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Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril from Tablet Dosage Form

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ABSTRACT

Atorvastatin Calcium (ATR) and Ramipril (RMP) are the is an ACE inhibitor type medication used to treat high blood pressure, heart failure, and diabetic kidney disease. A rapid, specific and economic UV spectrophotometric method has been developed using Methanol as a solvent for simultaneous determination of Atorvastatin Calcium (ATR) and Ramipril (RMP) content in bulk and pharmaceutical tablet dosage formulations. The absorbance values at 246 nm and 226 nm were used for the estimation of Atorvastatin Calcium (ATR) and Ramipril (RMP). The absorption maxima of Atorvastatin Calcium (ATR) and Ramipril (RMP) shown at 246 nm and 226 nm and methanol as used as solvent. This method obeyed Beer's law in the concentration range of 2–20 µg /ml for atorvastatin calcium and 1-6 µg /ml for ramipril. The Simultaneous Estimation method was developed and validated according to ICH guidelines for linearity, precision, accuracy, LOD and LOQ. Atorvastatin calcium found to be linear within concentration range of 2-20 µg/ml with regression coefficient of 0.998 and ramipril found to be linear within concentration range of 1-6 µg/ml with regression coefficient of 0.999. The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 8, 10 and 12 µg/ml of RMP and ATR. The average % recoveries for three different concentrations were found to be 90.14 %for ATR and RMP 99.05 % using proposed UV spectrophotometric method. The limit of detection and limit of quantification were found to be 0.31µg/ml and 0.1023µg/ml for Atorvastatin Calcium and 0.2805µg/ml and 0.85µg/ml for Ramipril respectively by proposed UV spectrophotometric method.The results of validation parameters indicates that the developed method was also found to be accurate, precise and sensitive and such simple & economic method can be used for the simultaneous estimation of atorvastatin calcium and ramipril. The obtained results proved that the method can be employed for the routine analysis of simvastatin and Ezetimibe in bulks as well as in the commercial formulation.

Keywords: Atorvastatin Calcium, Ramipril, Methanol, Method development, Simultaneous Estimation, UV spectrophotometry, ICH guidelines.

1.INTRODUCTION

Most of the pharmaceutical industries, are manufacturing multiple drug formulation to meet the market demand. It is a well known fact that a combination of drug has a wider range to treat ailment as compared to a single drug components. There are many method reported for simultaneous analysis of drug component of multiple component formulation. Almost all pharmacopoeial methods available for the analysis of such formulation are applicable only after prior separation of drug components. hence making them tedious and time consumimg. There is likely to be loss of accuracy and precision due to extraction and/or separation. In the pharmaceutical field, for assurance of the quality of drug formulation, it becomes necessary to develop analytical method which should have accuracy and precision. The accuracy and precision depend upon the relative and absolute errors. Errors will be less, if the method is simple. The method can be directly related to accuracy and precision. Therefore simplicity of method should be one of the prime considerations while developing the method of analysis. The instrument technique that can be utilized for analysis is uv-visible spectrometry. The aim of this research work is to develop a simple analytical method to solve the prolem of interference of additives or drugs in the analysis of individual drug without separating the drug in combination. An attempt is being made to develop simple, precise and accurate method for estimation of atorastin and ramipril in its pharmaceutical formulations. It was found that though individually these drugs have been analysed by many methods, no method has been reported for analysis of these drugs in combined dosage form. **Ramipril**, sold under the brand name **Altace** among others, is a medication used to treat high blood pressure, heart failure, also used to prevent cardiovascular disease in those at high risk. It is a reasonable initial treatment forhigh blood pressure. It is taken by mouth. Common side effects include headaches, dizziness, feeling tired, and cough. Serious side effects may include liver problems, angioedema, kidney problems, and high blood potassium. Use in pregnancy and breastfeeding is not recommended. It is an ace inhibitor and works by decreasing renin-angiotensin-aldosterone system activity. **Atorvastatin**, sold under the brand name **Lipitor** among others, is a statin medication used to prevent cardiovascular disease in those at high risk and treat abnormal lipid levels. For the prevention of cardiovascular disease, statins are a first-line treatment. It is taken by mouth.

The main objective of the study is to develop a rapid, specific and economic UV spectrophotometric method by using Methanol as a solvent for simultaneous determination of Atorvastatin Calcium and Ramipril content in bulk and pharmaceutical dosage formulations.

