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Validated Spectrophotometric Method of Estimation of Rosuvastatin By Using Hydrotropic Solubilization

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ABSTRACT

Concentrated aqueous hydrotropic solutions of sodium benzoate, sodium salicylate, urea, nicotinamide, sodium citrate and sodium acetate have been observed to enhance the aqueous solubilities of many poorly water-soluble drugs. Hydrotropic solubilization involves the addition of large amount of a second solute to increase the aqueous solubility of the first solute and precludes the use of organic solvents. These hydrotropes are economic and pollution-free. In the present investigation, 1.5M Urea solution was employed as hydrotropic solubilizing agent to solubilize poorly water-soluble drug Rosuvastatin for its spectrophotometric analysis. Rosuvastatin exhibits absorption maximum at 238nm .Beer's law was found to be obeyed in the concentration range of 2-10µg/mL. In this method, there is no interference from any common pharmaceutical additives and diluents. The correlation co-efficient ('*r* ' value) for Rosuvastatin was0.99955.The results of analysis have been validated as per ICH guidelines. The percentage recoveries obtained for Rosuvastatin Calcium ranges from 99.93 to 99.97 %. The method is accurate, precise and economical.

INTRODUCTION

Chemically Rosuvastatin (RSV) 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 (Fig 1). It is a lipid-lowering drug[1-2].The mechanism of action is the competitive inhibition of the enzyme HMG-CoA reductase, which catalyses the reduction of 3-hydroxyl-3-methylglutaryl coenzyme A to mevalonate, which is a rate limiting step in hepatic cholesterol synthesis. It is used in the treatment of hyper-cholesterolemia and dyslipidemia[3-4]. A review of literature showed that few solid phase extraction using tandem MS, LC-MS methods[5-7], one HPTLC method[8] and one Simple UV method [9] are reported for the estimation of RSV in pharmaceutical preparations and in biological fluids. Hydrotropy refers to the ability of a concentrated solution of a chemical compound to increase the aqueous solubility of another compound. Sodium benzoate, sodium salicylate, sodium acetate, sodium glycinate, sodium ascorbate, niacinamide, urea and sodium citrate are the most popular examples of hydrotropic agents which have been used to solubilize a large number of poorly water-soluble compound [10-30]. Maheshwari et al[10-18] analyzed various poorly water-soluble drugs, using hydrotropic solubilization phenomenon and mixed-solvency phenomenon including ibuprofen, salicylic acid, aspirin, frusemide, tinidazole, ketoprofen, cefixime, hydrochlorothiazide, cephalexin and piroxicam. UV/Visible absorption spectrophotometric method for the estimation of poorly water-soluble drug Rosuvastatin Calcium in pharmaceutical formulations has been developed. Aqueous solubility of RSV was enhanced to a great extent in 1.5 M Urea. The primary objective of the present investigation was to employ the hydrotropic solution to extract the drug from the dosage form and precludes the use of corrosive organic solvents.

MATERIALS AND METHODS

Instrument

Spectrophotometric analysis was carried out by using a double beam UV-visible Spectrophotometer (Shimadzu model UV-1700, Japan) with 1cm matched quartz cells .

Reagents and Chemicals

Rosuvastatin Calcium(RSV) supplied by Dr. Reddy's Laboratory, Hyderabad, India .All

chemicals were analytical grade obtained from SD fine chemicals. Water was purified by glass distillation apparatus.

Methods

Preliminary Solubility Study of the Drug

Solubility of Rosuvastatin Calcium was determined at 28±1°.

An excess amount of drug was added to 1.5M urea solution in vials. The vials were shaken mechanically for 12 h at 28±1°, in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hours, and then centrifuged for 5 minutes at 2000 rpm. The supernatant of each vial was filtered through Whatmann filter paper No. 41. The filtrates were diluted suitably, and analyzed spectrophotometrically against corresponding solvent blank.

Preparations of Standard Drug Solutions

For hydrotropic solubilisation, 50 mg of pure RSV was dissolved in 50 ml of 1.5 M urea solution and stirred for 15 minutes and the final volume was made up to 100 ml with distilled water [31].The solution was filtered through Whatmann filter paper No. 41 and was diluted with distilled water to prepare working concentrations of 500 µg/mL of RSV.This stock solution was further diluted suitably with distilled water to get a concentration of 10µg/ml and was then scanned in UV range 200-350nm.The spectrum showed an absorption maximum at 238 nm (Figure 2). From the spectra of the drug RSV (Figure 2) and 1.5M urea (Figure 3), it was found that the 1.5 M urea used does not interfere with the sampling wavelength. Therefore 1.5 M urea is used for the solubilization of drug. Aliquots of stock solution corresponding to 2-10 µg/mL were taken in a series of 100 mL volumetric flask and volume made up with distilled water. The absorbance measurements of these solutions were carried out at 238 nm against the blank prepared in the same manner omitting the drug. Calibration curve was prepared by plotting absorbance against concentration (Figure.4) and the data is given in Table.1.

