International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(2): March-April 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 05-03-2012; Accepted: 10-03-2012

SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM AND ASPIRIN IN PHARMACEUTICAL DOSAGE FORM BY UV SPECTROPHOTOMETRIC METHOD

Chintan P. Patel*, Rajesh R. Parmar, Dushyant A. Shah, Amit B. Gadhavi

Department of Quality Assurance, APMC College of Pharmaceutical Education and Research, College Campus, Motipura, Himmatnagar – 383001, India

Keywords:

Atorvastatin calcium, Aspirin, UV Spectrophotometric, Simultaneous equation

For Correspondence:

Chintan P. Patel

Department of Quality Assurance, APMC College of Pharmaceutical Education and Research, College Campus, Motipura, Himmatnagar – 383001, India

E-mail:

chintan_on2007@yahoo.com

ABSTRACT

A simple, precise, accurate, rapid and economical spectrophotometric method have been developed for simultaneous estimation of Atorvastatin calcium and Aspirin in pure and in combined capsule dosage form. Method-1 simultaneous equations and Method-2 Q-absorbance Ratio method by using 240 nm and 230 nm as absorbance maxima (λ max)for Atorvastatin calcium and Aspirin respectively and 290.5 nm (isoabsorptive point). A 0.1N NaOH was used as Solvent. Linearity was observed in the concentration range of 2-26 µg/ml for Atorvastatin calcium and 5-25 µg/ml for Aspirin respectively. The method was validated statistically and recovery study was performed to confirm the accuracy of the method.

INTRODUCTION

Atorvastatin calcium (ATOR) is chemically [R-(R*,R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5- (1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt trihydrate. Atorvastatin calcium is an inhibitor of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase. This enzyme catalyses the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis.

Aspirin (ASP) is chemically 2-acetoxybenzoic acid and used as an analgesic, antipyretic, antiinflammatory and antithrombic agent. Combined dosage forms of ATV and ASP are available in the market. Clinical trials showed that combination therapy when used in dyslipidaemic patient with coronary heart diseases reduced cardiovascular events.

Atorvastatin and Aspirin both drug are official in Indian Pharmacopeia. A survey of literature revealed that few chromatographic and Spectrophotometric methods are reported for determination Atorvastatin calcium and Aspirin individually and with other drug combination. The present work describe simple, precise, accurate and economical spectrophotometric method have been developed for simultaneous estimation of Atorvastatin calcium and Aspirin form combined dosage form.

MATERIAL AND METHOD

Instrument

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

Reagents and Chemicals

Reference Standards of ATORVASTATIN CALCIUM and ASPIRIN were obtained as gift samples from the Torrent Pharmaceutical. Ltd. The drug sample (capsules) ATORVA-ASP manufactured by Cadila were procured from market. All other reagents were of analytical grade for Spectrophotometric method.

Procedures

Preparation of Standard Stock Solution and Calibration curve:

Standard stock solution of pure drug containing 500 µg/ml of ATORVASTATIN CALCIUM and 500µg/ml of ASPIRIN were prepared separately in the 0.1N NaOH and final volume was

adjusted with same solvent to get 100 µg/ml of each drug. Working standard solution of 20 µg/ml were scanned in the entire UV range 400-200nm to determine the λ max of both drug. The λmax of Atorvastatin calcium and Aspirin is 240 nm and 230 nm respectively. Five working standard solution with concentration 2, 8, 14, 20, 26 µg/ml of Atorvastatin calcium and 5, 10, 15, 20, 25 µg/ml of Aspirin. The absorbance of resulting solution were measured at their respective λmax and plotted a calibration curve to get linearity and regression equation.

