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Spectrophotometric method for simultaneous determination of atenolol and atorvastatin in tablet dosage forms

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ABSTRACT

A UV spectrophotometric method was developed for simultaneous estimation of atenolol and atorvastatin in tablet dosage form. The method is based upon determination of atenolol and atorvastatin at their respective λ_{max} 225.0 nm and 241.0 nm, respectively. The linearity of atenolol and atorvastatin is established in the range of 5-30 µg/mL and 2-12 µg/mL, respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for atenolol and atorvastatin were found to be 0.1765 and 0.5823 µg/mL, 0.5823 and 0.2522 µg/mL, respectively. The recovery study confirmed accuracy of proposed method and low values of relative standard deviation confirmed precision of method. The results of the study showed that the proposed method is simple, rapid, precise and accurate, which can be applied for the routine determination of atenolol and atorvastatin in tablet dosage forms.

Key words: Atenolol, Atorvastatin, Simultaneous estimation method, Method validation

1. INTRODUCTION

Atrovastatin (ATV), a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor, has been used as a lipid lowering agent [1]. Chemically, ATV is (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid [2]. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methyl glutaryl-coenzyme A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cell increase the number of LDL receptor on the surface of the cells, which in turn increase the amount of LDL uptake by the hepatic cells and decrease the amount of LDL in the blood [3-5]. It is not official in I.P., B.P., and U.S.P. till date. ATV is reported to be estimated by spectrophotometry and HPLC individually or in combination with other drug [6-8].

Atenolol (ATL) is an antihypertensive, antianginal, and antiarrhythmic drug; chemically it is 4-(2-Hydroxy-3-isopropyl aminopropoxy)-phenyl acetamide [9,10]. It is mainly act by inhibition of rennin release and angiotensin-II and aldosterone production. The Indian Pharmacopoeia describes non-aqueous titration method for the assay of atenolol. Extensive literature search revealed that there is no validated spectrophotometric method reported for simultaneous estimation of ATV and ATL in combined dosage form and hence a successful attempt has been made in the present work to estimate these two drugs simultanously by spectrophotometric analysis.

2. MATERIALS AND METHODS

2.1 Reagents

Atenolol was obtained as gift sample from Cadila Pharmaceutical Ltd., Ahmedabad, India. Atorvastatin was procured from Cipla Ltd., Mumbai, India. All chemicals are of HPLC grade and were purchased from Qulaigens Fine Chemicals, Mumbai, India.

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2.2 Instrument

UV-Vis spectrophotometer 1601 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used.

2.3 Standard stock solution

An accurately weighed 10 mg of each of AVT and ATL was dissolved in 100 mL of methanol to obtain a concentration of 0.1 mg/mL or 100 µg/mL each. The working standard solution of AVT and ATL were prepared on the day of analysis by suitable dilution of the stock solution with methanol.

2.4 Determination of maximum wavelength

By appropriate dilution of two standard drug solution with 50% v/v aqueous methanol, solution containing 10 µg/mL of ATV and 10 µg/mL of ATL were scanned separately in the range of 200-350 nm to determine the wavelength of maximum absorption for the drug. The absorbance maximum of ATV and ATL was found to be 241 nm and 225 nm, respectively.

2.5 Simultaneous equation method

Two wavelengths selected for the method are 225 nm and 241 nm that are absorption maxima of ATL and ATV, respectively in 50% v/v aqueous methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solution of 5-30 µg/mL concentration for ATL and 2-12 µg/mL concentration for AVT. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%}_{1\,cm}$) for both the drugs were determined as mean of six independent determination. Concentration in the samples was determined by using following equation:

$$C_x = (A_2 ay_1 - A_1 ay_2)/(ax_2 ay_1 - ax_1 ay_2) \quad (1)$$

$$C_y = (A_1 ax_2 - A_2 ax_1)/(ax_2 ay_1 - ax_1 ay_2) \quad (2)$$

Where, A_1 and A_2 =Absorbance of mixture at 225 nm (λ_1) and 241 nm (λ_2)
ax_1 and ax_2 = Absorptivity of ATL at λ_1 and λ_2
ay_1 and ay_2 = Absorptivity of ATV at λ_1 and λ_2
C_x = Concentration of ATL
C_y = Concentration of ATV

2.6 Method validation

The method was validated in compliance with ICH guidelines [11]. The following parameters were used for the validation of the developed method.

