Research Article

Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Atorvastatin Calcium and Felodipine from Tablet Dosage Form

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Atorvastatin calcium (ATR) and felodipine (FEL) are beneficial in combination for elderly people in management of hypertension and atherosclerosis. Aim of present study is to develop simple, accurate, and precise method for simultaneous quantitative estimation of ATR and FEL from combined tablet dosage form. Method involves simultaneous equation, using acetonitrile—double distilled water (70 : 30)—common solvent showing absorption maxima at 245 and 268 nm. Calibration curves determination for both drugs has been carried out in 0.1 N HCl, phosphate buffer pH 6.8, and acetonitrile (ACN)—water (70 : 30 V/V). Linearity range was observed in the concentration range of 2 to 12 $\mu\text{g/mL}$ for FEL and 20 to 100 $\mu\text{g/mL}$ for ATR. Percent concentration estimated for ATR and FEL was 100.12 ± 1.03 and 99.98 ± 0.98 , respectively. The method was found to be simple, economical, accurate and precise and can be used for quantitative estimation of ATR and FEL.

1. Introduction

Atorvastatin (ATR) is chemically described as [R-(R*, R*)]-2-(4-fluorophenyl)-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid (Figure 1). It is a member of the drug class known as statins, used for lowering blood cholesterol [1]. It also stabilizes plaque and prevents strokes through anti-inflammation and other mechanisms. It inhibits HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. Inhibition of this enzyme stops the reduction of HMG-CoA to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases cholesterol synthesis and ultimately increases expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes [2, 3].

Felodipine (FEL) is a 1, 4 dihydropyridine derivative, that is, chemically described as ethyl methyl-1,4-dihydro-2,6-dimethyl-4-(2,3 dichlorophenyl)-3,5-pyridinedicarboxylate. It is a dihydropyridine calcium channel blocker used mainly

for the management of hypertension and angina pectoris like the other calcium channel blockers [4].

Literature survey reveals that spectrophotometric and chromatographic methods, and a stability-indicating LC method, have been reported for determination of ATR in pharmaceutical preparations in combination with other drugs [5–13]. Several chromatographic and spectrophotometric methods have been reported for felodipine assay [14–18]. However, most of the analytical methods developed for the quantization of ATR and FEL involve analysis of single component or combination with other drugs. No effective method has been reported for quantitative estimation of ATR and FEL from combined dosage form.

2. Material and Methods

Atorvastatin calcium and felodipine were kindly gifted by Cipla Ltd., Goa, India. Acetonitrile (Loba Chemie Pvt. Ltd., Mumbai, India) and other chemicals used are of analytical grade. Distilled water was prepared in laboratory.

TABLE 1: Absorbance values for calibration curves of FEL and ATR at 268 and 245 nm.

Concentration ($\mu\text{g/mL}$)	FEL		Concentration ($\mu\text{g/mL}$)	ATR	
	268 nm	245 nm		268 nm	245 nm
20	0.17069	0.05085	2	0.12878	0.02782
40	0.33971	0.10072	4	0.24756	0.05264
60	0.52023	0.15256	6	0.37096	0.08296
80	0.68275	0.21401	8	0.50512	0.11161
100	0.84833	0.25425	10	0.62368	0.13909
—	—	—	12	0.76792	0.16704

FIGURE 1: Structure of (I) atorvastatin, (II) felodipine.

2.1. Preparation of Bilayered Tablet. Bilayered tablets of total weight of 300 mg each, containing 150 mg immediate release layer of ATR (10 mg API) and 150 mg of sustained release layer of FEL (10 mg API), were prepared by initially adding FEL granules to die of RIMEK minipress (Karnavati engineering, Gujarat, India) and compressed, above which ATR blend was poured and allowed to undergo for the final compression to prepare the bilayered tablet using 8 mm flat faced punches.

