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

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ABSTRACT

Versatile, accurate, precise and economic method for simultaneous determination of Rosuvastatin calcium and Aspirin in fixed dose combination products was developed. The absorbance values at 242.0 nm and 297.0 nm and 287.5nm (isoabsorptive point) were used for the estimation of Rosuvastatin calcium and Aspirin, respectively without mutual interference. This method obeyed Beer's law in the concentration range of 2–26 µg /ml for Rosuvastatin calcium and 5-25 µg /ml for Aspirin. The results of analysIs have been validated statistically for linearity, accuracy and precision, LOD and LOQ of the proposed method.

INTRODUCTION

Rosuvastatin Calcium (RC) is official in Indian pharmacopoeia[1]. It is chemically (*E*)- (3*R*, 5*S*)- 7- {4- (4-fluorophenyl) -6-isopropyl- 2-{methyl(methylsulphonylamino)] pyrimidin-5-yl}-3,5 dihydroxyhepten-6-oic acid calcium. It is used as a lipid lowering agent act by inhibition of 3-hydroxy-3-methylglutaryl coenzymeA (HMG-CoA) reductase. Rosuvastatin is orally administered as calcium salt. Aspirin (ASP) is official in Indian pharmacopoeia[2]. It is chemically 2- (Acetyloxy) benzoic acid, which is best known as an anti-platelet drug. A formulation containing 75 mg of ASP and 10 mg of RC is available in market . A survey of literature revealed that few chromatographic and Spectrophotometric[8-14] ,HPLC[15-20] and HPTLC[21] methods are reported for determination of ASP and RC individually. However there is no method reported so far its simultaneous determination of ASP and RC from combine dosage form. The present work describes a validated, simple, precise and accurate spectrophotometric method for simultaneous estimation of ASP and RC from combined capsule dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Rosuvastatin calcium was obtained as a gift sample from Cadila Healthcare Ltd. Gujarat, India. Marketed formulation contains 10mg of rosuvastatin calcium(RC) and 75mg of Aspirin(ASP) . All other chemicals used were of analytical grade. 0.1N NaOH and caliberated glasswares were used throughout the work.

Apparatus

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. A Reptech electronic weighing analytical balance based on EMFC technology was used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of RC (100 mg) and ASP (100 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with distilled 0.1N NaoH to obtain standard solution having concentration of RC (1000 µg/ml) and ASP (1000 µg/ml).

CALIBRATION CURVE

A calibration curve was plotted over a concentration range of 2-26 µg/ml Rosuvastatin calcium(RC) 5- 25µg/ml Aspirin(ASP). Accurately measured standard stock solution of Rosuvastatin calcium (2, 8, 14, 20, & 26 mL) and standard stock solution of Aspirin (5, 10, 15, 20,& 25 mL) were transferred to a separate series of 100 mL of volumetric flasks and diluted to the mark with 0.1N NaoH . The absorbance of each solution was measured at the wavelengths 242.0 nm 297.0nm and 287.5.nm(isoabsorptive point).Calibration curves were constructed for Rosuvastatin calcium(RC) and Aspirin(ASP) by plotting absorbance versus concentrations at both wavelengths. Each reading was average of five determinations.

SELECTION OF ANALYTICAL WAVELENTH

For selection of analytical wavelength for the simultaneous estimation. The stock solutions of RC and ASP were separately diluted in 0.1N NaoH to get a concentration of 20 µg/ml of RC and 20 µg/ml of ASP respectively and scanned in the wavelength range of 200 -400 nm. From the overlay spectra of both drugs, wavelengths 287.5 nm (isoabsorptive point) , 242.0 nm (λ max of RC) and 297.0 nm(λ max of ASP) were selected.

