ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Stability Indicating Derivative Spectrophotometric Method for Simultaneous Determination of Amlodipine and Atorvastatin in Pharmaceutical Dosage Forms.

Maliheh Barazandeh Tehrani*, Mahin Shoorvazi, and Effat Souri.

Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran (14155-6451), Iran.

ABSTRACT

A simple, sensitive and selective derivative spectrophotometry has been developed for the determination of amlodipine and atorvastatin in their combined pharmaceutical tablet. In this method zero-crossing points for amlodipine and atorvastatin in the first to fourth order spectra were constructed in the range of 200-500 nm and the best optimum wavelengths were chosen for determination of binary drug mixture.The developed method was validated and found to be linear within the range 1-30 µg/ml for amlodipine and 5-60 µg/ml for atorvastatin with acceptable precision (CV %< 5.9) for both drugs in all selected concentrations.A comparative stability indicating study between the suggested procedure and HPLC method for determination of these compounds showed no significant differences between these two methods in alkaline and oxidation conditions. The developed method was applied successfully for the analysis of amlodipine and atorvastatin in dosage form. The validated method is simple, practical and cost effective for simultaneous determination of amlodipine and atorvastatin in dosage forms.

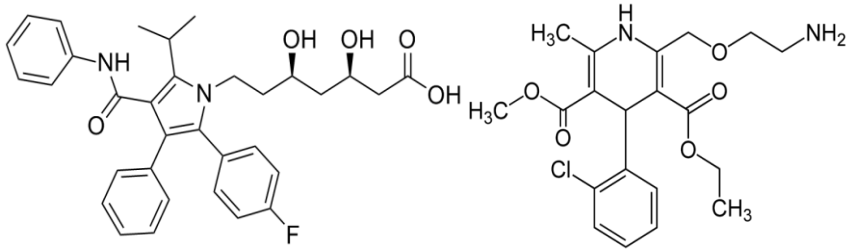
Keywords: Amlodipine, atorvastatin, derivative spectrophotometry, stability indicating method.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Amlodipine besylate, [2[(2-aminoethyoxy) methyl]-4-(2-chlorophenyl)-6-methy l-1, 4-dihydropyridine-3, 5-dicarboxylic acid, 3-ethyl-5- methyl ester] (Fig. 1) is a potent calcium channel blocker related to dihydropyridines. Amlodipine is used for the treatment of angina pectoris and mild to moderate hypertension. Atorvastatin calcium, (3*R*,5*R*)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1-H-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid (Fig. 1) is a synthetic and selective competitive inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A, (HMG-COA) reductase, which inhibits the hepatic cholesterol biosynthesis. Atorvastatin is used as a cholesterol and triglyceride lowering agent in the prevention of cardiovascular disorders (1).



Atorvastatin Amlodipine

Figure 1: Chemical structure of A) Atorvastatin B) Amlodipine

Hypertension and hypercholesterolemia are two of the most prevalent modifiable risk factors for cardiovascular disease. Amlodipine /atorvastatin, a combination of two drugs, have been supplied in different strength combinations and showed better results compared with monotherapy in patients with hypertension and dyslipidemia who requires simultaneous treatment with both drugs (2). These two classes have an additive or synergic effect, not only on new lesion formation but also on inhibiting the progression of established coronary atherosclerosis (3).

Amlodipine/atorvastatin is not official in USP and BP and there are few analytical methods for simultaneous determination of these drugs in dosage forms. There are several HPLC methods for the determination of either amlodipine (4-6) or atorvastatin (7-9) alone, or the combination of two drugs (10-12) in pharmaceutical dosage forms. Different spectrophotometric methods have also been reported for determination of amlodipine (13-15), atorvastatin (16) or amlodipine and atorvastatin in dosage forms (17-19).

In continuation to the recent works in our laboratory (20-24), in this study a stability indicating derivative spectrophotometric method is proposed for simultaneous determination of amlodipine and atorvastatin in dosage forms.

ISSN: 0975-8585

MATERIALS AND METHODS

Chemical

Pure amlodipine and atorvastatin were prepared from Osvah Pharmaceutical Company as gift sample. Methanol was of analytical grade and purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade and prepared from Merck.

Instrumentation

A Shimadzu Model 160 double-beam UV-visible spectrophotometer (Kyoto/Japan) with a fixed band width of 2 nm using l0 mm quartz cells were used for spectrophotometric measurements. The zero order spectra were obtained in the range of 200-500 nm. The derivative spectra were recorded in the same wavelength range at different slit width (Δλ).

