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Development and Validation of Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Rosuvastatin and Diltiazem in Combined Dosage Form

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ABSTRACT

A simple and economical dual wavelength spectrophotometric method has been developed for the simultaneous estimation of rosuvastatin and diltiazem in their combined dosage forms. The method was based on property of addivity of absorbances. At 295.6 rosuvastatin showed absorbance but diltiazem showed zero absorbance. The two wavelengths on rosuvastatin curve were found out where it showed same absorbance, which were 227 and 247.4 nm. Diltiazem showed adequate absorbances at these wavelengths. The method involved solving of an equation based on measurement of absorbances at three wavelengths 295.6, 227, and 247.4 nm. The proposed method was found to be simple, economical, accurate, and reproducible for routine analysis of both drugs in tablet dosage forms.

KEYWORDS

Rosuvastatin, Deltiazem, Spectrophotometric, Dual Wavelength.

INTRODUCTION

Rosuvastatin is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Chemically Rosuvastatin is (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid[1]. Diltiazem is widely used as antihypertensive agents, vasodilator agents, calcium channel blockers and cardiovascular agents. Chemically Diltiazem is (2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate[1]. Rosuvastatin is official in IP' 2007[2] while Diltiazem is official in IP' 2010[3], BP' 2009[4] and USP' 2005[5]. Literature survey reveals that number of methods such as spectrophotometric[6-8], HPLC[9], HPTLC[10], Capilary Zone electrophoresis[11] are reported for the estimation of rosuvastatin from its formulation or biological fluids. Similarly number of methods such as spectrophotometric[12], HPLC[13-16], HPTLC[17] are reported for the estimation of diltiazem from its formulation or biological fluids. There was no any method reported for the simultaneous estimation of rosuvastatin and diltiazem from their combined dosage form. So, present study was aimed to develop and validate[18] spectrophotometric method based on dual wavelength measurement for simultaneous estimation of rosuvastatin and diltiazem from their combined dosage form which would be simple, cost effective and easily adopted by small laboratories.

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MATERIAL AND METHOD

Instruments and Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a

spectral width of 2 nm, wavelength accuracy of 0.2 nm and pair of 1 cm matched quartz cells, Analytical Balance (CP224S, Sartorius, Germany), Ultrasonic Cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks, pipettes of borosilicate glass were used in the study, and Water Purification System (Millipore Bioscience Division Pvt. Ltd, India) was used during study.

Chemicals and Reagents

Kindly gifted reference standards of Diltiazem HCl (Devi's Laboratories, Gujarat, India), and Rosuvastatin calcium (Micro Labs Ltd) was used without further purification. The Pharmaceutical Tablet formulation containing 20 mg rosuvastatin and 120 mg diltiazem was prepared in the laboratory. LR grade 0.1N HCl (S.D. Fine Chem Ltd.) and Whatman filter paper no. 41 (Whatman International Ltd., England) was used for the study.

Preparation of Reagents and Solutions

Standard Stock Solution (1000 µg/ml)

Accurately weighed 10 mg of diltiazem and rosuvastatin standard was transferred in two separate 10 ml volumetric flask, were dissolved in 50 ml of 0.1N HCl and final volumes were made up to mark with same solvent.

Mixed Standard Stock Solution of Diltiazem and Rosuvastatin

From the above solutions of diltiazem and rosuvastatin, 10 ml aliquots were pipette out and transferred in two separate 100 ml volumetric flasks, diluted up to the mark with 0.1N HCl to obtain mixed standard stock solution diltiazem (100 µg/ml) & rosuvastatin (100 µg/ml).

Sample Solution Preparation

Tablets containing 20 mg rosuvastatin and 120 mg diltiazem were prepared using sodium starch glycollate, HPMC K-100M, carbopol, sodium bicarbonate, lactose, magnesium stearete and talc. Tablets were prepared by direct compression technique using Rimek Mini Tablet Press-II. Twenty such tablets (each tablet contains 20 mg rosuvastatin and 120 mg of diltiazem) were accurately weighed, their mean weight was determined, and were ground to fine powder. An amount of powdered mass equivalent to 4 mg of rosuvastatin and 24 mg of diltiazem was weighed and transferred to a 100 mL volumetric flask. The content was mixed with 0.1N HCl (70 mL) and sonicated for 20 min to dissolve the drug as completely as possible. The solution was then filtered through a Whatman filter paper no. 41 and residue was washed with 0.1N HCl. The volume was made up to mark with 0.1N HCl. One ml aliquot from above solution was transferred in 10 mL volumetric flask and the volume was adjusted up to the mark with 0.1N HCl**.** This solution was expected to contain theoretically 4 µg/ml of rosuvastatin and 24 µg/ml of diltiazem.