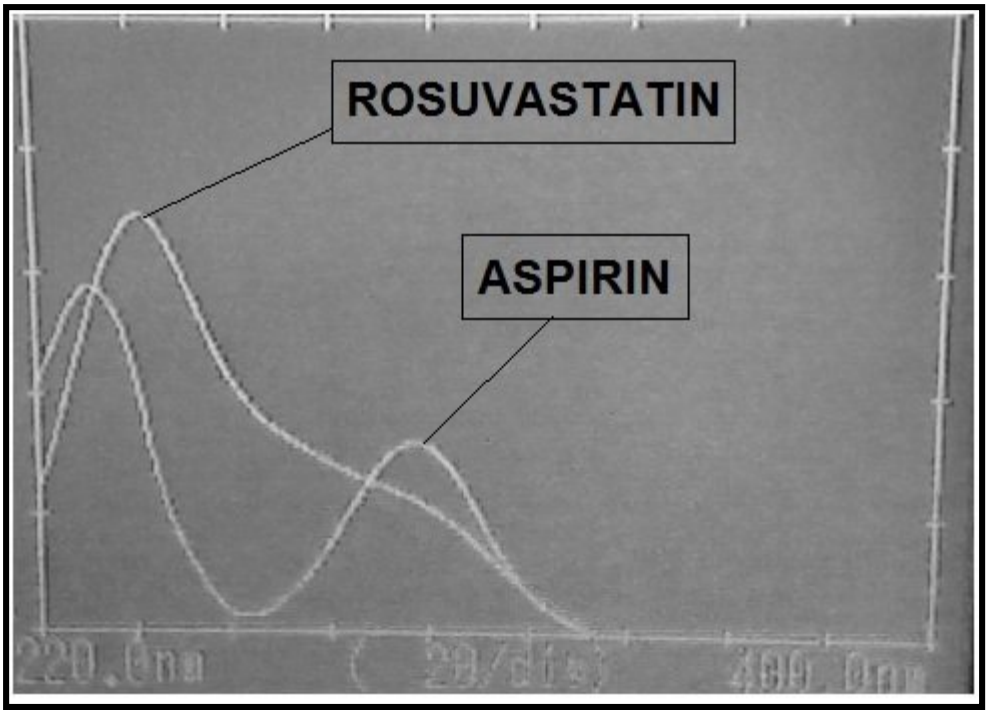

Figure-1 Overlain UV spectra of Rosuvastatin calcium(RC) and Aspirin (Asp).

Preparation of sample solution

Methods

Twenty capsules were accurately weighed and average weight per capsule was calculated. Powder equivalent to 75mg ASP and 10mg RC was accurately weighed and transferred to a 100ml volumetric flask containing 0.1N NaOH(25ml). The flask was sonicated for 10 min. The flask was shaken, and the volume was diluted to the mark with 0.1N NaOH. The above solution was filtered. The aliquot 30ml was transferred to 100ml volumetric flask and volume adjusted to the mark with 0.1N NaOH. Again the aliquot 10ml was transferred to 100 ml volumetric flask.

METHODS OF ESTIMATION

Method I (Simultaneous equation method)[3]

In simultaneous equation method (vierodt's method) two wavelengths were selected i.e. 242.0 nm and 297.0 nm which were absorbance maximas of Rosuvastatin calcium and Aspirin respectively. For calibration curves, stock solutions of Rosuvastatin calcium and Aspirin in the concentration of range of 2 – 26µg/ml and 5 – 25 µg/ml respectively. The absorbance of Rosuvastatin calcium and Aspirin were measured at 242.0 and 297.0 nm, calibration curves were plotted. The absoptivities of both the drugs at both the wavelengths were determined.The content of both ingredient in the sample were obtained by using following equations:

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

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

where,

A1 = Absorbance of the diluted sample at 297 nm

A2 = Absorbance of the diluted sample at 242 nm

ax1 = Absorptivity of Aspirin at 297 nm

ax_2= Absorptivity of Aspirin at 242 nm

ay_1= Absorptivity of Rosuvastatin calcium at 297 nm

ay2 = Absorptivity of Rosuvastatin calcium at 242 nm

Cx = Concentration of Aspirin in the diluted sample

Cy = Concentration of Rosuvastatin calcium in the diluted sample

Method II (Absorbance ratio - Q analysis method)[3]

In this method the ratio of absorbances at any two wavelengths is a constant value independent of concentration or wavelength. In the assay of the drug product under study which contains two active ingredient i.e. Rosuvastatin calcium and Aspirin the absorbances were measured at two wavelengths, one being the λmax of Rosuvastatin calcium (242 nm) and other being the wavelength of equal absorptivity of the two components i.e. an isosbestic point (287.5 nm). The content of both ingredient in the sample were obtained by using following equations :

$$Cx = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{ax_1}$$

$$Cy = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{ay_1}$$

A_1= Absorbance of the diluted sample at 287.5 nm

A_2= Absorbance of the diluted sample at 242.0 nm

ax1 = Absorptivity of Rosuvastatin calcium at 287.5 nm

ax_2= Absorptivity of Rosuvastatin calcium at 242 nm

ay_1= Absorptivity of Aspirin at 287.5 nm

ay_2= Absorptivity of Aspirin at 242 nm

$Q_M = A_2/A_1, Q_X = ax2/ax1, Q_Y = ay_2/ay_1$

VALIDATION OF THE PROPOSED METHOD:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[22].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-26 µg/ml and 5-25 µg/ml for RC and ASP respectively. Accurately measured standard solutions of RC (2, 8, 14, 20, and 26 ml) and ASP (5, 10, 15, 20 and 25 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with 0.1N NaOH. The absorbances of the solutions were measured at 242 nm of RC, 297 nm of ASP and 287.5nm(Isoabsorptive point) against 0.1N NaOH as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision

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 RC and ASP.

