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Development and Validation of First Derivative Method for Simultaneous Estimation of Rosuvastatin and Diltiazem in Combined Dosage Form

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ABSTRACT

A simple and economical first derivative spectrophotometric method has been developed for the simultaneous estimation of rosuvastatin and diltiazem in their combined dosage forms. The method involved determination of Zero Crossing points (ZCP) in their respective derivative spectra. By scanning first derivative spectra of rosuvastatin and diltiazem, ZCP for rosuvastatin was found to be at 240 nm and for diltiazem at 236.4 nm. For rosuvastatin 236.4 nm and for diltiazem 240 nm was chosen as an analytical wavelength. The method involved solving of an equation based on measurement of absorbances at wavelengths 240 and 236.4 nm. The proposed method was found to be simple, economical, accurate, and reproducible for routine analysis of both drugs in tablet dosage form.

KEYWORDS

Rosuvastatin, Deltiazem, Spectrophotometric, First Derivative.

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] first derivative spectrophotometric method 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.

Sample Stock 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.

Determination of the Zero Crossing Points (ZCP)

Working standard solutions having concentration 10 µg/mL of rosuvastatin and diltiazem were separately prepared and were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. It appeared that rosuvastatin showed zero crossing at 227.2 nm & 240 nm and while diltiazem showed zero crossing at 236.4 nm, & 223.6 nm. The absorbance values for diltiazem and rosuvastatin were found to increase concomitantly with concentration at 240 and 236.4 nm respectively. So these two wavelengths were decided as analytical wavelengths for estimation of rosuvastatin and diltiazem respectively.

Calibration Curve for Rosuvastatin and Diltiazem

Calibration curves were plotted over a wide concentration range of 4-40 µg/mL for rosuvastatin and diltiazem. Accurately measured standard stock solutions of rosuvastatin and diltiazem (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, and 4.0 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with 0.1N HCl, and first-derivative absorbances (D1) were measured at 236.4 nm for rosuvastatin and 240.0 nm for diltiazem. The calibration curves were constructed by plotting first-derivative absorbances vs concentrations. The correlation co-efficient, y- intercept, slope of regression line were calculated in linearity plot.

Analysis of Combined Tablet Dosage Form

Six replicates of sample solution were analyzed. The first-derivative absorbance of final sample solution was measured against 0.1N HCl as

blank at 240 nm and 236.4 nm. The amount of diltiazem and rosuvastatin was calculated using respective equation of calibration curve.

RESULTS AND DISCUSSION

Method Development

At 240.0 nm (zero crossing point of rosuvastatin), diltiazem gives some absorbance where as at 236.4 nm (zero crossing point of diltiazem), rosuvastatin gives some absorbance Hence the wavelengths 240.0 nm and 236.4 nm were selected as analytical wavelengths for determination of diltiazem and rosuvastatin, respectively. These two wavelengths were used for the estimation of diltiazem and rosuvastatin without any interference from the other drug and excipients in their combined formulation. The overlain spectra for standard rosuvastatin and diltiazem are shown in Figure-1. The overlain first derivative spectrum is shown in Figure-2. First-derivative spectra give good quantitative determination of both drugs at their respective ZCPs without any interference[19-21].

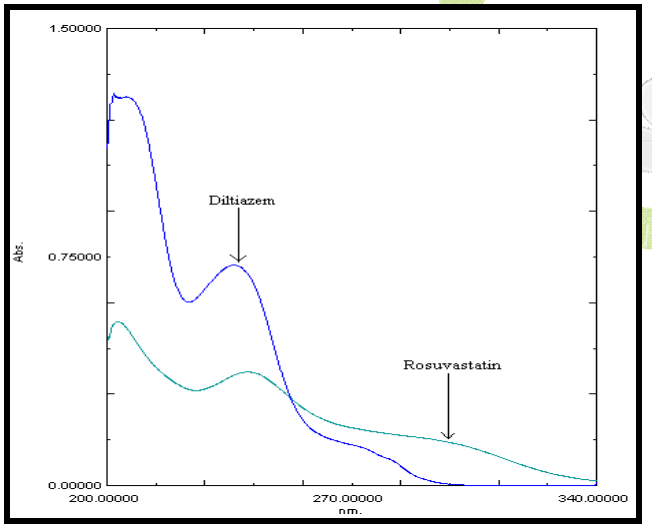

Figure 1: Overlain Zero Order Absorption Spectra of Standard Solution of Rosuvastatin and Diltiazem

Method Validation

The proposed method has been validated for the simultaneous determination of rosuvastatin and diltiazem in tablet dosage form using following parameters.