1.1 .MATERIALS AND METHOD

MATERIALS

The drug samples, Atorvastatin Calcium and Ramipril working standards were obtained as gift sample by Cipla Pvt. Ltd, Indore (MP) India. Stator- R 10 mg marketed tablets manufactured by Piramal Healthcare was procured from local market. Methanol, and water used were analytical grade and were purchased from Merck Specialties Private Limited, Mumbai, India.

INSTRUMENTATION:

Variable wavelength programmable UV detector UV1800 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L), Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic chemical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

2. EXPERIEMENTALS

2.1 PREPARATION OF STANDARED STOCK SOLUTION:

A. Preparation of standard stock solutions

Accurately weighed (10 mg) each of standard Atorvastatin Calcium (10 mg) and Ramipril (10 mg) were transferred to two separate 100 mL calibrated volumetric flasks (100 mL) dissolved in methanol which were further diluted with the methanol to obtain standard solutions of Atorvastatin Calcium and Ramipril (100 µg/mL).

B. Selection of working wavelength

Working standard stock solutions of both the drugs were diluted to obtain final concentration of Atorvastatin Calcium (10 µg/mL) and Ramipril (10 µg/mL). Solutions were scanned in the wavelength range of 200 - 400 nm. From the overlay spectra (Fig. 4.5) of the selected drugsin wavelength range of 210 -236, 237-246 nm were selected for the analysis. The overlayspectra of Atorvastatin Calcium and Ramipril are shows in figure 1.

2.2. DETECTION OF WAVELENGTH

Identification of the drug The UV spectra of Atorvastatin calcium and ramipril solution were taken individually and scanned in the range of 200-400 nm by UV Spectrophotometric method using Shimadzu Spectrophotometer UV-1800 (Shimadzu Corp. Japan). 10 mg of drug sample was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in sufficient amount of methanol and volume was made upto 100 ml with methanol. Exactly 10ml of the stock solution was pipetted out and was diluted to 100 ml with methanol (10 µg/ml). The spectrum was recorded in the range of 200-400 nm. The UV spectrum of Atorvastatin Calcium and Ramipril are shown in figure 2 and 3.

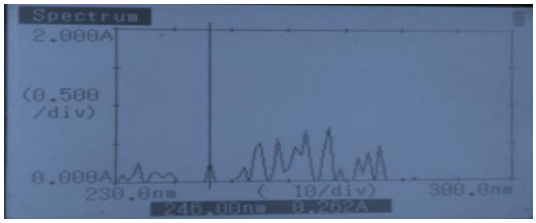

Figure 2. UV Spectra of Atorvastatin Calcium

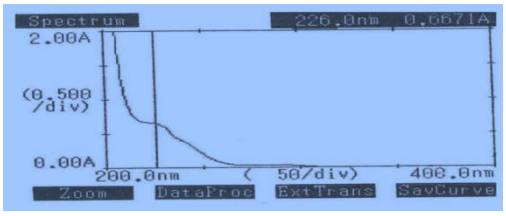

Figure 3. UV Spectra of Ramipril

2.3 CALIBRATION CURVE

Calibration curve of Atorvastatin Calcium and Ramipril was prepared in concentration range of 2-20 µg/ml for Atorvastatin Calcium and 1-6 µg/ml Ramipril with Methanol. The absorbance of each solution was measured at the wavelengths 246 nm and 226 nm. The absorbance values (mean of five determinations) with their standard deviation at different concentration in the range of 1-6 µg/ml and 2-20 µg/ml are tabulated. The drug obeys Beer's Lambert law in the concentration range. Linear regression analysis for all calibration curves of Atorvastatin Calcium and Ramiptil are given in Table 4.