Selection of Wavelength for Estimation of Diltiazem and Rosuvastatin

Absorbance spectrum of pure diltiazem was scanned in the spectrum basic mode. Using the cursor function, the absorbance corresponding to 295.6 nm (wavelength λ1, the wavelength of minimum absorbance (0.00) for diltiazem was noted from spectrum. At the λ1=295.6 nm rosuvastatin shows the absorbance in its spectrum, while diltiazem does not shows absorbance at this λ1, So in spectrum of mixture of both the drug absorbance obtained at λ1 was only due to rosuvastatin which is proportional to the concentration of rosuvastatin. Absorbance spectrum of pure rosuvastatin was also scanned in the spectrum basic mode. In spectrum of rosuvastatin the cursor function was moved along with peak curve until two wavelengths were obtained for which rosuvastatin showed equal absorbance. The wavelength obtained corresponding to such absorbance value were 227 nm and 247.4 nm (λ2 and λ3). Now, in the spectrum of mixture the difference in absorbance at 227 nm and 247.4 nm was due to the diltiazem which was found to increase concomitantly as concentration was increased.

Calibration Curve for Rosuvastatin and Diltiazem

Linearity was determined at seven levels over the range of 4–32 µg/ml of standard

concentration. Accurately measured mixed standard stock solutions (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 3.2 ml) of diltiazem and rosuvastatin were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with 0.1N HCl. The difference in absorbance between 227 nm and 247.4 nm was due to the diltiazem. This difference in absorbance was plotted against diltiazem concentration (µg/ml). The absorbance at 295.6 nm is due to rosuvastatin only and was plotted against rosuvastatin concentration (µg/ml).

Analysis of Combined Tablet Dosage Form

Six replicates of sample solution were analyzed. The absorbance of final sample solution was measured against 0.1N HCl as blank at 227 nm, 247.4 nm and 295.6 nm. The amount diltiazem and rosuvastatin was calculated using respective equation of calibration curve.

RESULTS

Method Development

The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. For elimination of the effects of an interfering component, two specific wavelengths were chosen:

(I) First wavelength λ1 at which zero absorbance of diltiazem and reasonable absorbance of rosuvastatin was observed. (295.6 nm)

(II) Two wavelengths λ2 and λ3 were selected at which the absorbance of rosuvastatin was same, and diltiazem was also having some absorbance at this wavelengths (227 nm and 247.6 nm).

The absorbance at these two wavelengths (λ2 and λ3) was found to be equal for rosuvastatin. The difference in absorbance at these two wavelengths (A227 – A247.6) cancels out the contribution of absorbance of rosuvastatin and the difference in absorbance was proportional to the concentration of diltiazem in the mixture. These two selected wavelengths were employed to determine the concentration of diltiazem from the mixture of diltiazem and rosuvastatin[19-20]. The overlain UV spectrum of these two drugs is depicted in Figure-1 while spectra of mixed standards containing rosuvastatin and deltiazem is shown in Figure-2.

Method Validation

Linearity

The difference in absorbance values at two wavelengths ($A_{227nm} - A_{247.6nm}$) was found to be linear in the range of 4 – 32 µg/ml of diltiazem with correlation coefficient 0.9980. Further the absorbance value at 295.6 was only due to rosuvastatin as diltiazem has zero absorbance at this wavelength. The absorbance values were found to be linear in the range of 4 – 32 µg/ml of rosuvastatin with correlation coefficient 0.9970. Regression parameters are mentioned in Table 1 and the calibration curves of diltiazem and rosuvastatin are shown in Figure-3 and Figure-4, respectively

