RESEARCH ARTICLE

Simultaneous Estimation of Amlodipine and Atorvastatin Using UV-Spectroscopy Method

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Received: 01st September, 2023; Revised: 07th December, 2023; Accepted: 04th May, 2024; Available Online: 25th June, 2024

ABSTRACT

The simple, precise and accurate UV spectroscopic method has been developed for simultaneous estimation of amlodipine besylate and atorvastatin calcium. The developed simultaneous equation method is reproducible and economical. The wavelengths selected for measuring the absorbance of both drugs were 365 and 246 nm, respectively. In a concentration range of 2 to 10 µg/mL, amlodipine besylate and atorvastatin calcium exhibited linearity at their respective λ_{max} of 365 and 246 nm as well as at the isoabsorptive point at 238 nm. Recovery studies showed recovery of >99.78% for amlodipine besylate and >99.36% for atorvastatin calcium, indicating method accuracy. The developed method is suggested for routine analysis because it is quick, easy, accurate, as well as sensitive and specific. The validation studies were carried out in accordance with the International Council of Harmonization (ICH) recommendations.

Keywords: Amlodipine besylate, Atorvastatin calcium, Simultaneous equation method, UV Spectroscopy.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.2.19

How to cite this article: Mali DP, Waghmare AV, Gaikwad DT. Simultaneous Estimation of Amlodipine and Atorvastatin Using UV-Spectroscopy Method. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):671-675.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Hypertension refers to elevated blood pressure that lasts for an extended period of time. While cardiac output is within the normal range, the majority of cases of hypertension (chronically raised blood pressure) are caused by increased arterial resistance. Some of the most potent antihypertensive medications actually work by reducing this resistance through vasodilatation.[1] One fundamental issue with treating hypertension is that, in 90% of cases, the cause of the chronically raised blood pressure is unknown, a condition known as primary or essential hypertension. Many of the interconnected regulation systems are changed, and lowering blood pressure frequently necessitates modifying two or more of these processes. Secondary hypertension is the term for hypertension that only occasionally results from a disease state. Naturally, treating the condition comes first, and doing so will typically reduce the secondary hypertension. In the absence of that, further direct involvement might be explored.[2]

2-[(2-aminoethoxy) methyl] amlodipine-4-(2-chlorophenyl)-1,4-dihydro-6-methyl acid carboxylate of 3,5-pyridine 3-ethyl 5-methyl ester) is a calcium antagonist that is derived from the dihydropyridine molecule. This medication is commonly used for treating chronic, stable angina and hypertension. Amlodipine, classified as class I in the Biopharmaceutics Classification System (BCS), is a calcium channel blocker belonging to the third generation of dihydropyridines. It is commonly employed in the treatment of hypertension, primarily acting by inducing relaxation of smooth muscle in blood vessels and subsequent dilation of the vasculature. The mechanism of action involves the inhibition of voltage-gated L-type calcium channels, so impeding the access of extracellular calcium into cardiac and vascular cells, which is responsible for the "slow" influx.[3] Amlodipine has a lower frequency of reflex tachycardia and additional adverse effects related to vasodilation compared to other derivatives of dihydropyridines. Additionally, prolonged duration of action and slow clearance shown by this drug enable the administration of a single daily dose. Amlodipine exhibits a delayed and near-complete absorption profile within the gastrointestinal tract.

As an HMG-CoA reductase inhibitor, the lipid-lowering medication atorvastatin calcium belongs to the BCS class II

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pharmacological category. Statins, including Atorvastatin calcium, are used to block the first and slowest stage in the cholesterol production process, the formation of mevalonate from HMG CoA. It raises HDL cholesterol and decreases LDL cholesterol and triglyceride levels.[4]

The dual action of amlodipine/atorvastatin comes from the calcium ion antagonist (slow-channel blocker) effect of amlodipine, whereas atorvastatin inhibits the HMG-CoA reductase. Amlodipine, one-half of amlodipine/Atorvastatin, reduces calcium influx into cardiac muscle and vascular smooth muscle by a process known as transmembrane influx inhibition. Amlodipine/Atorvastatin works by inhibiting the formation of mevalonate, a precursor of sterols like cholesterol, from HMG-CoA in a selective and competitive manner.[5]

Amlodipine besylate and Atorvastatin calcium are only available in fixed-dose combinations as pills in the market. As far as we are aware, there is no published simultaneous procedure for their determination. In this communication, we present a new, quick, accurate, and simple UV-spectrophotometric method for determining atorvastatin and amlodipine simultaneously.