Table 4 Data of standard calibration curve of Atorvastatin Calcium

S.No.	Concentration (µg/ml)	Absorbance (nm)
1.	0	0
2.	2	0.088
3.	4	0.16
4.	8	0.29
5.	12	0.419
6.	16	0.535
7.	20	0.645

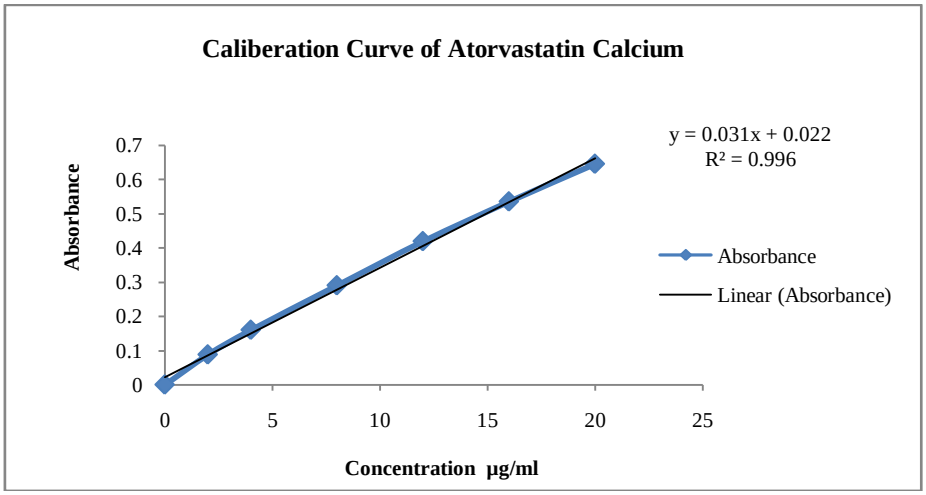

Figure 5 Calibration Curve of Atorvastatin Calcium

Table 6 Data of standard calibration curve of Ramipril

S.No.	Concentration (µg/ml)	Absorbance (nm)
1.	0	0
2.	1	0.102
3.	2	0.187
4.	3	0.276
5.	4	0.361
6.	5	0.441
7.	6	0.515

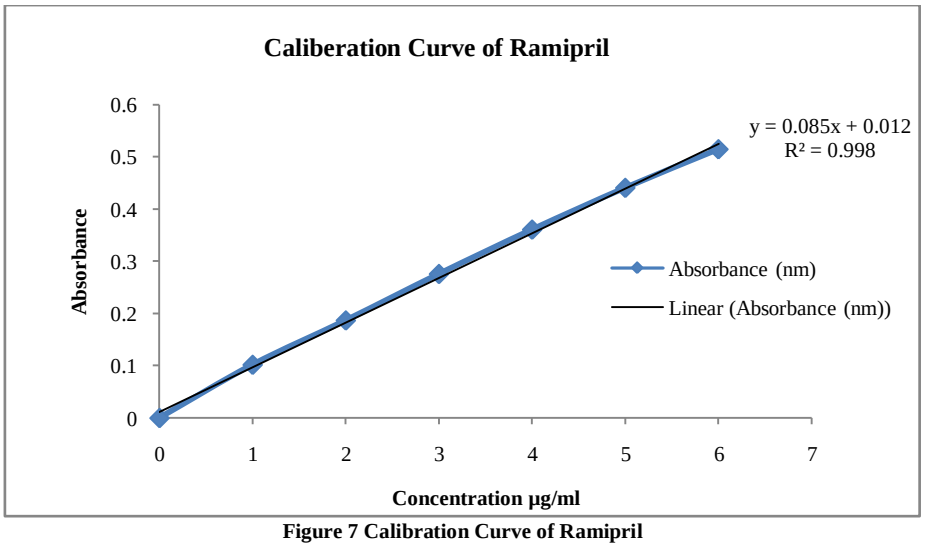

Figure 7 Calibration Curve of Ramipril

3. METHOD DEVELOPEMENT

SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM AND RAMIPRIL BY AREAUNDER CURVE METHOD

Area under curve method

In the simultaneous equation using AUC method, the area under curves of the recorded spectrums were measured at the selected wavelength ranges, 210- 236 nm and 237- 246 nm and calibration curves were plotted by taking concentration on x axis and AUC at 210- 236 nm or 237- 246 nm on Y-axis and the regression analysis of calibration curves and absorptivity values (X) of both these drugs are presented in Table 4.7 and Table 4.8. The 'X' values were determined as, X= Area under curve of component (210- 236 nm or 237- 246 nm)/concentration of the component in µg/ml. A set of two simultaneous equations framed using these 'X' values as follows,

$A1 = 0.085\ C_{RMP} + 0.012\ C_{ATR}$ ---------- (at λ 210- 236.0 nm) ----------(1)

$A2 = 0.031\ C_{RMP} + 0.022\ C_{ATR}$ ---------- (at 237- 246. 0 nm) ----------(2)

Where, C_{RMP} and C_{ATR} are the concentrations of RMP and ATR measured in 1-6 µg/ml and 2-20 µg/ml, in the sample solutions. A1 and A2 are the area under curve of sample solutions at the wavelength range 210 to 236 nm and 237 to 246.0 nm respectively.