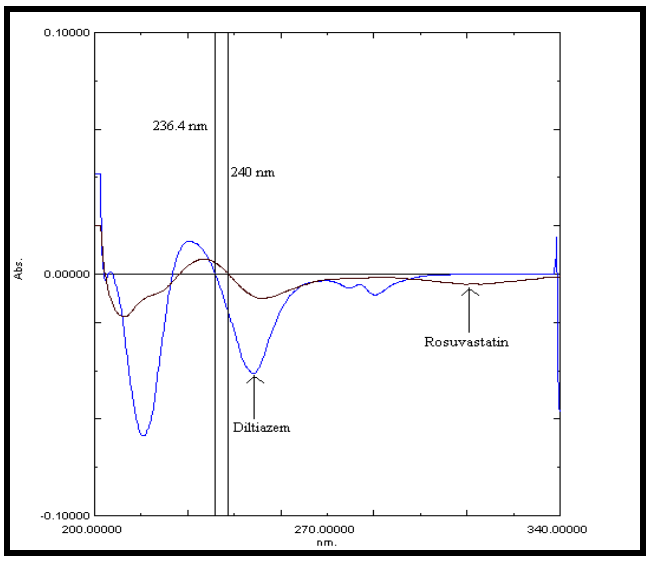

Figure: 2 Overlain First Derivative Absorption Spectra of Standard Solution of Rosuvastatin and Diltiazem

Linearity & Range

The first derivative absorbance values at wavelength 240 nm were found to be linear in the range of 4 – 40 µg/ml of diltiazem with correlation coefficient 0.9964. Further the first derivative absorbance values at 236.4 were found to be linear in the range of 4 – 40 µg/ml of rosuvastatin with correlation coefficient 0.9992. The calibration curves of diltiazem and rosuvastatin are shown in Figure-3 and Figure-4, respectively. This result confirms the suitability of the proposed method for the simultaneous determination of rosuvastatin and diltiazem from their mixture.

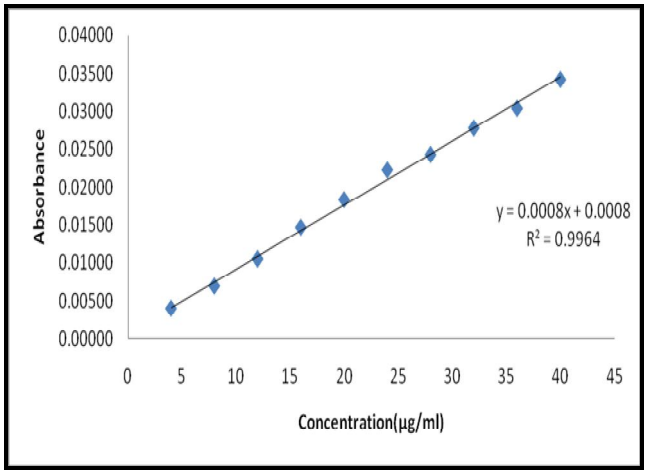

Figure: 3 Calibration Curve of Diltiazem at 240.0nm (ZCP of Rosuvastatin)