2.6.1 Linearity

Linear relationship between absorbance and concentration of the drugs was evaluated over the concentration range expressed in µg mL^{-1} by making five replicate measurements in the concentration range of 5-30 µg mL^{-1} for ATL and 2-12 µg mL^{-1} for ATV, respectively.

2.6.2 Precision

Precision of the developed method was studied by performing repeatability and intermediate precision studies. The sample application and measurement of absorbance was determined by performing six replicates measurement of the same band using a sample solution containing 20 µg mL^{-1} of ATL and 8 µg mL^{-1} of ATV each.

2.6.3 Recovery studies

Recovery studies were carried out by spiking three different known amounts of the standard substances to the drug product (standard addition method). Hence 16, 20 and 24 µg mL^{-1} of ATL and 8, 10 and 12 µg mL^{-1} of ATV were spiked to the dosage form that contained 20 and 10 µg mL^{-1} of ATL and ATV, respectively, after sample dilution.

2.6.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were calculated from the standard deviation of the y-intercept and slope of the calibration curves of ATL and ATV using the formula as given below.

$$\text{Limit of Detection} = \frac{3 \propto}{S}$$

$$\text{Limit of Quantification} = \frac{10 \propto}{S}$$

Where, α is the standard deviation of the y-intercept and S is the slope of the calibration curve.

2.6.5 Robustness

The effect of deliberate variations in the method parameters like wavelength and pH were evaluated in this study. The effect of these changes on the absorbance was evaluated by calculating the relative standard deviation (RSD) for each parameter.

2.7 Analysis of tablet dosage forms

Bi-layer tablets were prepared in our laboratory by compressing 50 mg of ATL and 10 mg ATV. Twenty tablets were taken and were crushed to a fine powder. The powder sample equivalent to 50 mg of ATL and 10 mg of ATV was transferred to a 10 mL volumetric flask and about 80 mL of 50% v/v aqueous methanol was added and sonicated to dissolve. The volume was made up to the mark with 50% v/v aqueous methanol. This solution was filtered through Whatman filter paper. 42.1 mL of this solution was diluted to

100 mL with 50% v/v aqueous methanol. The solutions were analyzed by multicomponent mode of analysis. The samples were analyzed six times. 50% v/v aqueous methanol was used as blank. Fig.1 shows overlay spectrum of ATL and ATV.

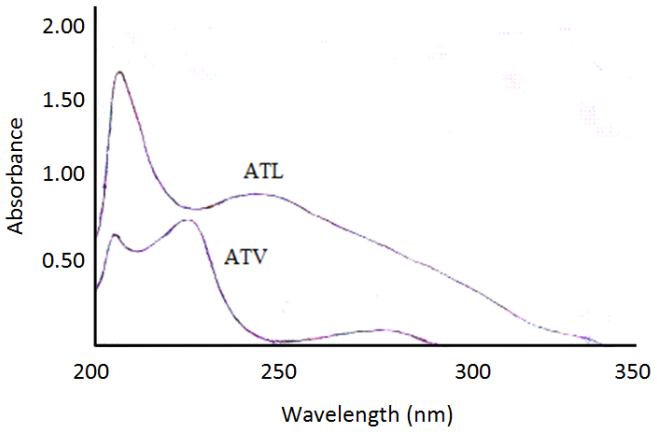

Fig.1. Overlain spectrum of atenolol (ATL) and atorvastatin (ATV) in aqueous methanol (50% v/v)

Table 1
Summary of linear regression and validation data

Parameters[a]	ATL	ATV
Wavelength (nm)	225.0	241.0
Linearity range (µg/mL)	5-30	2-12
Linear regression equation	Y=0.034X+0.0137	Y=0.0314X+0.0029
Slope ± SD	0.034±0.012	0.0314±0.0021
Intercept ± SD	0.0137±0.002	0.0029±0.0008
Correlation coefficient (r^2)	0.9994	0.9991
Limit of detection (LOD)	0.1765	0.0764
Limit of quantification (LOQ)	0.5823	0.2522
Repeatability (RSD)	0.730	0.725
Intra-day (RSD)	0.541	0.609
Inter-day (RSD)	0.267	0.990

[a] Represents average of six (n=6) estimations

Table 2
Recovery studies (Standard Addition Method)