Selection of working wavelength

From the overlay spectra (Fig. 6.2.1) of the selected drugs in wavelength range of 210 - 236, 237-246 nm were selected for the analysis. The obtained result is similar as given inthe references[68]. The overlay spectra of ATR and RMP are shows in figure 8.

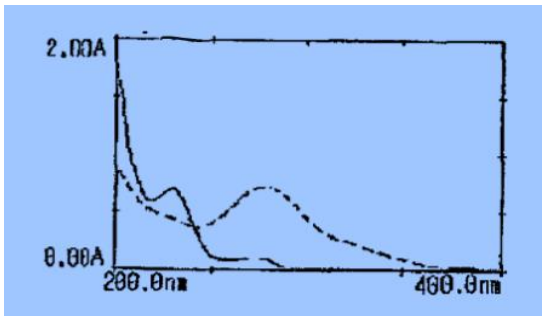

Figure 8 Overlay Spectra of Atorvastatin Calcium and Ramipril

4. VALIDATION

Validation of the developed method was done according to the USP 2006, Asian edition.

4.1 Linearity

Linearity was evaluated by analysis of working standard solution of Atorvastatin Calcium and Ramipril at six different concentrations. RMP found to be linear within conc. range of 1-6 µg/ml with regression coefficient of 0.998 and ATR was found to be linear within conc. range of 2-20 µg/ml with regression coefficient of 0.999 the results of regression analysis are summarized in (Table 9). Results show that within the concentration range mentioned above, there was an excellent correlation between absorbance and concentration of Atorvastatin Calcium and Ramipril (Figure 10 and 11).

Table 9: Regression analysis of calibration graphs of Atorvastatin Calcium and Ramipril for proposed UV Spectrophotometric method

Sr. No.	Parameters	Results (Atorvastatin Calcium)	Results(Ramipril)
1.	Absorption maxima	246 nm	226 nm
2.	Beer's range	2-20 µg/ml	1-6 µg/ml
3.	Regression equation	y = 0.032x + 0.012	y = 0.086x + 0.010
4.	Correlation coefficient	0.999	0.998
5.	Slope	0.032	0.086
6.	Intercept	0.012	0.010