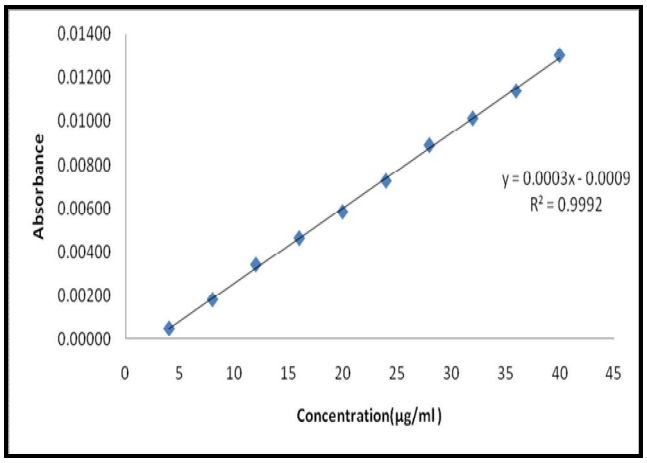

Figure: 4 Calibration Curve of Rosuvastatin at 236.4nm (ZCP of diltiazem)

Accuracy

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 1 suggested that method was accurate for the simultaneous estimation of rosuvastatin and diltiazem in their combined dosage forms.

Method precision (Repeatability)

The precision of the method was checked by repeated measurement of absorbance of solution of (n = 6) of rosuvastatin (4 µg/mL) and diltiazem (24 µg/mL) without changing the parameter of proposed method. Result of the repeatability studies are given in the Table 2.

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 over a period of 1 week for 3 different concentrations of standard solutions of rosuvastatin and diltiazem (12, 20 and 24 µg/ml). The results are reported in terms of Relative Standard Deviation (RSD) in Table 3.

LOD and LOQ

LOD and the LOQ of the drug were calculated using the following equations as per ICH guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Table 1: Recovery Data

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*
Diltiazem	0	8	0	7.9	---	----
	50%	8	4	11.80	3.90	97.54 ± 0.56
	100%	8	8	16.13	8.23	102.94± 0.79
	150%	8	12	19.75	11.85	98.75 ± 0.78
Rosuvastatin	0	8	0	8.17	---	----
	50%	8	4	12.16	3.99	99.72 ±1.19
	100%	8	8	16.08	7.91	98.87 ± 1.43
	150%	8	12	19.98	11.81	98.44 ± 0.75

* Mean % Recovery ± SD where n=6.

Table 2: Precision Data for Rosuvastatin and Diltiazem

Rosuvastatin(4 µg/ml) and Diltiazem (24 µg/ml)	**Absorbance at 236.4nm (ZCP of Diltiazem)**	**Absorbance at 240.0nm (ZCP of Rosuvastatin)**
1	0.00091	0.02248
2	0.00092	0.02237
3	0.00093	0.02220
4	0.00091	0.02231
5	0.00092	0.02235
6	0.00092	0.02245
Mean	0.00092	0.02236
S.D.	0.0002	0.0001
%RSD	0.820	0.458

Table 3: 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	0.01042±0.907	0.01042±1.344
	20	0.01823±0.592	0.01823±1.012
	28	0.02431±0.669	0.02433±0.975
Rosuvastatin	12	0.00344±0.889	0.00334± 1.53
	20	0.00585±0.691	0.00573± 1.406
	28	0.00882±0.398	0.00874± 1.08

Where, σ = The Standard deviation of the response

S = Slope of calibration curve.

LOD values for rosuvastatin and diltiazem were found to be 0.62 and 0.50 µg/ml, respectively and LOQ values for rosuvastatin and diltiazem were found to be 1.85 and 1.5 µg/ml, respectively. These data showed that proposed method was sensitive for the determination of rosuvastatin and diltiazem.

Table 4: Analysis of Sample by First Derivative 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	20.500	120.625	102.500	100.521
2	20	120	20.000	119.188	100.000	99.323
3	20	120	19.833	118.813	99.167	99.010
4	20	120	19.667	121.313	98.333	101.094
5	20	120	20.333	119.688	101.667	99.740
6	20	120	20.167	120.688	100.833	100.573
Mean			20.083	120.052	100.417	100.043
S.D.			0.312	0.973	1.559	0.811

Assay of the pharmaceutical formulation

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 4.

CONCLUSION

The proposed method is based on first derivative data processing and only requires measurement of absorbance at selected wavelengths i.e. zero crossing points. The values of % RSD were 0.820 and 0.458 for determination of rosuvastatin and diltiazem respectively, showing repeatibility 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.

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