Standard solutions

Stock standard solutions of amlodipine besylate (1 mg/ml) and atorvastatin (4 mg/ml) were prepared separately in methanol/water (50:50). Calibration solutions of amlodipine besylate in the presence of fixed concentration of atorvastatin (20 μg/ml) were prepared in the range of 1-30 μg/ml (1, 2, 5, 10, 15, 20, 25 and 30 μg/ml). Also calibration solutions of atorvastatin in the range of 5-60 μg/ml (5, 10, 15, 20, 30, 40, 50 and 60 μg/ml) were prepared in the presence of fixed concentration of amlodipine besylate (5 μg/ml).

Spectrophotometric method

The zero order spectra of amlodipine besylate (50 μg/ml) and atorvastatin (60 μg/ml) solution were separately obtained in the range of 200-500 nm. Because of remarkable overlapping, the first to fourth order derivative spectra were constructed in the range of 200-500 nm to find out the zero-crossing points for both drugs.

Linearity

Eight series of calibration solutions (1-30 μg/ml for amlodipine and 5-60 μg/ml for atorvastatin) were prepared and the absorbance value was determined at zero-crossing wavelengths obtained. The calibration curves were constructed and statistical data were calculated.

Precision and accuracy

To study the within-day and between-day precision and accuracy three different concentration values of amlodipine besylate solutions (1, 15 and 30 μg/ml) in the presence of atorvastatin (20 μg/ml) were prepared and the absorbance value was measured in the zero-

crossing points for atorvastatin. The concentration of amlodipine besylate calculated using the calibration curve. The results of three analyses were used for within-day precision and accuracy. The analysis was repeated for three consecutive days to find out the between-day precision and accuracy. The same procedure was performed for varying concentration of atorvastatin (5, 20 and 60 μg/ml) in the presence of fixed concentration of amlodipine besylate (5 μg/ml). The same procedure was repeated for three consecutive days to find out the between-day precision and accuracy of the method.

Analysis of pharmaceutical formulation

Ten tablets of amlodipine/atorvastatin (Lipivas®) from Alborz Daru Pharmaceutical Company containing 5 mg amlodipine and 20 mg atorvastatin were accurately weighed and finely powdered. Proper amount of powder equivalent to 5 mg amlodipine and 20 mg atorvastatin was weighed and dissolved in methanol by shaking in ultrasonic bath for 20 min. The solution was filtered and reached to the volume in a 100 ml volumetric flask. Working solutions of 12.5 μg/ml amlodipine and 50 μg/ml atorvastatin were prepared in methanol by appropriate dilution and the absorbances were measured according to the suggested procedure.

Stability studies

The degradation studies of amlodipine and atorvastatin was carried out under acid/base hydrolytic and oxidative stress conditions according to a previously published article (10). For acid hydrolysis of drug substances, solutions of amlodipine and atorvastatin were treated with 0.1 M HCl on a water bath at 65°C for 2h. Alkaline hydrolysis of drug substances also was conducted with 0.1 M sodium hydroxide at 65°C for 2h. For oxidative stress, sample solutions of amlodipine and atorvastatin in 3% hydrogen peroxide were kept at 65°C for 2h. After degradation, the acidic and alkaline solutions were neutralized by adding proper amounts of sodium hydroxide and HCl and diluted with solvent to yield a concentration of 20 μg/ml for both amlodipine and atorvastatin.

RESULTS AND DISCUSSION

Derivative spectrophotometric method

The zero order spectra of amlodipine besylate and atorvastatin are presented in Figure 1 which shows significant overlapping. Therefore derivative spectrophotometric method based on zero-crossing technique was used for simultaneous determination of this product. The first to fourth order derivate spectra of standard solutions of amlodipine besylate and atorvastatin were recovered and deeply examined to find out a suitable spectrum to be used for simultaneous determination of these drugs. As it is observed in Fig. 2 at the first, second, third and fourth order spectra the acceptable zero-crossing points for atorvastatin and amlodipine besylate were obtained. The results are summarized in Tables 1 and 2 for atorvastatin and amlodipine. The best selected zero-crossing point would be the one which shows a better linear

calibration curve with acceptable correlation coefficient and lower intercept. Therefore zero-crossing points of amlodipine at λ = 294, 335 and 342 nm in the first, second and fourth order were chosen respectively. The zero-crossing points for atorvastatin were 390.5, 421.5 and 372.5 nm in the first, third and fourth order spectra respectively.