MATERIALS AND METHODS

Instrument

A double-beam UV-visible spectrophotometer (Shimadzu-1800) and two matched quartz cells (1-cm) were utilized for recording the absorbance of the solution.

Materials

Amlodipine besylate and atorvastatin calcium were procured as gift samples from Unichem Pharmaceutical and Amoli Labs, respectively.

Solvents

Methanol was used as the solvent. Each and every one of the chemicals and reagents utilized were of analytical quality.

Determination of λ_{max} of Amlodipine Besylate Along With Atorvastatin Calcium

In order to identify the wavelength of maximum absorption for each drug, solutions comprising 10 µg/mL of amlodipine besylate and 10 µg/mL of atorvastatin calcium were appropriately diluted with methanol and then individually screened in the wavelength range of 200 to 400 nm. Amlodipine and atorvastatin both displayed absorbance maxima (Figures 1 and 2). Amlodipine shows at 365 and 238 nm, and Atorvastatin peak was at 246 nm. The overlaid spectra displayed the maximum concentrations of both medicines and iso-absorptive sites at 238 nm.[6,7]

Preparation of Standard Drug Solution

Accurately weighed 10 mg of atorvastatin calcium and amlodipine besylate were dissolved in 10 mL methanol in volumetric flasks to obtain a solution concentration as 1000 µg/mL. About 1-mL aliquot from this was diluted 10 mL with methanol in a volumetric flask to obtain

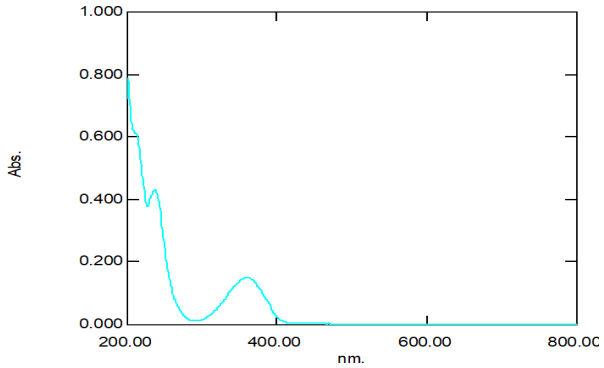

Figure 1: λ_{max} of amlodipine besylate

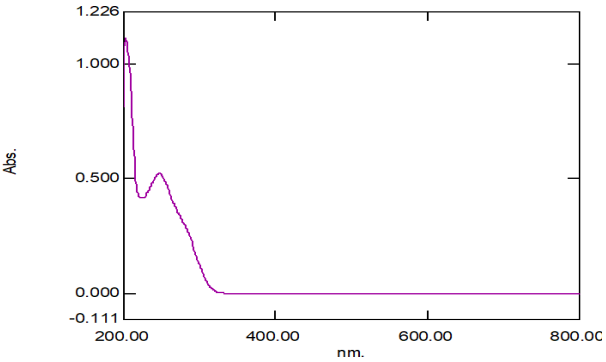

Figure 2: λ_{max} of atorvastatin calcium

100 µg/mL solution. From these, further dilutions were made so as to obtain solution ranges from 2 to 10 µg/mL.[8-9]

Preparation of Sample Solution

Withdraw 1-mL from the mid-stock solution of amlodipine besylate and atorvastatin calcium in 10 mL volumetric flask and measure the absorbance (Figure 3).[10-11]

Simultaneous Equation Method

The absorption maxima of amlodipine besylate and atorvastatin calcium in methanol were recorded and were found to be at wavelengths of 365 and 246 nm, respectively. These wavelengths were chosen for the simultaneous analysis. By using stock solutions of both drugs, the series of standard solutions having concentrations of 2 to 10 µg/mL were prepared individually using methanol as solvent. The absorbance at each concentration was recorded at the chosen wavelengths, and the absorptivities (A 1%, 1-cm) were calculated for both medicines by taking an average of triplicate determinations.

$$Cx=A_2(ay_1)-A_1(ay_2)/\ ax_2\ ay_1 - ax_1\ ay_2$$
$$Cy=A_2(ax_1)-A_1(ax_2)/\ ax_2\ ay_1 - ax_1\ ay_2$$

