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Simultaneous estimation of amlodipine besylate and atorvastatin calcium in pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of amlodipine besylate (AM) and atorvastatin calcium(AT) in tablet dosage form. A Eurosphere-100 C_{18}, 250×4.6mm, 5µm particle size in isocratic mode, with mobile phase methanol: Water (55:45v/v) was used. The flow rate was 1.0mL/min and individual component were measured at 207nm. The retention time of amlodipine besylate and atorvastatin were found to be 2.49 and 5.14 min respectively. Linearity for AM and AT was in range of 2.5-12.5µg/mLand 5-25µg/mL with correlation coefficient values 0.9978 and 0.9995 respectively. Amount found for AM and AT is 5.04g and 10.07g and percentage recovery obtained were 100.87% and 100.74% respectively. The proposed method is precise, selective and rapid for simultaneous estimation of amlodipine besylate and atorvastatin calcium.

KEYWORDS

Amlodipine besylate;
Atorvastatin calcium;
RP-HPLC;
Method validation.

INTRODUCTION

Multidrug administration is often associated with clinically significant interaction, especially narrow therapeutic index drugs, either at pre-absorption or post-absorption stage[1,2]. This can limit the desired therapeutic effect of either of drug molecule. The present study was aimed to develop simple, rapid and precise analytical method for simultaneous estimation of amlodipine besylate (AM) and atorvastatin calcium (AT).

Amlodipine[3,4] is chemically 3-ethyl-5methyl (4RS)-2-[(2-aminoethoxy) methyl-4- (2-chlorophenyl)-6-methyl] 1,4-dihydropyridine-3, 5-dicarboxylate benzenesl phonate. AM used as antiangianl and antihypertensive agent.

Various analytical methods have been reported in literature for estimation of amlodipine in single and combination spectrophotimetric methods[5-11], HPLC[12-17] and HPTLC[18-21].

Atorvastatin[22] is chemically 7-[[2-(4-fluorophenyl) -5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrol -1-yl]]-3,5-dihydroxy heptanoic acid. AT is used as antihyperlipoproteinemic agent. It is not yet official in any pharmacopoeia.

Very few analytical methods have been reported in literature for estimation of atorvastatin in single and combination spectrophotimetric methods[23-25] and HPLC [26-29].

Fixed dose combination containing AM and AT is available only in tablet form in market. This combina-

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tion dosage form was recently introduced and only one analytical method is reported for simultaneous estimation of these dugs by using Raman spectroscopy[30]. The aim of this work was to develop a simple, rapid, precise and accurate reverse phase- HPLC method for the estimation of AM and AT from tablet dosage form.

MATERIAL AND METHOD

Instrument

High performance liquid chromatography system Chemito LC 6600 equip with universal injector with injection volume 20µL, Ultra-Visible(UV-Vis) detector.

A Eurosphere 100 C_{18} column (250×4.6mm) 5µm particle size forms the stationary phase.

Chemicals and reagents

Standard drug samples AM and AT were received as gift samples from M/S Dr. Reddy's Laboratories Ltd., Hyderabad and M/S Blue Cross Laboratories Ltd., Nasik respectively. Tablet formulation containing AM (5mg) and AT(10mg) was procured from the market. All reagents used were of analytical grade: HPLC grade methanol and HPLC grade water were obtained from qualigens.

Mobile phase

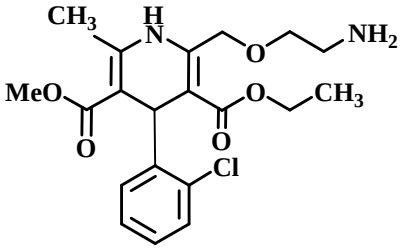
Methanol: Water (55:45v/v), mix. of HPLC grade methanol (550mL) and water (450mL) was prepared and then subjected to filtration and degassing.