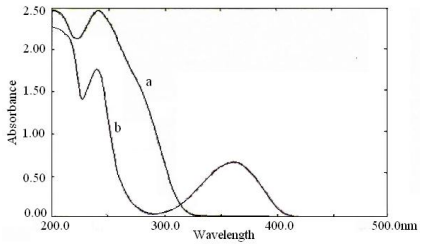


Figure 2: Zero-order spectra of atorvastatin (a) and amlodipine (b)

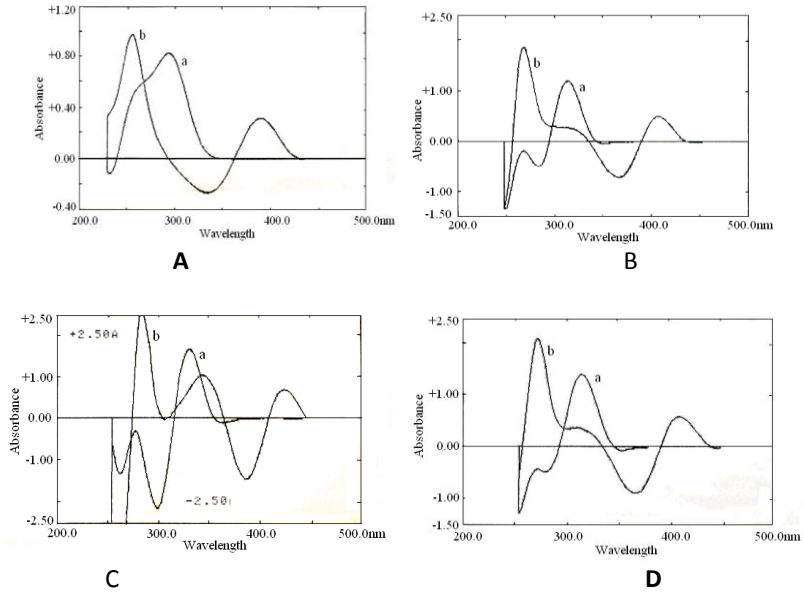


Figure 3: (A) First, (B) second, (C) third and (D) fourth order derivative spectra of atorvastatin(a) and amlodipine(b)

Linearity

Under the optimized conditions six series of calibration curves were constructed at the specified wavelengths. The statistical data from Tables 1 and 2 shows that Beer's law is obeyed within the concentration ranges of 5-60 and 1-30 µg/ml for atorvastatin and amlodipine respectively.

Accuracy and precision

Synthetic mixtures of amlodipine and atorvastatin were prepared as mentioned in the experimental section. The within-day and between-day precision and accuracy at three

ISSN: 0975-8585

different concentrations (5, 20 and 60 µg/ml for atorvastatin and 1, 15 and 30 µg/ml for amlodipine) are demonstrated in Tables 3 and 4. The CV values obtained were in the range of 0.29-4.16% for both drugs in all three selected concentrations.

Table 1: Statistical data of calibration curves of atorvastatin in the presence of amlodipine (5 µg/ml)

Parameters	Derivative order		
	^{1}D(Δλ=20) (λ=294nm)	^{2}D (Δλ=28) (λ=335)	^{4}D (Δλ=21) (λ=342)
Linearity range	5-60 µg/ml	5-60 µg/ml	5-60 µg/ml
Regression equation	Y=0.01304X+0.00635	Y=0.00385X+0.00209	Y=0.00631X+0.00223
SD of slope	0.00012	0.00008	0.00010
RSD of slope (%)	0.92	2.01	1.52
SD of intercept	0.0031	0.0015	0.0024
Correlation coefficient	0.9994	0.9981	0.9980

Table 2: Statistical data of calibration curves of amlodipine in the presence of atorvastatin (20 µg/ml)

Parameters	Derivative order		
	^{1}D (Δλ=20) (λ=390.5nm)	^{3}D (Δλ=31.5) (λ=421.5nm)	^{4}D (Δλ=21) (λ=372.5nm)
Linearity range	1-30 µg/ml	1-30 µg/ml	1-30 µg/ml
Regression equation	Y=0.00742X+0.00229	Y=0.01220X+0.00293	Y=0.00837X+0.00125
SD of slope	0.00006	0.00015	0.00013
RSD of slope (%)	1.049	1.196	1.555
SD of intercept	0.00097	0.00121	0.00141
Correlation coefficient	0.9999	0.9998	0.9993

Table 3: Accuracy and precision data for determination of atorvastatin in the presence of amlodipine (5 µg/ml) by different order derivative spectrophotometry