Where, A_1 and A_2 are absorbances of mixture at 365 and 246 nm, respectively, ax_1 and ax_2 are absorptivities of atorvastatin calcium at λ_1 and λ_2, respectively and ay_1 and ay_2 are absorptivities of amlodipine besylate at λ_1 and λ_2, respectively. Cx and Cy are concentrations of atorvastatin calcium and amlodipine besylate, respectively.[12]

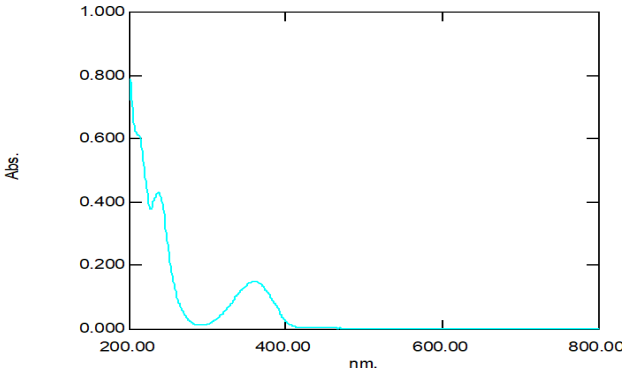

Figure 3: Combined UV spectra

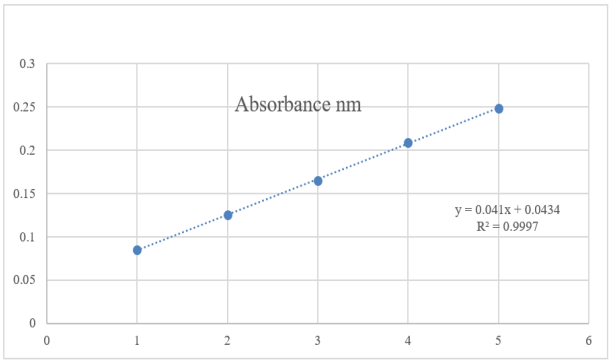

Figure 4: Calibration curve of amlodipine besylate

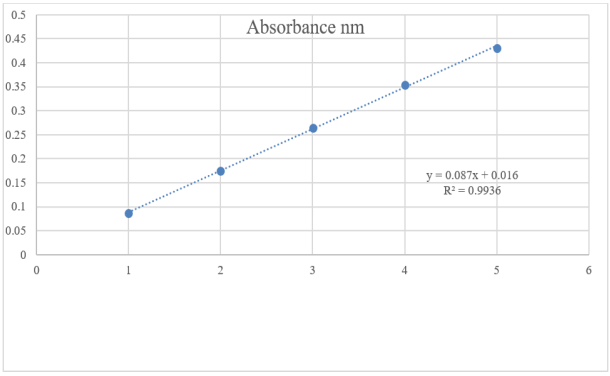

Figure 5: Calibration curve of atorvastatin calcium

RESULT AND DISCUSSION

Method Validation Parameters

Accuracy

Accuracy estimation was carried out by measuring atorvastatin calcium and amlodipine besylate recovery using the traditional addition method. 0.8, 1.0, and 1.2 mL of working standard solution (100 µg/mL) of each drug was added to 1-mL of the test's working sample solution (100 µg/mL of both) in methanol and subsequently diluted to 10 mL. The absorbance of the solution was determined for atorvastatin and amlodipine at a selected wavelength. Triplicate determinations were carried out.

Table 1: Percentage recovery of added substances

Name of drug	*%*	*%recovery of added substances**	*%RSD*
Amlodipine	80	102.23 ± 1.47	1.065
	100	100.56 ± 0.65	
	120	99.78 ± 0.06	
Atorvastatin	80	101.98 ± 0.98	1.208
	100	100.48 ± 0.08	
	120	99.36 ± 0.5	

**Values expressed Mean ± SD, (n = 3)*

Table 2: %Purity of drug of intra-day precision

Name of drug	*Concentration (µg/mL)*	*%Purity of drug*	*%RSD*
Amlodipine	4	99.65 ± 0.47	1.065
	6	101.74 ± 0.52	
	8	98.71 ± 0.08	
Atorvastatin	4	99.78 ± 0.95	1.208
	6	100.87 ± 0.06	
	8	99.58 ± 0.54	

Table 3: %Purity of drug of inter-day precision

Name of drug	*Concentration (µg/mL)*	*%Purity of drug*	*% RSD*
Amlodipine	4	98.54 ± 0.25	0.807
	6	100.25 ± 0.12	
	8	98.561 ± 0.12	
Atorvastatin	4	99.01 ± 0.98	0.351
	6	99.24 ± 0.04	
	8	98.40 ± 0.43	