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of RC and ASP by the standard addition method. Known amounts of standard solutions of RC and ASP were added at 80, 100 and 120 % level to prequantified sample solutions of RC and ASP (100µg/ml for RC and 750 µg/ml for ASP). The amounts of RC and ASP were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.

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.

RESULT AND DISSCUSSION

The solubility of ROSUVASTATIN CALCIUM(RC) and ASPIRIN (ASP) was studied and 0.1N NaOH was selected as a choice of solvent. Rosuvastatin calcium and Aspirin showed well defined λmax at 242.0 nm and 297.0 nm respectively. The two drugs also show an isoabsorptive wavelength at 287.5 nm, where both the drugs have same absorptivity value. The wavelengths 242.0 and 297.0 nm were considered for development of Simultaneous Equation Method where as 287.5 and 242.0 nm for absorbance ratio method.The two drugs individually and in their mixture were found to follow Beer-Lambert's law over the concentration range of 2-26 µg/ml and 5-25 µg/mL for RC and ASP respectively.

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.

TABLE:1 OPTICAL CHARACTERISTICS DATA

Parameters	Method I Simultaneous Equation Method			
	RC	**RC**	**ASP**	**ASP**
Wavelength (nm)	242	297	242	297
Beer's law limit (µg /ml)	2-26	2-26	5-25	5-25
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.0371x + 0.0197 0.0371 0.0021	y = 0.013x + 0.0022 0.01302 0.0165	y = 0.0206x + 0.007 0.0206 0.007	y = 0.0176x - 0.004 0.0176 -0.004
Correlation coefficient (r^2)	0.9993	0.9993	0.9994	0.9989
LOD (µg/ml)	0.47	0.26	0.59	0.39
LOQ(µg/ml)	1.42	0.79	1.81	1.20
Precision(% RSD,n=3) Interday Intraday	 2.49 0.7-1.3	 3.05 1.2-1.9	 1.50 0.7-1.5	 1.89 1.1-1.8

TABLE:2 OPTICAL CHARACTERISTICS DATA

Parameters	Method II Absorbance ratio - Q analysis method			
	RC	**RC**	**ASP**	**ASP**
Wavelength (nm)	242	287.5	242	287.5
Beer's law limit (µg /ml)	2-26	2-26	5-25	5-25
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 0.0371x + 0.0197 0.0371 0.0021	y = 0.0147x + 0.0031 0.0147 0.0197	y = 0.0206x + 0.007 0.0206 0.007	y = 0.0142x - 0.0053 0.0142 -0.0053
Correlation coefficient (r^2)	0.9993	0.9987	0.9994	0.9990
LOD (µg/ml)	0.47	0.47	0.59	0.36
LOQ(µg/ml)	1.42	1.43	1.81	1.11

Precision(% RSD) (n=3) Interday	2.49	1.98	1.50	2.01
Intraday	0.7-1.3	0.8-1.3	0.7-1.5	0.9-1.4

TABLE:3 ANALYSIS OF CAPSULE FORMULATION

Method	Capsule sample	Label claim (mg/casule)	% Label claim	SD
I	RC	10 mg	99.83%	±0.92
	ASP	75 mg	99.78%	±0.35
II	RC	10 mg	99.46	±1.45
	ASP	75 mg	100.32	±0.67

*SD = Standard deviation

TABLE:4 RECOVERY RESULT OF ROSUVASTATIN CALCIUM AND ASPIRIN

Method	Recovery Level	% Recovery	SD	% Recovery	SD
		ROSUVASTATIN CALCIUM		ASPIRIN	
I	80%	100.55	±1.27	99.77	±0.68
	100%	101.33	±0.67	101.21	±0.60
	120%	99.81	±0.32	100.5	±0.65
II	80%	99.42	±1.88	99.89	±0.52
	100%	100.94	±1.80	99.49	±0.91
	120%	100.82	±2.27	99.65	±1.50

*SD = Standard deviation

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