Difference Spectrophotometric Method Development and Validation For Simultaneous Estimation of Rosuvastatin Calcium and Telmisartan in Bulk and Combined Dosage Form

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ABSTRACT

Rosuvastatin calcium and Telmisartan combination is used in the treatment of coronary heart diseases. UV spectrophptometric and chromatographic methods were reported in the literature for the estimation of Rosuvastatin calcium and Telmisartan. Therefore a simple and highly sensitive difference spectrometric method for the estimation of Rosuvastatin calcium and Telmisartan in bulk and in combined dosage form is described. The proposed method is based on the principle that both the drugs can exhibit two different forms in acidic and basic medium that differs in their absorption spectra in acidic and basic medium. In the difference spectrum, Rosuvastatin calcium showed maxima at 248nm and minima at 237.5nm and Telmisartan showed maxima at 309nm and minima at 286nm. Linearity range was observed in the concentration range of 20-60μg/ml for Rosuvastatin calcium and Telmisartan. Percentage purity and recovery study were in the limit of 98-102% and precision was less than 2 for both drugs. Limit of Detection for Rosuvastatin calicium and Telmisartan were found to be 0.0635μg/ml and 0.1693μg/ml respectively. Limit of Quantitatin for Rosuvastatin calcium and Telmisartan were found to be 0.1925 μg/ml and 0.5129μg/ml respectively. The proposed method can be successfully used for the analysis of pure drug and marketed formulation. The method is found to be precise, simple, accurate and can be applied for the routine estimation of Rosuvastatin calcium and Telmisartan.

KEY WORDS: Difference spectroscopy, rosuvastatin calcium, telmisartan, 0.1N hydrochloric acid, 0.1N sodium hydroxide

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INTRODUCTION

Rosuvastatin calcium[1] and Telmisartan[2] is a fixed dose combination containing Rosuvastatin 10 mg as Lipid Lowering agent and Telmisartan 40 mg as Anti Hypertensive agent. Chemically Rosuvastatin is bis[(E)-7-[4(4-fluorophenyl)-6-isopropyl-2[methyl (methylsulfonyl) amino] pyrimidin-5-yl](3R,5S)3,5- dihydroxyhept-6-enoic acid] calcium salt[1]. Chemically Telmisartan is 4'-[(1,4'-dimethyl-2'-propyl [2,6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid[1,2]. Pharmacologically Rosuvastatin Calcium is is a lipid lowering agent. It is a competitive inhibitor of HMG-Co A reductase. It catalyses the reduction of 3-hydroxyl-3-methylglutaryl coenzymeA to mevalonate, which is a rate limiting step in hepatic cholesterol synthesis.

 Mevalonate is a small molecule used in the synthesis of cholesterol and other mevalonate derivatives. In this way, it lowers the amount of cholesterol and LDL- cholesterol. Pharmacologically[3] Telmisartan interferes with the binding of angiotensin II to the angiotensin II AT1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance. Telmisartan does not inhibit the angiotensin converting enzyme, other hormone receptors, or ion channels. This is a new combination is market and so far no analytical methods have been reported for simultaneous analysis of both the drugs together so following experiment was performed.

A detailed literature survey for rosuvastatin calcium revealed several methods based on varied technique, viz, UV/Visible spectrophotometry, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography[1,3-10]. Similarly, detailed literature survey for Telmisartan revealed several methods based on varied technique, viz, UV/Visible spectrophotometry, Difference Spectroscopy, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography[1,2,11-15]. Till date, two analytical methods are reported for the combination of these two drugs, viz, UV/Visible Spectrophotometry and high Performance Liquid Chromatography[16,17].

MATERIALS AND METHODS:

APPARATUS:

UV/Visible Spectrophotometer: ELICO SL218 (ELICO Ltd., Hyderabad)

Analytical Balance: Shimadzu AX 200 (Shimadzu Corporation, Kyoto, Japan)

Sonicator: Ultrasonic Cleaner FS_4 (Frontline Electronics and Machinary Pvt. Ltd., Ahmedabad)

CHEMICALS AND REAGENTS:

Rosuvastatin Calcium: Gift sample from Zydus Cadila Pharmaceuticals Ltd., Ankleshwar

Telmisartan: Gift sample from Nivika Chemo Pharma Pvt. Ltd., Ankleshwar

Formulation of Rosuvastatin Calcium and Telmisartan : TELROSE, Micro Labs Pvt Ltd, Banglore: 10mg Rosuvastatin Calcium+40 mg Telmisartan

Solvent: Methanol

Diluent: 0.1N Sodium Hydroxide, 0.1N Hydrochloric acid

METHOD DEVELOPMENT AND OPTIMIZATION:

PREPARATION OF STANDARD STOCK SOLUTION:

Rosuvastatin calcium standard stock solution (100µg/ml):

Accurately weighed quantity of rosuvastatin calcium 10 mg was transferred into 100 ml volumetric flask dissolved it and diluted up to mark with Methanol. This will give a stock solution having strength of 100µg/ml.