Drug	Recovery level (%)	Initial amount (µg/mL)	Amount added (µg/mL)	% Recovery[a]	% RSD[a]
ATL	80	20	16	98.24±0.03	0.031
	100	20	20	98.15±0.01	0.010
	120	20	24	99.66±0.06	0.060
ATV	80	10	8	99.96±0.05	0.050
	100	10	10	100.96±0.02	0.019
	120	10	12	101.04±0.07	0.069

[a]Represents average of three (n=3) estimations at each level

3. RESULTS AND DISCUSSION

A UV-spectrophotometric, multicomponent mode of analysis, method was developed for the simultaneous estimation of ATL and ATV in tablet dosage form. Solvent used was 50% v/v aqueous methanol. The absorbance was recorded at 241 and 225 nm. The overlain spectrum was shown in Fig.1. Statistical evaluation of analytical data and recovery studies was performed to determine the suitability of the proposed method for the simultaneous estimation of ATL and ATV in tablet dosage form.

3.1 Method validation

3.1.1 Linearity

Absorbance was found to have better linear relationship with the concentration. For ATL, the r^2 was found to be 0.9994, and for the ATV the r^2 was 0.9991. Calibration graphs were constructed in the concentration range of 5-30 µg/mL for ATL and 2-12 µg/mL for ATV. The correlation coefficients, y-intercepts and slopes of the regression lines of the two drugs were calculated and are presented in Table 1.

3.1.2 Precision

Repeatability and intermediate precision of the developed method was expressed in terms of relative standard deviation (RSD) of the absorbance. The results showed that the repeatability, intra- and inter-day variation of the results at concentration of 20 µg/mL for ATL and 10 µg/mL for ATV were within the acceptable range. The coefficients of variation for both the inter-day and intra-day precision of the method was found was found to be less than 1% for both drugs (Table 1).

3.1.3 Accuracy/recovery studies

The recovery studies were carried out at 80%, 100% and 120% of the test concentration as per ICH guidelines. The percentage recovery of ATL and ATV at all the three levels was found to be satisfactory (Table 2). For ATL, the % recovery was found between 98.15% and 99.66% and for ATV between 99.96% and 101.04%, respectively.

3.1.4 Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection and quantification were found to be 0.1765 and 0.5823 µg/mL for ATL and 0.0764 and 0.2522 µg/mL, respectively, indicating the sensitivity of the developed method.

3.1.5 Robustness of the method

The robustness of the method evaluated by assessing the effect of variations in method parameters on absorbance showed low RSD values (less than 1.0%) indicating robustness of the method (Table 3).

Table 3
Robustness studies

Drug	Parameters		Target Conc. (µg/mL)	Mean Conc[a] (µg/mL)	SD[a]	%RSD[a]
ATL	Wavelength	227 nm	20	19.71	0.201	0.200
		225 nm		19.88	0.110	0.113
		223 nm		19.69	0.200	0.198
	pH	1.4		19.72	0.220	0.220
		1.2		19.77	0.150	0.151
		1.0		19.91	0.004	0.005
ATV	Wavelength	243 nm	10	10.09	0.005	0.005
		241 nm		10.03	0.001	0.002
		239 nm		9.90	0.004	0.004
	pH	1.4		10.09	0.005	0.006
		1.2		9.84	0.006	0.007
		1.0		9.64	0.018	0.020

[a]Represents average of three estimations (n=3) at each level

Table 4
Assay results of the tablet dosage form

Drug	Label claim	% Mean assay[a]	SD[a]	% RSD[a]
ATL	50 mg	100.10	0.359	0.368
ATV	10 mg	100.48	0.606	0.603

[a]Represents average of six (n=6) determination

3.2 Analysis of tablet formulation

The bi-layer tablets prepared in our laboratory using 50 mg of ATL and 10 mg ATV was analysed using the developed method. The content of ATL and ATV was calculated by comparing the absorbance of sample with that of the standard (Table 4). The low value of RSD indicates that the method is precise and accurate.

4. CONCLUSIONS

A concomitant drug treatment of ATL and ATV may require for the treatment of hypertension and hypercholesterolemia that is frequently coexists. As there are no methods for their simultaneous estimation, a suitable UV-spectrophotometric method was developed and validated for the simultaneous determination of ATL and ATV in co-formulations. The developed method was found to be simple, rapid, selective, sensitive and suitable for simultaneous estimation of ATL and ATV. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The newly developed method can be used for routine analysis as method for the simultaneous estimation of ATL and ATV in tablet dosage forms.

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