Method-1 (Simultaneous Equation Method)

The Simultaneous Equation Method of analysis based on the absorption of the drugs Atorvastatin calcium and Aspirin at their λmax. Two wavelength selected for the development of Simultaneous Equation are 240nm (λ1) and 230nm (λ2). absoptivities of both the drugs at both the wavelengths were determined.The equations obtained for the estimation of concentration were,

$$Cx = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} \quad a_{x_1} a_{y_2}}$$

$$Cy = \frac{A_1 a_{x_2} \quad A_2 a_{x_1}}{a_{x_2} a_{y_1} \quad a_{x_1} a_{y_2}}$$

Where A1 and A2 are absorbance of Sample solution at 240 and 230 nm respectively.

ax1= Absorptivity of Atorvastatin calcium at 240 nm

ax2 = Absorptivity of Atorvastatin calcium at 230 nm

Ay1 = Absorptivity of Aspirin at 240 nm

Ay2= Absorptivity of Aspirin at 230 nm

C_X and C_Y are concentration of Atorvastatin and Aspirin in sample solution.

Method-2 (Q-Absorbance OR Absorbance Ratio Method)

The absorbance ratio method of analysis is based on the absorbance at two selected wavelengths; one is an isosbestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Figure-1) wavelength 290.5nm (isosbestic point) and 240nm (λ max of Atorvastatin) are selected for Q-Absorbance equation (3 & 4).

Cx = (Qm-Qy) x A1/(Qx-Qy) x ax1 ……(3)

Cy = (Qm-Qx) x A1/(Qy-Qx) x ay1 …….(4)

Where A1 and A2 are absorbance of sample solution at 290.5 nm and 242.0 nm respectively, ax1 = Absorptivity of Atorvastatin calcium at 290.5 nm,ax_2= Absorptivity of Atorvastatin calcium at 240nm,ay_1=Absorptivity of Aspirin at 290.5nm,ay_2=Absorptivity of Aspirin at 240nm C_X and C_Y are concentration of Atorvastatin and Aspirin in sample solution.

Procedure for capsule formulation

Twenty capsules were accurately weighed, and contents were removed. Average weight of the content per capsule was calculated. The contents of a capsule were reduce to fine powder. A quantity of capsule powder equivalent to 10mg of Atorvastatin calcium and 75mg of Aspirin was transferred to 100ml volumetric flask and dissolved in 0.1N NaOH with sonicated for 20 min, was then filtered through Whatman filter. The Aliquot portion of filtrate was further diluted to get a final concentration of about 3µg/ml Atorvastatin calcium and 22.5µg/ml of Aspirin. For Method-1(simultaneous equation method)The absorbance of sample solution was measured at 240nm and 230nm in 1cm cell against the black and For Method-2(Q-absorbance method) The absorbance of sample solution was measured at 290.5nm and 240nm in 1cm cell against the blank. The content of Atorvastatin calcium and Aspirin in a capsule was calculated by the simultaneous equation method and Q-absorption method.