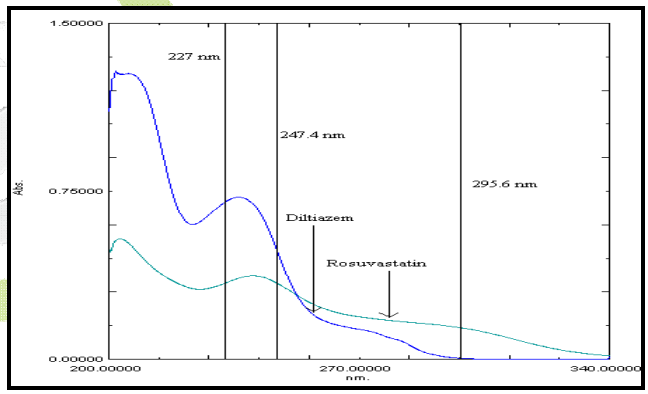

Figure 1: Overlain Spectra of Diltiazem and Rosuvastatin

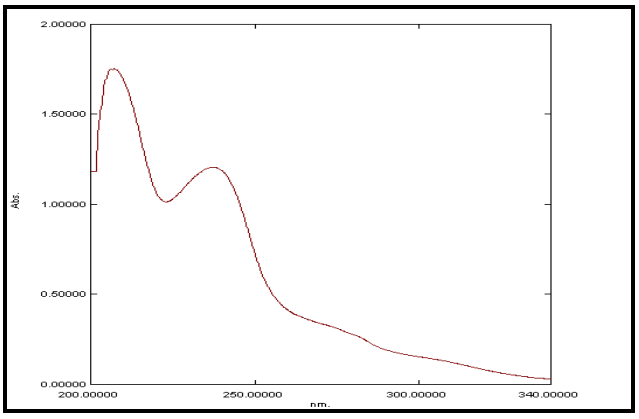

Figure 2: Spectra of Mixed Standards of Diltiazem and Rosuvastatin

Table 1: Linearity of Rosuvastatin and Diltiazem

Sr. No.	Concentration of Rosuvastatin and Diltiazem (µg/ml)	Absorbance at 295.6nm (for Rosuvastatin)	Absorbance difference 227nm – 247.4nm (for Diltiazem)
1	4	0.03345	0.05078
2	8	0.07715	0.07984
3	12	0.11685	0.12211
4	16	0.15808	0.16174
5	20	0.21008	0.20696
6	24	0.24304	0.24671
7	32	0.31836	0.32653
Correlation coefficient		0.997	0.998
Slope of regression line		0.010	0.010
Y-intercept		0.005	0.004

Accuracy (%Recovery)

Accuracy was checked by recovery study at 3 different concentration levels, i.e., a multilevel recovery study. The tablet samples were spiked with an extra 50, 100, 150% of standard rosuvastatin and diltiazem, and the mixtures were analyzed by proposed method. Results of the recovery study are shown in table 2 suggested that method was accurate for the simultaneous estimation of rosuvastatin and diltiazem in their combined dosage forms.

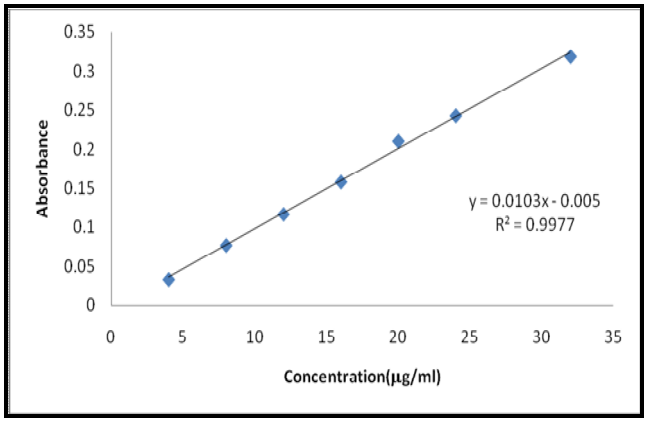

Figure 3: Calibration Curve for Rosuvastatin

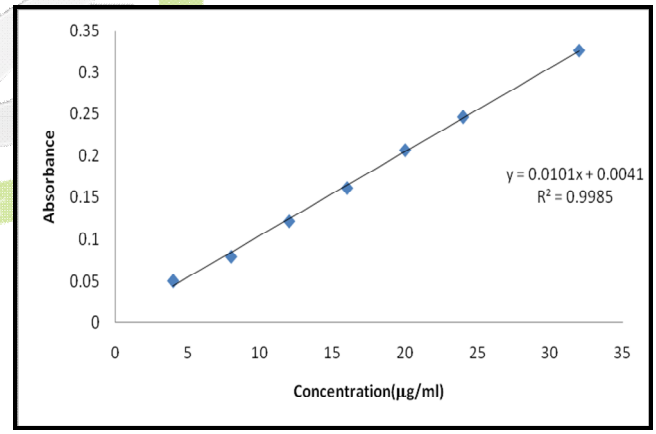

Figure 4: Calibration Curve for Diltiazem

Precision

Method Precision (Repeatability)

The Method precision of the proposed method was determined by analyzing mixed standard solution of diltiazem and rosuvastatin at (4 µg/ml for rosuvastatin and 24 µg/ml for diltiazem) 6 times without changing the parameters of measurement. The results are reported in terms of relative standard deviation (RSD) in table 3.