Standard stock solution

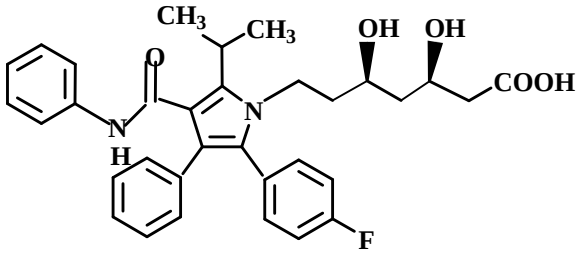
Standard AM(25mg) was accurately weighed and transferred to a 25ml volumetric flask and dissolved with methanol. The flask was shaken and volume was made up to the mark with methanol to get a solution of AM (1000µg/ml). Standard AT (25mg) was accurately weighed and transferred to a 25ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to get a solution of AT(1000µg/ml).

Working standard solution

From standard stock solutions dilution were made to get AM (5µg/ml) and AT (10µg/ml) solutions using methanol as solvent.



Amlodipine



Atorvastatin

Figure 1 : Chemical structure of amlodipine and atrovastatin

TABLE 1 : Result of RP- HPLC assay

Formulation	Actual concentration (mg)		%AM	% AT
	AM	AT	(n =3)	(n=3)
Tablet	5.0	10.0	99.83	100.18
	S.D		0.031	0.098

Sample solution

Twenty tablets were weighed accurately and finely powdered. The powder equivalent to AM(5mg) and AT(10mg) was accurately weighed and transferred to volumetric flask of 100ml capacity containing methanol (50ml). Then the content was shaken for 30min. and volume was made up to the mark with methanol. The above solution was filtered through Whatman filter paper no.1. This solution was again filtered through 0.45µ millipore membrane filter. 1ml of this solution was diluted to 10ml with mobile phase to get the AM(5µg/ml) and AT (10µg/ml) solution (theoretical values).

Assay

20µl of the test and standard solutions (n=3) were injected separately to an injector of liquid chromatography and chromatograms were recorded for 15min. From the area, the amounts of both the drugs were calculated. The values are given in TABLE 1.

Assay result

In replicate analysis(n=3) of two drug by proposed method showed, the content of amlodipine besylate and

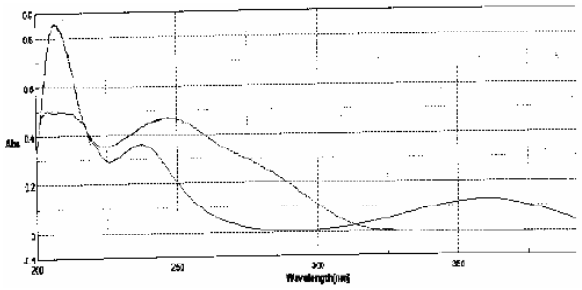


Figure 2 : Overlay spectra of amlodipine besylate and atorvastatin calcium

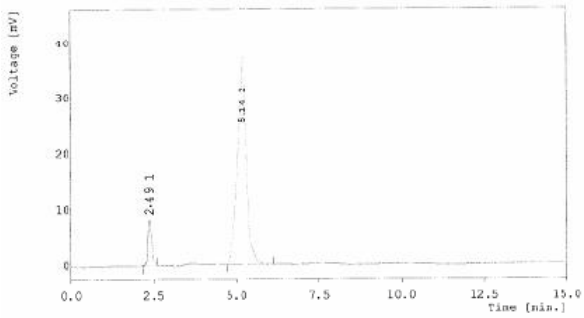


Figure 3 : Typical chromatogram of the sample solution containing amlodipine besylate and atorvastatin calcium at retention time of 2.49 and 5.14min. respectively

atorvastatin calcium were found as 5.04mg/tablet and 10.07mg/tablet respectively.