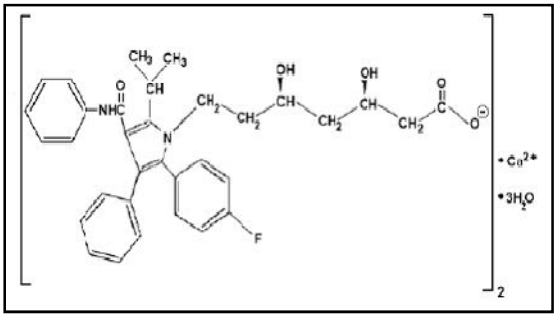

Figure-1 Atorvastatin calcium

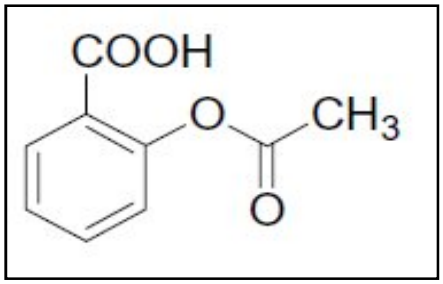

Figure-2 Aspirin

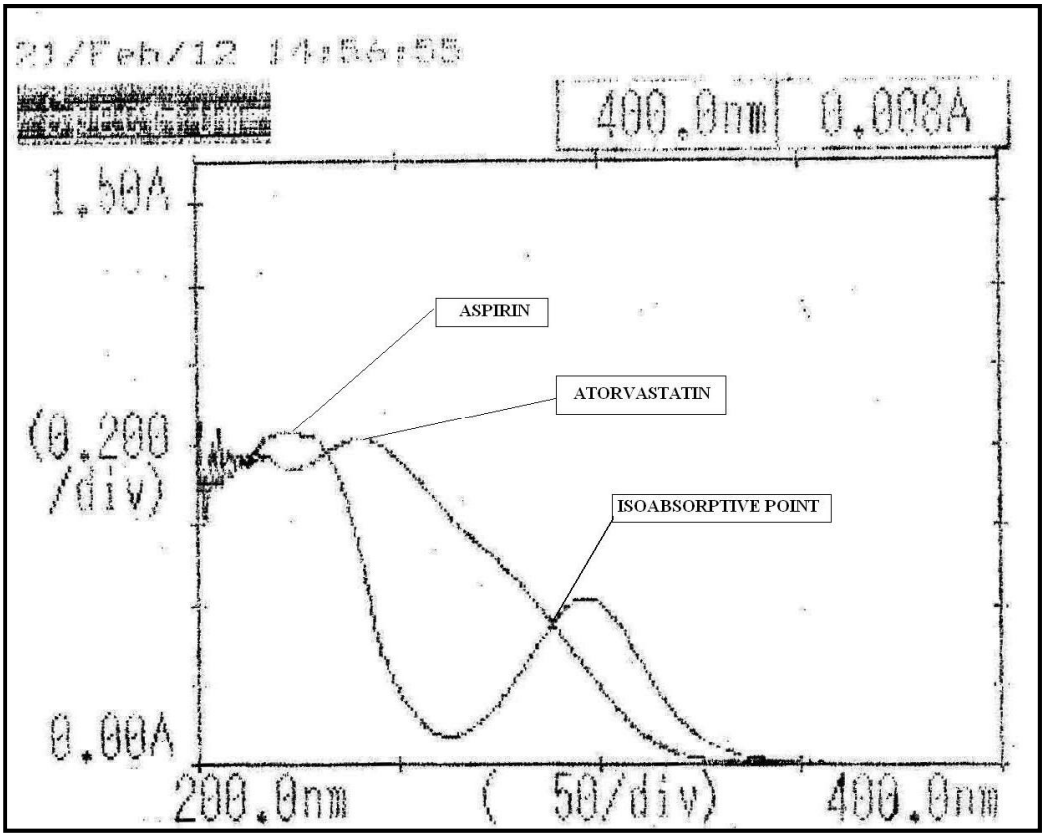

Figure-3 Overlain spectra of Atorvastatin calcium (20µg/ml) and Aspirin (20µg/ml)

Table-1 Optical Characteristic:

Method-1(simultaneous equation method)				
Parameters	**Atorvastatin calcium**		**Aspirin**	
Wavelength (nm)	240	230	240	230
Beer's law limit (µg /ml)	2-26	2-26	5-25	5-25
Regression equation (y = a + bc)	y =0.034x + 0.008	y =0.0320x + 0.006	y = 0.025x - 0.011	y = 0.036x - 0.016
Slope (b)	0.034	0.0320	0.025	0.036
Intercept (a)	0.008	0.006	0.011	0.016
Correlation coefficient (r^2)	0.9998	0.9996	0.9996	0.9997
LOD (µg/ml)	0.18	0.27	0.25	0.21
LOQ (µg /ml)	0.56	0.83	0.77	0.63

Method-2(Q-Absorbance method)				
Parameters	**Atorvastatin calcium**		**Aspirin**	
Wavelength (nm)	240	290.5	240	290.5
Beer's law limit (µg /ml)	2-26	2-26	5-25	5-25
Regression equation (y = a + bc)	y =0.034x + 0.008	y = 0.014x + 0.003	y = 0.025x - 0.004	y = 0.016x - 0.007
Slope (b)	0.034	0.014	0.025	0.016
Intercept (a)	0.008	0.003	0.004	0.007
Correlation coefficient (r^2)	0.9995	0.9996	0.9996	0.9994
LOD (µg/ml)	0.20	0.21	0.29	0.25
LOQ (µg /ml)	0.61	0.63	0.88	0.76

