5000 µg/mL of Atorvastatin. Made five (5) replicate injections and recorded % RSD. Calculated S/N ratio of 0.05 % to establish LOQ. See Table 5.

CONCLUSION

The Isocratic RP-LC method developed for the analysis of binary mixtures of Amlodipine and Atorvastatin calcium in their pharmaceutical preparations is precise, accurate but with a little higher run time. This method is capable to detect both the drug components of Amlodipine and Atorvastatin calcium in presence of their degradation products (Amlodipine Imp-A and Atorvastatin calcium Impurity-D, F, G and H) with detection level of 0.05 %.

The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is stability-indicating, separates degradants and

can be conveniently used by the quality control department to determine Impurity assay of pharmaceutical preparations and also for stability sample analysis.

ACKNOWLEDGEMENT

The authors wish to thank the management of InvaGen Pharmaceuticals, Inc. for supporting this work. Co-operation from colleagues of Quality & Analytical Research and Development departments of InvaGen Pharmaceuticals, Inc. is much appreciated.

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