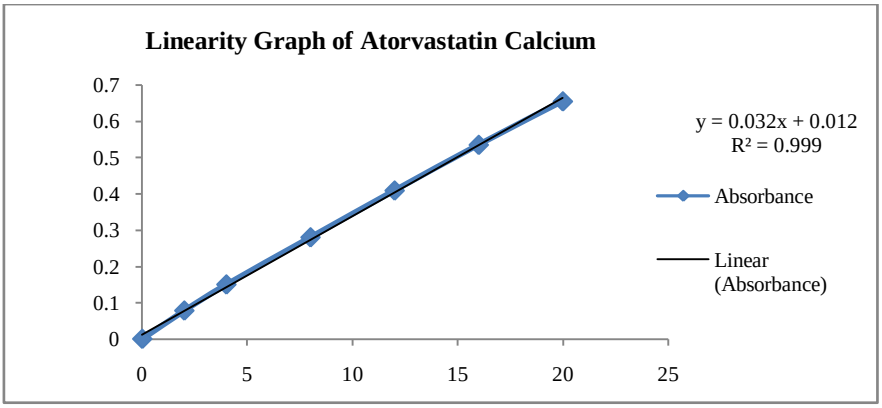

Figure 10 Regression (Linearity) analysis for Atorvastatin Calcium

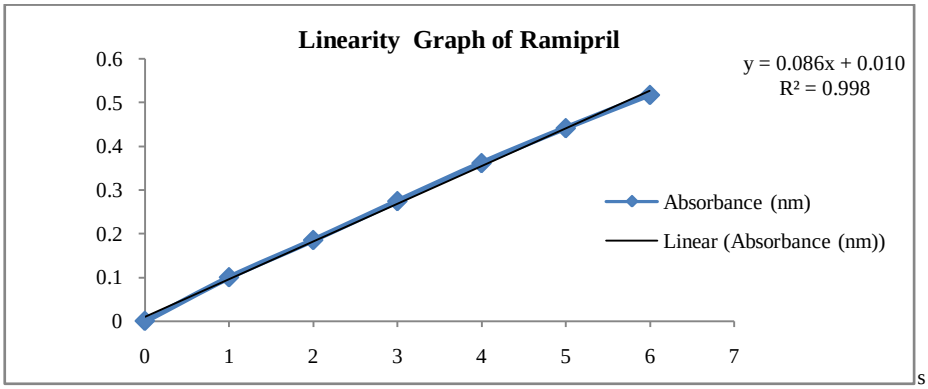

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Figure 11 Regression (Linearity) analysis for Ramipril

4.2 Precision

The repeatability is expressed as percentage relative standard deviations (% RSD) for the ATR at the concentration of 2, 4 and 8 µg/ml and their average % RSD value were0.350, 0.168 and 0.286 while for the RMP the concentration of 1,2 and 3 µg/ml and their average % RSD value were 0.387, 0.170 and 0.282.

Table 12 Result of Intraday (Repeatability) Precision studies

S.no	Parameters	% Amt. found (Atorvastatin Calcium)			% Amt. found (Ramipril)		
		2 µg/ml	4 µg/ml	8µg/ml	1µg/ml	2 µg/ml	3 µg/ml
1.	Morning	99.89	99.65	99.48	99.93	99.56	99.35
2.	Afternoon	99.69	99.69	100.05	99.66	99.97	99.98
3.	Evening	99.21	99.21	99.72	99.28	99.22	99.78
4.	Mean	99.59	99.79	99.75	99.48	99.84	99.76
5.	S.D.	0.349	0.168	0.286	0.385	0.170	0.282
6.	% R.S.D.	0.350	0.168	0.286	0.387	0.170	0.282

4.3 Accuracy

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 8, 10 and 12 µg/ml of RMP and ATR. The average % recoveries for three different concentrations were found to be 90.14 %for ATR and RMP 99.05 % using proposed UV spectrophotometric method. The higher values indicate that the proposed method is accurate for the determination of ATR and RMP in pharmaceutical dosage form. Results of recovery studies are summarized in (Table 13)

Table 13 Recovery (Accuracy) analysis for Atorvastatin Calcium and Ramipril

S.no	Recovery Level	Standard Concentration µg/ml	Concentration added µg/ml	Concentration Found	%Recovery	% Mean Recovery
	Atorvastatin Calcium					
1.	80 %	10	8	7.96	99.50	99.14
2.	100%	10	10	9.86	98.60	
3.	120%	10	12	11.92	99.33	
	Ramipril					
1.	80 %	10	8	7.94	99.25	99.05
2.	100%	10	10	9.90	99.00	
3.	120%	10	12	11.87	98.90	

4.4 LOD and LOQ

The limit of detection and limit of quantification were found to be 0.31µg/ml and 0.1023µg/ml for Atorvastatin Calcium and 0.2805µg/ml and 0.85µg/ml for Ramipril respectively by proposed UV spectrophotometric method. Results of LOD and LOQ are summarized in (Table 14).

Table 14 limit of detection (LOD) and limit of quantification (LOQ) of Atorvastatin Calcium and Ramipril

S.no	Parameters	Method(Simultaneous estimation method)	
		Atorvastatin Calcium	Ramipril
1.	LOD(µg/ml)	0.1023	0.2805
2.	LOQ(µg/ml)	0.31	0.85

CONCLUSION

It can be concluded that the Simultaneous estimation and Method development for both the drugs was performed and it gave good results. The best result was given by methanol solvent.

As method development procedure, validation studies were also performed for the same, but due to limited quantity of the compound only few parameters were observed as Linearity, limit of detection, Limit of quantitisation, Precision and linearity.

The validation procedure followed were as per the ICH guidelines. Atorvastatin Calcium & Ramipril both gave excellent results. Since there were no reference results for this study, so the results were not compared to any standard.

The linearity was achieved with methanol solvent, Linearity, Accuracy and precision were satisfactory and the limit of detection (LOD), limit of quantitation achieved was also satisfactory. Hence we conclude that the simple, rapid, less-time consuming, cost effective and precise method was developed and validated by UV-spectroscopy with the simultaneous estimation of Atorvastatin Calcium & Ramipril.

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