Table-2 Results of the recovery studies

Level of recovery %	**Amount of pure drug added (µg/ml)**		**Simultaneous equation method % recovery**		**Q-absorbance method % recovery**	
	ATOV	**ASP**	**ATOV**	**ASP**	**ATOV**	**ASP**
80	2.4	18	100.30	100.51	100.16	100.64
100	3	22.5	100.44	100.30	100.21	100.14
120	3.6	27	100.08	100.27	100.29	100.21
Mean % recovery			100.27	100.36	100.22	100.13
SD*			0.666	0.532	0.419	0.536
RSD**			.0665	0.534	0.418	0.535

*SD = Standard deviation ** RSD=Relative Standard deviation

Table-3 Results of analysis of capsule formulation

Drugs	**Simultaneous equation Method**	**Q-Absorbance method**
	%Assay ± SD(n=6)	
Atorvastatin calcium(10mg)	100.4% ± 0.95	99.87 ± 0.64
Aspirin(75mg)	100.2% ± 0.45	99.93 ± 0.56

Validation of the Method according to ICH Guidelines

Validation of the method was done according to ICH guidelines for Simultaneous Equation method.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. ATR was linear with the concentration range of 2-26 µg/ml at 240 nm. ASP showed the linearity in the range of 5 – 25 µg/ml at 230 nm.

Precision(repeatability)

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration.

Intermediate precision(reproducibility):

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of ATOR and ASP.

Accuracy (recovery study):

Accuracy of proposed methods, recovery studies carried out at 80%, 100%, and 120% of the test concentration as per ICH Guideline. The recovery study was performed three times at each level.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$,

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

In this method, two wavelengths were used for the analysis of the drugs. 240 nm (λmax of ATOR) and 230 nm (λmax of ASP) are the wavelengths at which calibration curves were prepared for both the drugs. The two drugs also show an isoabsorptive wavelength at 287.5 nm, where both the drugs have same absorptivity value.

Linear correlation was obtained between absorbances and concentrations of ATOR and ASP in the concentration ranges of 2-26 µg/ml and 5-25 µg/ml for both drugs respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. (Method-1)LOD and LOQ values for ATOR were found to be 0.18 and 0.27 µg/ml and 0.56 and 0.83 µg/ml at 240 and 230 nm respectively. LOD and LOQ values for ASP were found to be 0.25 and 0.21 µg/ml and 0.77 and 0.63 µg/ml at 240 and 230 nm respectively.(Method-2)LOD and LOQ values for ATOR were found to be 0.020 and 0.21 µg/ml and 0.61 and 0.63 µg/ml at 290.5 and 240nm respectively. LOD and LOQ values for ASP were found to be 0.29 and 0.25 µg/ml and 0.88 and 0.76 µg/ml at 290.5 and 240 nm respectively. These data show that method is sensitive for the determination of ATOR and ASP. Both drugs showed good regression values at their respective wavelengths and at isoabsorptive point, and the results of a recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method. The proposed validated method was successfully applied to determine ATOR and ASP in their combined dosage form. The results obtained for ATOR and ASP were comparable with the corresponding labeled amounts (Table-3).

Comparison between method-1 and method-2

The proposed analytical methods were compared using statistical analysis. The Student's t - test and F-test was applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated t-value and F-value was found to be less than the critical t-value and F-value (tcrit=2.228, Fcrit=5.05) at 5% significance level respectively.

CONCLUSION

The proposed methods are simple, rapid and validated in terms of linearity, precision, accuracy, reproducibility, and can be used successfully for routine simultaneous estimation of Atorvastatin calcium and Aspirin in pure and capsule dosage forms.

ACKNOWLEDGEMENT

The authors are thankful to Torrent Pharmaceutical Ltd. Gujarat, India for providing gift sample of ATOR, ASP for research. The authors are highly thankful to APMC College of Pharmaceutical Education and Research, Himatnagar, Gujarat, India for providing all the facilities to carry out the work.

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