Telmisartan standard stock solution (100µg/ml):

Accurately weighed quantity of telmisartan 10 mg was transferred into 100 ml volumetric flask dissolved it and diluted up to mark with Methanol. This will give a stock solution having strength of 100µg/ml.

SELECTION OF WAVELENGTH:

From the standard stock solution of each drug, 10µg/ml solutions were prepared separately by using 0.1N HCl and 0.1N NaOH. Solution was scanned between 200-400 nm. Wavelengths were selected from the difference spectra of rosuvastatin calcium and telmisartan.

CONSTRUCTION OF CALIBRATION CURVE:

Calibration curve for rosuvastatin calcium:

Different aliquots were taken from their standard stock solution and diluted with 0.1N HCl and 0.1N NaOH separately to prepare a series of concentrations from 20-60µg/ml as reference and test solutions, respectively. Difference spectrum was recorded by placing Rosuvastatin calcium in 0.1N HCl in reference cell and 0.1N NaOH in sample cell. Difference in absorbance between Maxima and Minima was calculated to find out the amplitude. The calibration curve was prepared by plotting amplitude versus concentration.

CALIBRATION CURVE FOR TELMISARTAN:

Different aliquots were taken from their standard stock solution and diluted with 0.1N HCl and 0.1N NaOH separately to prepare a series of concentrations from 20-60µg/ml as reference and test solutions, respectively. Difference spectrum was recorded by placing Telmisartan in 0.1N HCl in reference cell and 0.1N NaOH in sample cell. Difference in absorbance between Maxima and Minima was calculated to find out the amplitude. The calibration curve was prepared by plotting amplitude versus concentration.

ESTIMATION OF ROSUVASTATIN CALCIUM AND TELMISARTAN IN COMBINED TABLET DOSAGE FORM:

Twenty Tablets were weighed and their average net weight was calculated. The Tablets were crushed and the powder was made to a fine powder. The powder equivalent to 10mg of Rosuvastatin calcium and 40mg of Telmisartan were weighed and transferred in to 100 ml volumetric flask. Dissolved in Methanol, sonicate for 20 min and made up to the volume with the same. The solution was filtered through Whatman filter paper No.41. From the stock solution, 10µg/ml and 40µg/ml solutions were prepared separately by using 0.1N HCl and 0.1N NaOH for ROS and TEL, respectively. The amplitude was calculated by measuring the absorbance of the equimolar concentrations at maxima and minima in the difference spectrum. The amount of Rosuvastatin calcium and Telmisartan was calculated.

METHOD VALIDATION[18]:

LINEARITY AND RANGE:

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 20-60µg/ml for both Rosuvastatin calcium and Telmisartan. Plot the calibration curve of amplitude v/s concentration and determine correlation coefficient and regression line equations for Rosuvastati calcium and Telmisartan.

ACCURACY:

PREPARATION OF SAMPLE SOLUTION:

Twenty tablets were powdered. Powder equivalent to 10mg of Rosuvastatin calcium and 40 mg of Telmisartan were weighed and transferred into 100ml of volumetric flask, sonicate it for 20 minutes and diluted up to mark with Methanol. The solution was filtered using Whatman filter paper no.41 and first few drops of filtrate were discarded. (100µg/ml of Rosuvastatin calcium and 400µg/ml of Telmisartan)

From the above solution, take 1ml in 10ml volumetric flask and dilute it up to the mark with 0.1N HCl and 0.1N NaOH to get the final concentration of 10µg/ml of Rosuvastatin calcium and 40µg/ml of Telmisartan . To 5ml of above solution, 5ml of increasing concentration of working standard solution (8, 10, 12µg/ml of Rosuvastatin calcium and 32, 40 and 48 µg/ml of Telmisartan) were added. Absorbances of solutions were measured at selected wavelengths for Rosuvastatin calcium and Telmisartan. The amount of Rosuvastatin calcium and Telmisartan were calculated at each level and % recoveries were computed.

PRECISION:

INTRADAY (REPEATABILITY):

Solutions containing 10µg/ml of Rosuvastatin calcium and 40µg/ml of Telmisartan were analyzed two times on the same day and %R.S.D were calculated.

INTERDAY (INTERMEDIATE):

Solutions containing 10µg/ml of Rosuvastatin calcium and 40µg/ml of Telmisartan were analyzed on the different day and %R.S.D were calculated.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):

The LOD and LOQ of Rosuvastatin Calcium and Telmisartan by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\ \sigma\ /S$ respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 2.