Linearity and calibration

From the standard drug solution five working standards (2.5,5.0,7.5,10.0,12.5µg/mL) of AM and five working standard(5,10,15,20,25µg/mL) of AT were prepared. The two drugs were evaluated with UV-VIS detector at 207nm. Peak area was recorded for all the peaks. The plot of area Vs respective concentrations of AM and AT was found to be in the linear range of 2.5-12.5µg/mL and 5-25µg/mL with correlation coefficient values r =0.9978 and r=0.9995 respectively. The values are given in TABLE 2.

Recovery study

To ensure the reliability and accuracy of the method recovery studies were carried out by mixing a known quantity of standard drug with preanalysed sample and content were analyzed by proposed method.

The lower values of relative standard deviation (RSD) indicate the method is precise and accurate. The mean recoveries of amlodipine besylate and atorvastatin calcium were 100.87% and 100.74% respectively and there is no positive or negative interference of exipients present in the tablet. Values are given in TABLE 3

TABLE 2 : Statistical data for linearity and calibration range

Parameters	AM	AT
Linear range ((µg/ml)	2.5-12.5µg/ml	5-25µg/ml
Slope	149.28	470.13
Intercept	133.09	970.81
SD of slope	3.99	5.97
Correlation coefficient (r)	0.9978	0.9995

TABLE 3 : Recovery study

Amount of sample		Amount of drug added		Amount recovered		% Recovery	
AM µg/ml	AT µg/ml	AM µg/ml	AT µg/ml	AM µg/ml	AT µg/ml	AM %	AT %
5.0	10.0	4.0	8	4.01	8.09	100.31	101.16
5.0	10.0	5.0	10	5.04	10.02	100.84	100.23
5.0	10.0	6.0	12	6.09	12.10	101.46	100.83
		Mean				100.87	100.74
		R.S.D				0.58	0.47

TABLE 4 : Repeatability data for AM and AT

Sr. No.	Conc. (µg/ml)	Area of AM	Area of AT	t_R of AM	t_R of AT
1	5:10	875.42	5486.11	2.49	5.14
2	5:10	876.15	5486.83	2.48	5.15
3	5:10	875.51	5485.48	2.50	5.13
4	5:10	875.87	5487.01	2.51	5.14
5	5:10	876.62	5485.26	2.47	5.17
6	5:10	877.04	5485.63	2.48	5.14
7	5:10	876.76	5487.24	2.49	5.12
8	5:10	875.32	5485.20	2.52	5.14
9	5:10	876.08	5486.57	2.51	5.15
10	5:10	875.60	5485.33	2.48	5.13
Mean		876.04	5486.07	2.49	5.14
S. D.		0.603	0.788	0.016	0.014
RSD		0.069	0.014	0.656	0.267

Method validation

The analytical method was validated as per the recommendations of USP[31] and ICH[32] guidelines for the parameters like accuracy, precision, ruggedness and repeatability. All tests were carried out for both the drugs alone and in combination.

Accuracy

Accuracy is the measure of how close the experimental value is to be the true value. Accuracy studies were performed by standard addition method at the 80, 100 and 120% levels. The results are shown in TABLE 3

Precision

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Precision is the measure of how close the data values to each other for number of measurements under the same analytical conditions. ICH has defined the precision to contain three components: repeatability, intermediate precision and reproducibility.

Repeatability

Standard mixture solution containing AM (5µg/ml) and AT(10µg/ml) were injected and chromatograms were recorded each time. Area and retention time(t_R) was measured and RSD was calculated. Results are shown in TABLE 4

Intra and inter day precision

Variation of results within the same day(intra day) and variation of results between days(inter day) were analyzed. Intra day precision was determined by analyzing AM and AT for three times in the same day. Inter day precision was determined by analyzing both the drugs daily for three days. The results are shown in TABLE 5 and 6.

Ruggedness

It is defined as the reproducibility in the results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, chemicals, solvents, and sources of reagents. The results are shown in TABLE 7

RESULT AND DISCUSSION

Amlodipine besylate is antianginal and atorvastatin calcium is antihyperlipoproteinemic. The marketed survey revealed that amlodipine besylate (AM) and atorvastatin calcium (AT) in combination is recently introduced in the market. It is available in market as film coated tablet formulation. It is indicated for the treatment of co-existing essential hypertension and hyperlipidemia.