Table 2: Recovery Data of Rosuvastatin and Diltiazem

Drug	Level	Amount of sample (µg/ml)	Amount of standard spiked (µg/ml)	Total Amount recovered (µg/ml)	Amount of standard recovered (µg/ml)	%Recovery ± RSD*
Rosuvastatin	0	8	0	8.16	---	----
	50%	8	4	12.21	4.05	101.28 ± 0.26
	100%	8	8	16.08	7.92	98.96 ± 0.14
	150%	8	12	19.98	11.82	98.51 ± 0.16
Diltiazem	0	8	0	7.95	---	----
	50%	8	4	11.96	4.01	100.25 ± 0.25
	100%	8	8	15.91	7.96	99.50 ± 0.15
	150%	8	12	19.92	11.97	99.75 ± 0.20

*Each value is the mean of three determinations.

Table 3: Precision Data for Rosuvastatin and Diltiazem

Rosuvastatin (4 µg/ml) & Diltiazem(24 µg/ml)	Absorbance at 295.6nm (for Rosuvastatin)	Absorbance difference 227nm – 247.6nm (for Diltiazem)
1	0.03338	0.24685
2	0.03341	0.24654
3	0.03365	0.24656
4	0.03348	0.24684
5	0.03340	0.24742
6	0.03323	0.24695
Mean	0.03343	0.24665
SD	0.0001	0.0007
RSD	0.411	0.290

Table 4: Intraday and Interday Precision Data

Drug	Conc. of drug(µg/ml)	Intraday precision Mean abs ± %RSD (n=3)	Interday precision Mean abs ± %RSD (n=3)
Diltiazem	12	0.12223 ± 0.263	0.12228 ± 0.709
	20	0.20642 ± 0.276	0.20632 ± 0.458
	24	0.24623 ± 0.242	0.24643 ± 0.337
Rosuvastatin	12	0.11669± 0.211	0.11626 ± 0.474
	20	0.21027 ± 0.139	0.21086 ± 0.322
	24	0.24287 ± 0.239	0.24338 ± 0.225

Intermediate Precision

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solutions of diltiazem and rosuvastatin at 3 different concentrations (12, 20, and 24 µg/ml for both diltiazem and rosuvastatin) 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation (RSD) in table 4.

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response,

S = slope of the calibration curve.

LOD and LOQ for diltiazem were found to be 0.41 µg/ml and 1.23 µg/ml respectively. LOD and LOQ for rosuvastatin were found to be 0.40 µg/ml and 1.2 µg/ml.

Estimation of Diltiazem and Rosuvastatin in Tablet Dosage Form

The proposed validated method was successfully applied to the simultaneous determination of diltiazem and rosuvastatin in tablet dosage form. The results of analysis of tablet formulation are shown in Table 5.

CONCLUSION

The proposed method is based on dual wavelength data processing and only requires measurement of absorbance at selected wavelengths. The values of % RSD were 0.411 and 0.290 for determination of rosuvastatin and diltiazem respectively, showing repeatability of the method. Interference studies revealed that the common excipients and other additives usually present in the tablet dosage forms did not interfere in the proposed method for estimation of both drugs. The proposed method was found to be simple, rapid, economical, accurate and precise. It can be useful for routine in-process quality control and simultaneous estimation of rosuvastatin and diltiazem from their combined tablet dosage forms.

Table 5: Analysis of Sample by Dual wavelength Method (n = 6)

Sample No.	Label Claim		Amount Found		% Label Claim	
	Rosuvastatin (mg/tab)	Diltiazem (mg/tab)	Rosuvastatin (mg/tab)	Diltiazem (mg/tab)	Rosuvastatin (mg/tab)	Diltiazem (mg/tab)
1	20	120	20.167	120.813	100.833	100.677
2	20	120	20.333	119.500	101.667	99.583
3	20	120	19.833	119.063	99.167	99.219
4	20	120	19.500	121.000	97.500	100.833
5	20	120	20.167	119.875	100.833	99.896
6	20	120	19.667	121.188	98.333	100.990
Mean			20.083	120.052	19.944	120.240
S.D.			0.312	0.973	0.328	0.880

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