RESULTS AND DISCUSSION:

The solubility of Rosuvastatin calcium and Telmisartan was studied and methanol was selected as a choice of solvent. Rosuvastatin Calcium and Telmisartan showed well defined maxima at 248nm and 309nm respectively and minima at 237.5nm and 286nm respectivaly, therefore these wavelengths were considered for development of difference spectroscopic method (Figure 3). Two drugs individually followed Beer-Lambert's law over the concentration range of 20-60µg/ml for Rosuvastatin calcium and Telmisartan. Coefficient of correlation for Rosuvastatin calcium and Telmisartan were found to be 0.998 and 0.998 respectively with RSD <2. The values of correlation coefficient suggest the level of precision of the method. Drug content in tablet (amount present) was directly found from the above mentioned regression equations for both drugs. Standard deviations, RSD were calculated and are given in table 2. Percentage estimation in tablet dosage form was 99.23% and 99.88% (%RSD < 2) for Rosuvastatin calcium and Telmisartan respectively (Table 3). The method was validated according to International Conference on Harmonization

guidelines for validation of analytical procedures. Linear regression equations (intercepts and slopes) for Rosuvastatin calcium and Telmisartan were established. The high values of the correlation coefficients and the values of Y-intercepts close to zero indicate the good linearity of the calibrations. The values of slope, intercept and correlation coefficient values are given in Table 1. Limit of detection (LOD) and limit of quantitation (LOQ) were determined by using the formula and are mentioned in Table2. To study the validation parameters accuracy, reproducibility, reliability and interference, recovery experiment was carried out by standard addition. The recovery of added standard was calculated at different concentration levels. From the total amount of drug found, the percentage recovery was calculated which was between 98-102 % (RSD < 2.0).

CONCLUSION:

The proposed method is simple, precise, and accurate for the rapid for simultaneous determination of Rosuvastatin calcium and Telmisartan in combined tablet dosage forms and this method may be successfully applied in control laboratories for their determination in combined dosage form.

Table 1 Data of optical characteristics

Parameters		Observed Value	
		ROS	TEL
Beer's Law Limit (µg/ml)		20-60µg/ml	20-60µg/ml
Correlation coefficient (r^2)		0.998	0.998
Regression Equation (y=mx + c)	Slope	0.014	0.005
	Intercept	-0.127	0.031

Table 2 Data of validation parameters

Parameters	Observed Value	
	ROS	TEL
Accuracy (%Recovery)	99.20	98.84
Intraday precision (%RSD)	0.3020	0.2772
Interday precision (%RSD)	0.1929	0.3111
Linearity (r^2)	0.998	0.998
LOD (µg/ml)	0.0635	0.1693
LOQ (µg/ml)	0.1925	0.5129

Table 3: Recovery data

Level of Standard Addition (%)	% Recovery ± SD*	
	Rosuvastatin Calcium	Telmisartan
80	99.82±0.2678	99.10±0.2602
100	98.21±0.2857	98.27±0.2021
120	99.58±0.2728	99.14±0.1879

Table 4 : Analysis of tablet formulation

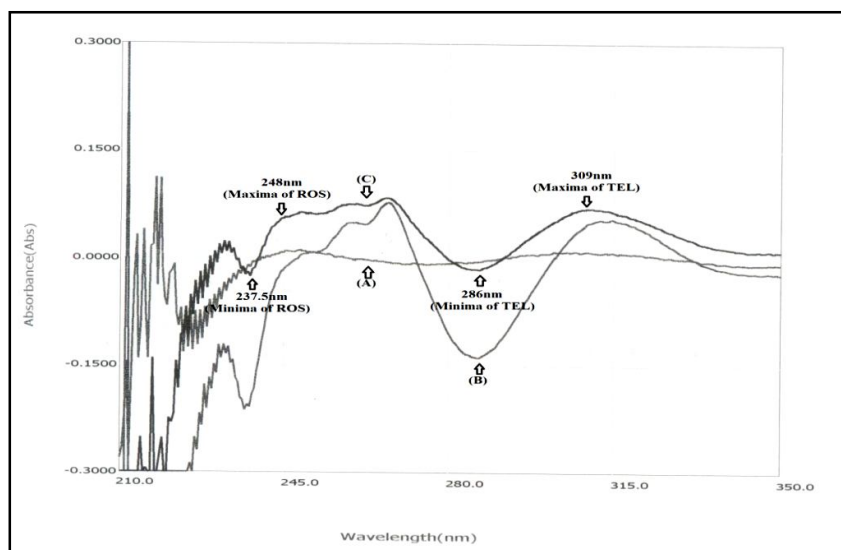| Tablet Components	Labelled Claim (mg/tab)	Amount Found (mg/tab)	% Purity	SD	%RSD
Rosuvastatin Calcium	10.0	9.92	99.23	0.2887	0.2909
Telmisartan	40.0	39.95	99.88	0.2566	0.2568

Fig. 1: Overlain Spectra (A) ROS (20μg/ml) (B) TEL (20μg/ml) (C) Mixture of ROS (20μg/ml) and TEL (20μg/ml)

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