Added (µg/ml)	Within-day (n = 3)			Between-day (n = 9)		
	Found (µg/ml)	CV (%)	Error (%)	Found (µg/ml)	CV (%)	Error (%)
^{1}D (Δλ=20)						
5.00	4.87±0.055	1.12	-2.68	4.81±0.09	-3.74	1.83
20.00	20.17±0.103	0.51	0.87	20.06±0.12	0.29	0.57
60.00	58.97±0.031	0.05	-1.71	59.16±0.16	-1.40	0.26
^{2}D (Δλ=28)						
5.00	4.89±0.079	1.62	-2.21	4.97±0.2	3.94	-0.57
20.00	19.44±0.14	0.70	-2.78	19.51±0.23	1.19	-2.46
60.00	58.54±0.26	0.45	-2.44	58.78±0.79	1.34	-2.04
^{4}D (Δλ=21)						
5.00	4.84±0.092	1.91	-3.24	4.95±0.16	3.30	-0.93
20.00	20.04±0.48	2.40	0.19	19.98±0.43	2.14	-0.12
60.00	58.70±0.35	0.59	-2.16	58.97±0.36	0.61	-1.72

ISSN: 0975-8585

Table 4: Accuracy and precision data of determination of amlodipine in the presence of atorvastatin (20 μg/ml) by different order derivative spectrophotometry

Added (μg/ml)	Within-day (n = 3)			Between-day (n = 9)		
	Found (μg/ml)	CV (%)	Error (%)	Found (μg/ml)	CV (%)	Error (%)
^{1}D(Δλ= 20)						
1	0.989± 0.029	2.96	-1.10	0.986± 0.041	4.16	-1.40
15.00	15.13± 0.048	0.32	0.85	15.09±0.13	0.88	0.62
30.00	29.84± 0.049	0.17	-0.53	29.89± 0.14	0.48	-0.36
^{3}D (Δλ=31.5)						
1.00	0.97±0.008	0.81	-2.9	0.968± 0.034	3.46	-1.35
15.00	15.26±0.15	1.01	1.72	15.065± 0.18	1.22	0.43
30.00	30.01±0.21	0.69	0.04	29.943±0.12	0.4	-0.19
^{4}D (Δλ=21)						
1.00	1.03±0.027	2.59	2.70	1.011± 0.029	2.82	1.15
15.00	15.16±0.22	1.46	1.06	15.14± 0.28	1.88	0.93
30.00	30.15±0.38	1.29	0.50	29.87±0.35	1.17	-0.44

Relative recovery

The recovery was checked by addition of known amounts of standard solutions of atorvastatin and amlodipine to a solution obtained from the pharmaceutical solution. The standard solution, the pharmaceutical solution and the resulting mixture were assayed according to the proposed method. The percentage recoveries in different derivative conditions were between 98.0-100.8 percent with maximum standard deviation of 1.57 and 99.6-102.2 with maximum standard deviation of 1.67 for amlodipine and atorvastatin respectively.

Application

The validated method was used for simultaneous determination of amlodipine and atorvastatin in a commercial formulation. The results showed acceptable agreement with the labeled content for both drugs and no interferences from the excipients were observed. This analysis technique was compared with a reported HPLC method (11) which showed no significant difference between these two methods (Table5).

Stability Studies

In order to find out the usefulness of the spectrophotometric method for determination of amlodipine and atorvastatin in the presence of their degradation products, degradation studies were conducted according to a previously reported method (10). Sample solutions of amlodipine and atorvastatin were exposed to different forced degradation conditions, i.e. 0.1M HCl, 0.1M NaOH and 3% H_2O_2 in water bath at 65°C. The resulted solutions were determined by spectrophotometry in different derivative orders and compared with the HPLC method. The results are shown in Table 6 which shows good agreement between the HPLC method and the third derivative UV/visible spectrophotometric method in all stress conditions for amlodipine.

ISSN: 0975-8585

There were no significant differences between the spectrophotometry and HPLC method in alkaline and oxidant degradation results for atorvastatin too. In acidic media the HPLC results have shown 58.7 percent degradation while in UV/visible derivative method the maximum degradation was 8.3 percent. According to the previously reported study by Shah R.P. et al (25), the degradation product in acidic media shows the same absorbance pattern to atorvastatin which is not distinguishable by spectrophotometric method.