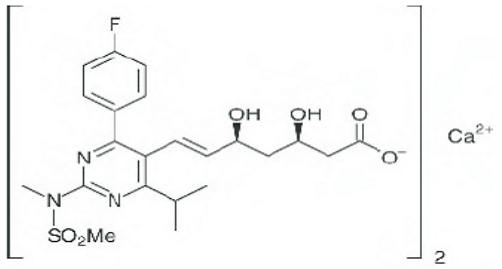

Fig.1: Chemical Structure of Rosuvastatin Calcium

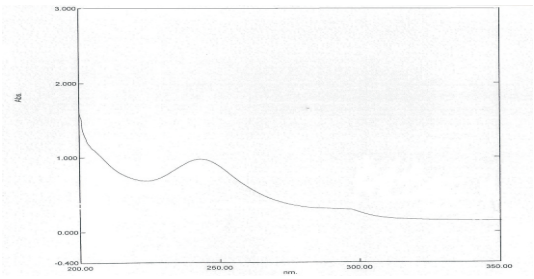

Fig.2: UV Spectrum of Rosuvastatin Calcium

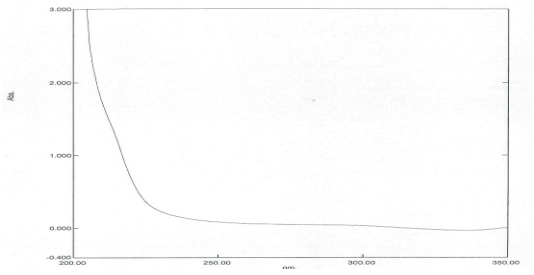

Fig.3: UV Spectrum of 1.5M Urea

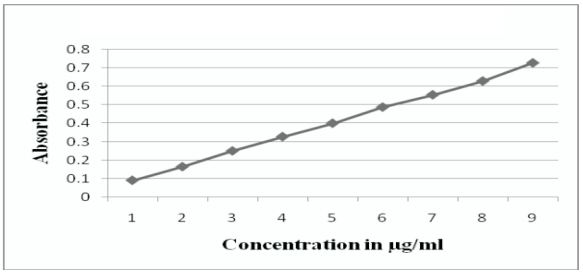

Fig. 4: Caliberation plot of Rosuvastatin

Table No.1: Data for Calibration Plot

Sl No:	Volume of RSV stock solution(ml)	Concentration of RSV in final solution (µg/ml)	Absorbance at 238 nm
1	0.4	2	0.0901
2	0.6	3	0.1638
3	0.8	4	0.24948
4	1.0	5	0.3264
5	1.2	6	0.3992
6	1.4	7	0.4865
7	1.6	8	0.5532
8	1.8	9	0.6277
9	2.0	10	0.7269

The data reveals that Beer's law is obeyed from 2 – 10 µg/ml

Table No.2: Optical Characteristics of Rosuvastatin for the developed method

No	Parameters	
1	Beers law limit	2-10µg/mL
2	Correlation coefficient	0.99955
3	Y= ax + C	Y= 0.0784406x-0.0680573
4	Molar absorptivity	7.8441x 10^4L/mol/cm.
5	Sandell's sensitivity	0.0127 (µg/cm^2 / 0.001 absorbance unit)

Procedure for analysis of tablet formulation

Two commercial formulations, Rozavel (M/s Sun Pharma) and Rosuvas (M/s Ranbaxy Ltd.) were purchased from local market. The average weight of each tablet was calculated by weighing 20 tablets and were powdered finely in a glass mortar. Powder equivalent to 50 mg each of RSV was weighed and transferred to two 100 ml volumetric flasks, 70 ml of 1.5M urea solution was added to both the flask and stirred for 15 min to dissolve the drug and the final volume was made up to 100 ml with distilled water. The solutions were filtered through Whatmann

Table No.4: Data of Recovery studies

No	Brand Name	Drug added (spiked) (mg)	Amount found, mg*	% Recovery estimated (Mean±S.D) (n=6)
1	Rozavel	10	59.982	99.97±0.01639
2	Rosuvas	10	59.955	99.93±0.0225

*Average of six determinations

Table No.3: Result of Analysis of tablets

Brand Name	Concn. µg/mL	Absorbance *	% Label claim	Active content per tablet (mg)	Mean% Label claim	Standard deviation	Standard Error
Rozavel	3	0.1637	99.93	9.99	100.01	0.00067	0.000384
	5	0.3267	100.09	10.09		0.00045	0.000260
Rosuvas	3	0.1631	99.57	9.957	99.90	0.00026	0.000153
	5	0.3259	99.85	9.985		0.00040	0.000233