Literature survey revealed that there is only one method for simultaneous estimation of amlodipine besylate and atorvastatin calcium by Raman spectroscopy. Hence, an attempt has been made to develop simple, rapid and accurate method for estimation of amlodipine besylate and atorvastatin calcium by ultraviolet spectroscopy and high performance liquid chromatography.

TABLE 5 : Determination of precision for AM

Conc. (µg/ml)	Intra-day(n =3)	CV	Inter-Day(n=3)	CV
2.5	495.018±0.228	0.046	492.510±0.601	0.122
5.0	905.806±0.367	0.040	898.961±0.370	0.041
7.5	1256.120±0.514	0.041	1247.648±0.451	0.036

TABLE 6 : Determination of precision for AT

Conc. (µg/ml)	Intra-day (n =3)	CV	Inter-Day (n=3)	CV
5.0	3421.250±1.464	0.043	3379.851±0.435	0.013
10.0	5591.810±1.120	0.020	5584.945±0.439	0.008
15.0	7956.292±0.896	0.011	7944.736±0.573	0.007

TABLE 7 : Results of ruggedness testing

	% Label claim estimated	
	AM	AT
Analyst I	99.87	98.91
Analyst II	98.94	98.96
Mean	99.41	99.94
SD	0.465	0.025
%RSD	0.47	0.03

TABLE 8 : System suitability test parameters

System suitability parameters	Proposed method	
	AM	AT
Retention time (t_R)	2.49min	5.14min
Capacity factor (k')	1.50	4.16
Theoretical plate number (N)	3259	6806
Tailing Factor (As)	1.40	1.19
Resolution Factor (Rs)	--	6.362

A reverse phase HPLC method was developed for simultaneous estimation of amlodipine besylate(AM) and atorvastatin calcium(AT) in tablet formulation. The separation was achieved by Eurosphere-100 C_{18} column and methanol: water(55:45 v/v) as mobile phase, at a flow rate of 1.0ml/min. The detection was carried out at 207nm. The retention time of AM and AT was found to be 2.49 and 5.14min respectively. Linearity was assessed by a plot of concentration versus area. The graphs were found to be linear in range of 2.5-12.5µg/ml for AM and 5-25µg/ml for AT with correlation coefficient values 0.9978 and 0.9995 respectively. On the basis of the parameters fixed, the method of estimation was validated, for following parameters:

- **Accuracy:** Three replicate injections, each of three different test concentrations in the range of 80 to 120 % of labeled claim of tablet under study has yielded the results within 100 to 101% of true concentration of each drug. These results indicate the accuracy of the method.
- **Precision:** Precision studies were carried out us-

ing parameters like different days, repeatability. Results showed that the % RSD is in the range of 0.1-0.03, i.e. less than 2 for different days and 0.04-0.6.(TABLE 4,5,6)

- **Ruggedness:** Ruggedness studies were carried out using different analyst parameter. Results showed that the % RSD is in the range of 0.03-0.4, i.e. less than 2 for different analyst studies. This study signifies the ruggedness of the method under varying conditions of its performance (TABLE 7).

System suitability test

As per USP-24 system suitability test was carried out on freshly prepared standard stock solutions of amlodipine besylate and atorvastatin calcium. 20µL of the both drugs were injected under the optimized chromatographic conditions and following parameters were studied to evaluate the suitability of system.

(1) Number of theoretical plates (N)
(2) Tailing factor (As)
(3) Capacity factor (k')
(4) Resolution (Rs)

The values of system suitability test were shown in TABLE 8

CONCLUSION

The proposed RP-HPLC method is simple, accurate, rapid and selective. High percentage of recovery shows that the method is free from interferences of the exipients used in the formulations. Therefore method can be useful in routine quality control analysis of these drugs.

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