Table 5: Assay results of containing atorvastatin (20 mg) and amlodipine (5 mg) using derivative spectrophotometry and HPLC method

Derivative Order		Amlodipine (5µg/ml)		Atorvastatin (20µg/ml)	
		Mean±SD	(%)Recovery	Mean±SD	(%)Recovery
UV/VIS	^{1}D (Δλ= 20)	5.15± 0.05	103.0	19.56±0.12	97.8
	^{2}D (Δλ= 28)	----	----	19.33±0.54	96.7
	^{3}D (Δλ=31.5)	5.22± 0.05	104.4	----	----
	^{4}D (Δλ= 21)	5.26± 0.13	105.2	19.27±0.21	96.4
HPLC		5.22±0.03	104.4	19.20±0.15	96.0

Table 6: The results of the stress degradation tests using different condition after 2h at 65°C

Stress condition	% degradation		
Solvent	0.1 M HCl	0.1 M NaOH	3% H_2O_2
Amlodipine			
HPLC	11.22	17.82	41.96
UV/VIS			
^{1}D (Δλ= 20)	4.02	14.29	37.05
^{3}D (Δλ=31.5)	9.42	17.58	39.12
^{4}D (Δλ=21)	3.21	10.22	38.98
Atorvastatin			
HPLC	58.70	6.20	13.5
UV/VIS			
^{1}D (Δλ= 20)	8.30	8.76	8.90
^{2}D (Δλ= 28)	7.80	4.19	13.77

CONCLUSION

The results obtained from the degradation studies of atorvastatin showed, the best results for degradation studies obtained from the first and second order spectrophotometry. The validated method is rapid, simple, reliable, practical and cost effective for simultaneous determination of amlodipine and atorvastatin without interference from the excipients in dosage form and could be used in quality control laboratories without laborious sample preparation.

ISSN: 0975-8585

ACKNOWLEDGMENT

This study was part of a M.D. thesis supported by Tehran University of Medical Sciences (grant No: 17776).

REFERENCES

[1] Brunton L, Chabner A, Knollmann C. Goodman and Gilman's. The Pharmacological Basis of Therapeutics. Twelfth Edition, Mc Graw-Hill Medical Publishing Division; 2010.
[2] Mckeage K, Siddiqui M, Asif A. Am J Cardiol Drugs 2008; 8(5):1-67.
[3] Jukema JW, van der Hoorn JW. Expert Opin Pharmacother 2004; 5(2):459-68.
[4] Male Suik MD, Cardoso SG, Bajerski L, Lanzanova FA. J AOAC Int 2006; 89 (2):359-64.
[5] Kawabe Y, Nakamura H, Hino E, Suzuki S. J Pharm Biomed Anal 2008; 47: 618-624.
[6] Alsarra IA. J Chromatogr Sci 2009; 47(10): 863-7.
[7] Erturks S, Aktas ES, Ersoy L, Ficicioglu S. J Pharm Biomed Anal 2003; 33:1017-1023.
[8] Stanisz B, kania L. Acta Pol pharm 2006; 63(6):471-6.
[9] Khedr A. K AOAC Int 2007; 90(6):1547-53.
[10] Mohammadi A, Rezanour N, Ansari Dogaheh M, Ghorbani Bidkorbeh F, Hashem M, Walker RB. J Chromatogr B 2007; 846:215-221.
[11] Chaudhari BG, Patel NM, Shab PB. Chem Pharm Bull 2007; 55(2):241-246.
[12] Sivakumar T, Manavalan R, Muralidharan C, Valliappan K. J Sep Sci 2007; 30: 3143-3153.
[13] Ragno G, Garofalo A, Vetuschi C. J Pharm Biomed Anal 2002; 27: 19-24.
[14] Rahman N, Hoda MN. J Pharm Biomed Anal 2003; 31:381-392.
[15] Rahman N, Singh M, Hoda MN. Farmaco 2004; 59(11): 913-9.
[16] Ashour S, Bahboud M, Khateeb M. Spectrochim Acta A 2011; 78:913-917.
[17] Chaudhari BG, patel AB. Int J Chem Tech Res 2010; 2(1):633-639.
[18] Rao L, Rajeswari K R, Sankar GG. Res J Pharm Biol Chem Sci 2010; 1 (2): 66-69.
[19] Ramesh D, Ramakrishna S. Indian J Pharm Sci 2010; 2(4): 215-219.
[20] Souri E, Jalalizadeh H, Farsam H, Ghadiri R, Amanlou M. Chem pharm Bull 2005; 53(8):949-951.
[21] Souri E, Jalalizadeh H, Farsam H, Rezwani H, Amanlou M. DARU 2005; 13(1):11-16.
[22] Souri E, Amanlou M, Farsam H, Afshari A. Chem Pharm Bull 2006; 54(1):119-122.
[23] Souri E, Amanlou M. E-J Chem 2010; 7(S_1): 5197-S202.
[24] Barazandeh Tehrani M, Namadchian M, Fadaye Vatan S, Souri E. DARU J Pharm Sci 2013; 21: 29.
[25] Shah RP, Kumar V, Singh S. LC-MS/MS studies on identification and characterization of hydrolytic products of atorvastatin. 2007; Proceedings of 12th ISMAS symposium cum.