*Mean of three determinations

Table No.5: Data for repeatability

Concentration (µg/ml)	Absorbance at 238nm	Mean value	Standard deviation	Standard Error	Coefficient of variation
	0.1644				
3	0.1635	0.1637	0.000665	0.0003844	0.003322
	0.1631				
	0.3272				
5	0.3267	0.3267	0.0004509	0.0002603	0.001126
	0.3263				
	0.4856				
7	0.4851	0.4852	0.0004042	0.000233	0.000680
	0.4848				

filter paper No. 41 and the first few ml were rejected. The filtrates were diluted suitably with distilled water to get 3 µg/mL and 5 µg/mL each of Rosuvastatin Calcium. The absorbance at 238 nm was measured and the amount of drug present in the sample solutions were obtained from the slope and intercept values obtained from the calibration curve (Table No.1). The experiments were repeated three times to check its reproducibility. The results of analysis of tablet formulations are recorded in Table No.3.

Method Validation

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision, robustness and accuracy for the analyte [32]. To evaluate the validity and reproducibility of the method, known amount of pure drug was added to the analyzed sample of tablet powder and the mixture was analyzed for the drug content using the proposed method. For this recover study the tablet powder equivalent to 50 mg drug was taken in two 25 ml volumetric flask. 10 mg of pure drug (spiked drug) was transferred to each flask and 20 ml of 1.5 M urea solution was added and the flasks were shaken for about 10 min. Then, volume was made up to the mark with distilled water and filtered through Whatman filter paper No. 41. The solutions were diluted appropriately with distilled water and analyzed for drug content against blank. The percentage recovery was found to be within the range (Table 4).

Repeatability expresses the precision under the same operating conditions over a short interval of time. The precision of an analytical procedure is usually expressed as the standard deviation of a series of measurements. The reproducibility of the method was studied using three different concentrations of RSV (3, 5 and 7µg/ml) which were prepared from stock solution. The absorbance was measured at 238nm against 1.5 M urea as blank for three times and their mean values were calculated and the data is given in table.5.The intra-day and inter-day precision studies

Table No.6: Intra-day precision

Concentration (µg/ml)	Absorbance at 238 nm			RSD %
	0 hr	1.5 hr	3 hr	
3	0.1644	0.1621	0.1612	1.02
5	0.3272	0.3258	0.3241	0.48
7	0.4856	0.4831	0.4825	0.34

Table No.7: Inter-day precision

Concentration (µg/ml)	Absorbance at 238 nm			RSD %
	1st day	2nd day	5th day	
3	0.1644	0.1609	0.1591	1.67
5	0.3272	0.3228	0.3198	1.15
7	0.4756	0.4610	0.4706	1.58

of RSV were carried out by estimating the corresponding responses three times on the same day and on three different days (1st, 2nd and 5th day) for three different concentrations of RSV (3, 5 and 7µg/ml) and the results are reported in terms of relative standard deviation in table.6 and table.7

Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD of RSV by the proposed method was found to be 0.5µg/ml.

Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample, which can be quantitatively determined with a suitable level of precision and accuracy.The LOQ of RSV by the proposed method was found to be 2µg/ml.

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are proportional to the concentration of analyte in the sample. The calibration curve of RSV was linear over the range of 2 - 10g/ml.

RESULTS AND DISCUSSION

Quantitative estimation of poorly water-soluble drugs involves use of organic solvents. Major drawbacks of organic solvents include high cost, volatility and toxicity. In the present investigation, hydrotropic solubilization is employed to enhance the aqueous solubility of poorly water-soluble drug like Rosuvastatin Calcium in tablet dosage forms. The results of solubility studies indicated that enhancement in aqueous solubility of Rosuvastatin Calcium in 1.5 M urea solution was more than 5 folds as compared to their solubility in distilled water. Therefore, this solution was employed to extract Rosuvastatin Calcium from the fine powder of tablet formulation. By proper choice of hydrotropic agents, the use of organic solvents in analysis may be discouraged to a large extent. It is evident that there is good agreement between the amount estimated and those claimed by the manufacturers. The mean percentage label claims 99.90 and 100.01for Rosuvas and Rozavel respectively (Table 3) are very close to 100 with low values of standard deviation and standard error which confirms the accuracy of the proposed method. Accuracy, reproducibility and precision of the proposed method were further confirmed by the mean percentage recovery values (99.93 to 99.97), which were close to 100 with low values of standard deviation (Table.4). The proposed method for determination of RSV showed molar absorptivity of 7.8441x 10^4L/mol/cm and Sandell's sensitivity of 0.0127 (µg/cm^2/0.001 absorbance unit). Linear regression of absorbance on concentration gave the equation y = 0.0784406x-0.0680573 with a correlation coefficient (r) of 0.99955(Table 2). Moreover the developed method is economic, simple, precise and rapid, hence can be employed for routine analysis for the estimation of Rosuvastatin from marketed formulations and in biological fluids.

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