2.2. Standard Stock Solution

2.2.1. In 0.1N HCl. Standard stock solution was prepared by dissolving 10 mg of ATR and 10 mg of FEL separately in 100 mL of volumetric flask containing 10 mL of 0.1 N HCl. Then, the final volume of the solution was made up to 100 mL with 0.1 N HCl to get stock solution of 100 $\mu\text{g/mL}$. Adequate quantities were sampled out from the standard stock solution in 10 mL volumetric flask to get concentration of 10, 20, 30, 40, 50, and 60 $\mu\text{g/mL}$ ATR and 2, 4, 6, 8, 10, and 12 $\mu\text{g/mL}$ FEL. Then, the absorbances of the solution was measured at 268 nm (λ max of FEL) and 245 nm (λ max of ATR) using double beam UV visible spectrophotometer against 0.1 N HCl as blank.

2.2.2. In Phosphate Buffer pH 6.8. All the above procedure was repeated using phosphate buffer pH 6.8 instead of 0.1 N HCl. Calibration curve of ATR and FEL in 0.1 N HCl and phosphate buffer (PB) pH 6.8 are shown in Figures 2 and 3, respectively. Absorbance values are shown in Table 1.

FIGURE 2: Calibration curve of ATR in 0.1 N HCl and phosphate buffer (PB) pH 6.8.

2.3. UV Method Development and Optimization

2.3.1. Selection of Common Solvent. ACN—double distilled water (70 : 30% V/V)—was selected as common solvent for developing spectral characteristics of drugs. The selection was made after assessing the solubility of both drugs in different solvents.

2.3.2. Preparation of Standard Drug Solution

(1) ATR Standard Stock Solution (100 $\mu\text{g/mL}$). Accurately weighed quantity of Atorvastatin calcium (10 mg) was transferred into 100 mL volumetric flask dissolved in 60 mL of

FIGURE 3: Calibration curve of FEL in 0.1N HCl and phosphate buffer (PB) pH 6.8.

ACN—water (70 : 30% V/V)—and diluted up to mark with same solvent. This will give a stock solution having strength of 100 µg/mL.

(2) *FEL Standard Stock Solution (100 µg/mL)*. Accurately weighed quantity of felodipine (10 mg) was transferred into 100 mL volumetric flask dissolved in 60 mL of ACN—water (70 : 30% V/V)—and diluted up to mark with same solvent. This will give a stock solution having strength of 100 µg/mL.

2.3.3. Construction of Calibration Curve

(1) *Calibration Curve for ATR*. Different aliquots were withdrawn from the standard stock solution and diluted with appropriate quantity of ACN—water (70 : 30% V/V)—to get a series of concentration ranging from 20 to 100 µg/mL. Absorbance was measured at different concentrations and the calibration curve was prepared by plotting absorbance versus concentration.

(2) *Calibration Curve for FEL*. Different aliquots were withdrawn from the standard stock solution and diluted with appropriate quantity of ACN—water (70 : 30% V/V)—to get a series of concentration ranging from 2 to 12 µg/mL. Absorbance was measured at different concentrations and the calibration curve was prepared by plotting absorbance versus concentration.

2.3.4. *Selection of Wavelength*. By appropriate dilutions of two standard drug solutions with ACN—double distilled water (70 : 30% V/V)—solutions containing 10 µg/mL of ATR and 10 µg/mL of FEL were scanned separately in the range of 200–400 nm to determine the wavelength of maximum absorption for both drugs (Figure 4).

2.3.5. *Selection of Method and Wavelength*. For quantitative estimation of ATR and FEL, simultaneous equation method

employing 245 nm and 268 nm as analytical wavelength was used. The two wavelengths were chosen from the overlain spectra of ATR and FEL. Overlain spectra of ATR and FEL are shown in Figure 5.

2.4. *Procedure for Calculating Absorptivity of Both the Drugs at Selected Wavelengths*. From standard drug solutions, six works in standard solutions with concentration of 20, 40, 60, 80, and 100 µg/mL for ATR and 2, 4, 6, 8, 10, and 12 µg/mL for FEL were prepared and scanned separately on the selected wavelengths for both the drugs. The absorptivity at selected wavelength was calculated.