The simultaneous equation methodology, the absorbance correction method, and the percentage recoveries were used to calculate the dosage of amlodipine and atorvastatin at each level (Table 1).[13]

Precision

The level of repeatability of the procedure was determined using an appropriate statistical analysis. The concentration of both drugs was examined three times on a single day for an intra-day investigation and also for inter-day. The standard deviation (SD) as well as the relative standard deviation (RSD) were estimated. The results are shown in the Tables 2 and 3.[14]

Linearity

In order to build calibration curves and find the regression equations, the concentrations of amlodipine besylate and atorvastatin calcium at their respective absorption maxima were plotted against absorbance (Table 4). The calibration curves for amlodipine and atorvastatin drugs were plotted for the solutions of the concentration range 2 to 10 µg/mL individually (Figures 4, and 5) as well as in combined form (Figure 6).[15]

Limit of detection (LoD) and limit of quantitation (LoQ)

For an analytical method, LoD is the concentration at which an instrument signal (signal-to-noise ratio of 3) differs

Table 4: Linearity of drugs

Parameters	*Simultaneous equation method (Amlodipine besylate)*	*Simultaneous equation method (Atorvastatin calcium)*	*Simultaneous equation method (Combined)*
λ_{max} (nm)	365	246	238
Beers law limit (µg/mL)	2–10	2–10	2–10
Correlation coefficient	0.9997	0.9936	0.9998
Slope	0.041	0.087	0.2839
Intercept	0.0434	0.016	0.4885
Regression equation	y = 0.041x + 0.0434 R^2 = 0.9997	y = 0.087x + 0.016 R^2 = 0.9936	y = 0.2839x - 0.4885 R^2 = 0.9998

Table 5: LoD and LoQ of drugs

Drug	*LoD*	*LoQ*
Amlodipine besylate	0.145	0.152
Atorvastatin calcium	0.096	0.124

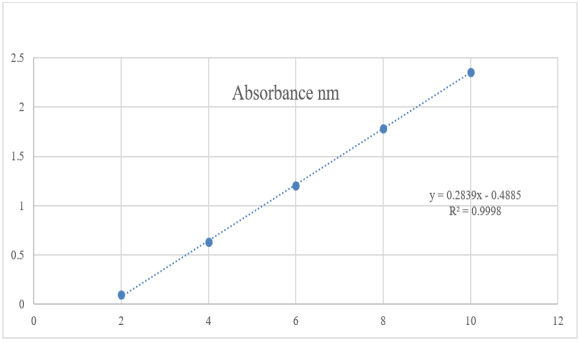

Figure 6: Calibration curve of combined drugs

substantially from the blank (contains a concentration). The LoQ is the concentration that can be accurately and precisely quantifiable reliably with a specific level of signal-to-noise ratio (SNR) (10).

LoD and LoQ were established based on experimental standard deviation and slope (Table 5).[16]

LoD = 3.3 * (σ / S) and

LoQ = 10 * (σ / S)

Where:

σ is the standard deviation

S is the slope of the calibration curve.

Application of developed UV- spectroscopy method

The developed UV- spectroscopy method for simultaneous estimation of amlodipine besylate and atorvastatin calcium can be used to find out the drug content uniformity of drugs and also for the percentage cumulative drug release.[17]

CONCLUSION

The technique was successful in estimating the concentrations of amlodipine besylate and atorvastatin calcium in a synthetic combination that contained 2.5 mg of amlodipine besylate and 10 mg of atorvastatin calcium. The quantity of both drugs was directly calculated using the method's equations. Standard deviations and the coefficient of variation values were calculated. The lower values of standard deviation indicated the methods' repeatability, accuracy, and reproducibility. Recovery experiments also supported reproducibility, dependability, and interference. Thus, it can be said that the created method was straightforward, precise, sensitive, and accurate. The results obtained after examination of pharmaceutical formulations show that the suggested method is suitable for the simultaneous determination of both substances with almost little interference from common additives used in pharmaceutical formulations. As a result, the method listed above can be used to estimate atorvastatin calcium and amlodipine besylate simultaneously in commercial finished products.

ACKNOWLEDGMENT

The authors are thankful to Principal Dr. H. N. More, Bharati Vidyapeeth College of Pharmacy, Kolhapur, for providing the necessary facilities for the research work.

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