2.5. *Analysis of Tablet Formulation*. Twenty tablets were powdered. Tablet formulation containing ATR 10 mg and FEL 10 mg was analyzed using this method. From the triturates of 3 tablets, an amount equivalent to 10 mg of ATR and 10 mg of FEL was weighed and dissolved in 10 mL of ACN—water (70 : 30% V/V)—and sonicated for 10 min. Then, the solution was filtered through Whatman filter paper number 41 and then final volume of the solution was made up to 100 mL with ACN—double distilled water (70 : 30% V/V)—to get a stock solution containing 100 µg/mL of ATR and 100 µg/mL FEL.

Appropriate aliquots of ATR and FEL within Beer's law limit were taken and analyzed by the proposed method using the procedure described earlier. The concentration of ATR and FEL present in the sample solution was calculated by using the simultaneous equation,

$$\begin{aligned} C_y &= \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}, \\ C_x &= \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}, \end{aligned} \quad (1)$$

where C_y is the concentration of ATR in gm/lit, C_x is the concentration of FEL in gm/lit, A_1 is the absorbance of sample solution at 268 nm, A_2 is the absorbance of sample solution at 245 nm, a_{x1} is the absorptivity of FEL at 268 nm, a_{y1} is the absorptivity of ATR at 268 nm, a_{x2} is the absorptivity of FEL at 245 nm, and a_{y2} is the absorptivity of ATR at 245 nm.

2.6. Method Validation

2.6.1. *Linearity*. In quantitative analysis, the calibration curve was constructed for both ATR and FEL after analysis of consecutively increased concentrations.

2.6.2. *Recovery Studies*. Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet samples within the analytical concentration range of the proposed method at three different sets at levels of 80, 100, and 120%. The added quantities of the individual drugs were estimated by above method. Intraday precision and interday precision have also been carried out.

TABLE 2: Absorptivity values for ATR and FEL.

Concentration ($\mu\text{g/mL}$)	Absorptivity of FEL		Concentration ($\mu\text{g/mL}$)	Absorptivity of ATR	
	268 nm	245 nm		268 nm	245 nm
2	0.00603	0.001399	20	0.08533	0.0254
4	0.00618	0.00148	40	0.08539	0.0266
6	0.00638	0.001462	60	0.08548	0.0282
8	0.00654	0.001495	80	0.08562	0.0299
10	0.00673	0.001516	100	0.08586	0.0331
12	0.00698	0.001543	—	—	—
Mean	0.00759	0.001465	Mean	0.08553	0.0286
S.D.	0.44	1.03	S.D.	1.39	0.95

(a)

(b)

FIGURE 4: UV spectra of (a) ATR, (b) FEL in ACN: water (70 : 30% V/V).

FIGURE 5: Overlain spectra of ATR and FEL.

2.6.3. Interday Precision. Analysis of drug was performed on two different days and the deviation in the results was observed. Results are shown in Table 4.

2.6.4. Intraday Precision. Analysis of drug was performed on the same day in morning and evening, and the deviation in the results was observed. Results are shown in Table 5.

2.6.5. Ruggedness. Ruggedness of the method was confirmed by the analysis of formulation that was done by the different analysts, using similar operational and environmental conditions.

3. Result and Discussion

Calibration curve of ATR and FEL was plotted by measuring the absorbance of prepared dilutions of the aforesaid different

TABLE 3: Results of analysis of laboratory samples.

Label claim (mg/tab)	% concentration estimated*
ATR	100.12 $\pm$ 1.03
FEL	99.98 $\pm$ 0.98

* Indicates $\pm$ SD ($n = 9$).

concentrations at their respective wavelength of maximum absorbance (Figures 6 and 7).

3.1. Linearity. Linear regression data showed a good linear relationship over a concentration range of 2 to 12 $\mu\text{g/mL}$ for FEL and 20 to 100 $\mu\text{g/mL}$ for ATR, whereas, Rajesh et al. demonstrated that linearity was within the range of 2–10 $\mu\text{g/mL}$ for each atorvastatin calcium and felodipine [19]. Six point regression data at both wavelengths (268 nm 245 nm) were generated for FEL (Table 2).

3.2. Tablet Analysis. See Table 3.

3.3. Recovery Study. The added quantities of added drug were estimated by simultaneous equation (Table 4).

3.4. Interday Precision. Interday precision study was performed and method was found to be precise.

3.5. Intraday Precision. Intraday precision study was performed and method was found to be precise. Recoveries obtained for the two drugs do not differ significantly from 100%, which showed that there was no interference from

TABLE 4: Result of recovery studies and interday precision.

Day	Label claim (mg/tablet)	Amount added (%)	Total amount added (mg)	Concentration recovered* (mean ± SD)	% recovery estimated* (mean ± %SD)
Day 1	ATR 10	80	8	8.02 ± 0.34	100.22 ± 1.12
		100	10	10.90 ± 0.78	98.10 ± 0.98
		120	12	12.23 ± 0.89	103.91 ± 1.23
	FEL 10	80	8	8.17 ± 1.45	101.70 ± 1.18
		100	10	10.71 ± 1.78	101.68 ± 1.24
		120	12	12.99 ± 1.25	99.93 ± 0.98
Day 2	ATR 10	80	8	8.04 ± 1.11	101.11 ± 1.25
		100	10	10.08 ± 1.45	101.60 ± 1.17
		120	12	12.03 ± 0.34	100.64 ± 1.11
	FEL 10	80	8	8.17 ± 1.71	101.70 ± 0.65
		100	10	10.22 ± 1.12	97.76 ± 0.34
		120	12	12.54 ± 0.34	103.60 ± 0.32

*Indicates ± SD (n = 3).

TABLE 5: Result of intraday precision.

Day 1	Label claim (mg/tablet)	Amount added (%)	Total amount added (mg)	Concentration recovered* (mean ± SD)	% recovery estimated* (mean ± %SD)
Morning	ATR 10	80	8	8.04 ± 0.30	100.22 ± 1.12
		100	10	10.10 ± 0.34	97.10 ± 0.57
		120	12	12.13 ± 0.44	102.79 ± 1.52
	FEL 10	80	8	8.27 ± 1.47	101.80 ± 1.74
		100	10	10.56 ± 1.78	101.32 ± 1.04
		120	12	12.90 ± 1.28	99.99 ± 0.91
Evening	ATR 10	80	8	8.08 ± 1.18	101.64 ± 1.17
		100	10	10.04 ± 1.55	101.40 ± 1.87
		120	12	12.05 ± 0.28	101.55 ± 1.13
	FEL 10	80	8	8.11 ± 1.54	101.28 ± 0.45
		100	10	10.14 ± 1.17	99.68 ± 0.56
		120	12	12.99 ± 1.98	99.46 ± 0.98

*Indicates ± SD (n = 3).

FIGURE 6: Calibration curve of ATR in ACN—water (70 : 30 v/v)—at 268 and 245 nm.

common excipients used in the formulation indicating accuracy and reliability of the method (Table 5).

FIGURE 7: Calibration curve of FEL in ACN—water (70 : 30 v/v)—at 268 and 245 nm.

3.6. *Ruggedness.* Ruggedness of the method was tested using different chemical sources of acetonitrile and effects on

TABLE 6: Ruggedness of the method.

	Label claim (mg/tablet)	% recovery estimated* (mean $\pm$ %SD)	
		ATR	FEL
Source I	100/10	102.74 $\pm$ 0.35	101.18 $\pm$ 0.31
Source II	100/10	103.12 $\pm$ 0.35	103.18 $\pm$ 0.62

*Indicates $\pm$ SD ($n = 3$).

results were observed and shown in Table 6. From the result, it was found that method has ruggedness.

4. Conclusion

In present study, from the observation of the validation parameters, it can be concluded that the developed method is simple, accurate, reliable, and economical for the simultaneous quantitative estimation of atorvastatin calcium and felodipine from combined dosage form using UV spectrophotometric method.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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