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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF CLOPIDOGREL BISULFATE AND ROSUVASTATIN CALCIUM IN BULK AND FORMULATION.

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Abstract

Two simple spectrophotometric methods have been developed for simultaneous estimation of Rosuvastatin Calcium (RS) and Clopidogrel bisulphate (CP) from tablet dosage form. Method (I) is simultaneous equation method, wavelengths selected are 243 and 230 nm for RS and CP respectively. Method (II) is the absorbance ratio method, based on the determination of graphical absorbance ratio at two selected wavelengths, one being the iso-absorptive point 227 nm and other being the absorption maxima of RS at 243 nm. The linearity ranges for were found RS and CP to be 2-10 µg/mL-1 and 5-25 µg/ml respectively. The accuracy and precision were determined and found to comply with ICH guidelines. Both the methods showed good reproducibility and recovery with % RSD in the desired range. The proposed methods can be successfully applied for the routine analysis of both the drugs from tablet dosage form. Proposed method is comparatively cheap and can easily apply for routine analysis

INTRODUCTION:-

Clopidogrel:-

(CP) is an analogue of ticlopidine, chemically known as, methyl-2-chlorophenyl- (4, 5, 6, 7 tertra hydro thieno [3,2-c] pyridine-5yl) acetate bisulphate [1,2]. It is an antiplatelet agent, it is used for the long term prevention of atherothrombotic events (myocardial infarction, stroke, peripheral arterial disease, acute coronary syndrome, cardio-vascular death) it is used in the treatment of cardiovascular diseases. (drugbank: DB00758)

Rosuvastatin:-

(RS) is the calcium salt of (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(Nmethylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5dihydroxyhept-6-enoic acid. Rosuvastatin is a competitive inhibitor of the enzyme HMG-CoA reductase, having a mechanism of action similar to that of other statins. RS is official in IP whereas CP is official in USP. The chemical structures of RS & CP are shown in Fig.1. Combination drug products of RS and CP are widely marketed and used in the treatment of cardiac disorders (drug bank: DB01098).

Analytical methods like UV spectrophotometry, HPLC, HPTLC have been reported for estimation of RS & CP by single drug and also by combining with other drugs. However no method has been reported till date for the simultaneous estimation of RS & CP using the UV spectrophotometric method where drug solubalized by Hydrotrophy. The proposed methods are optimized and validated as per the ICH guidelines Harmonized Tripartite Guideline Q2b (R1).

MATERIALS AND METHODS:-

Instrumentation & Reagents:-
SHIMADZU double beam UV visible spectrophotometer (model 1800). Shimadzu ATX 224 balance. Double distilled water and Whatman filter paper , Urea, Active pharmaceutical ingredient (API) of Clopidogrel Bisulphate (CP) and Rosuvastatin Calcium (RS) and test samples Rosufit-CV (capsule with composition CP-75 mg and RS-5 mg) were procured from the local market Vadodara, India.

Preparation of standard solution:-
Standard stock solutions (1000 µg/ml) of RS and CP were prepared separately by dissolving 100 mg of powder respectively with 100 ml double distilled water in volumetric flasks & known quantity of 2M Urea were an added and diluted with same solvant to get final concentrations in range of 2-10 µg/ml and 5-25 µg/ml for both the drugs respectivly. The solution was scanned in the range of 200 to 400 nm against double distilled water as blank, the excitation wavelengths were found to be 243 nm for RS and 230nm for CP and Iso-absorptive point at 227 nm. The calibration curve was obtained by plotting absorbance values at 230nm & 243nm & at 230nm & 227 nm against amount of standard drug in µg/ml.

Preparation of sample solution:-
Twenty Capsule labeled as containing 5 mg of RS and 75 mg of CP together with excipients, weighed and emptied and weight of the powder equivalent to one capsule content (390 mg) was accurately weighed then transferred to a clean 100 ml volumetric flask, known quantity of 2M Urea and 20 ml double distilled Water was added, and the flask was attached to a rotary shaker for 10 min to disperse the material completely. The mixture was then sonicated for 10 min and diluted to volume double distilled Water to give a solution containing 50 µg/ml concentration of RS and 750 µg/ml concentration of CP. This solution was filtered through Whatmann filter paper, the sample solutions of required concentration are prepared and diluted with same solvent.

Method I (Simultaneous equation method):-
Simultaneous equation method uses the absorbances at two selected wavelengths, both being the λmax of the two drugs. Working standard solutions were scanned in the range of 200-400 nm to determine the λmax of both the drugs. The λmax of CP and RS were found to be 230 nm and 243 nm respectively (fig.1 & 2). standard solutions having concentrations 5-25 µg/ml and 2-10 µg/ml for CP & RS were prepared in known quantity of 2M Urea with Double distilled Water. The absorbances of resulting solutions were measured at 230 nm and 243 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficient of these two drugs was determined using the calibration curve equation. Cx=(A2ay1-A1ay2)/(ax2ay1-ax1ay2) and Cy=(A1ax2-A2ax1)/(ax2ay1- ax1ay2). (Beckett et al 2002)

Method II (Absorbance ratio method):-
Absorbance ratio method uses the ratio of absorbances at two selected wavelengths one at iso-absorptive point and other being the λmax of one of the two components. From the overlay spectra of two drugs, it is evident that CP and RS show an iso-absorptive point at 227 nm and the second wavelength used was 243 nm which is λmax of RS. Standard solutions having concentrations 5-25 µg/ml and 2-10 µg/ml of CP & RS respectively were prepared with known quantity of 2M Urea and diluted with double distilled Water. The concentration of sample mixture can be calculated using equations **Cx = [(Qm-Qy)/(Qx-Qy)] xA1/ ax1 and Cy=[(Qm-Qx)/(Qy-Qx)] x A1/ay1.**

VALIDATION PARAMETERS
(International Conference On Harmonization Q2 a(R1):-

Precision
Precision of the methods was determined by performing inter day variation, intra day variation and repeatability studies. In inter day variation, the absorbance of sample solutions of CP and RS (10 µg/ml) were measured on three consecutive days. In intraday variation, the absorbances were measured three times in a day. In repeatability study, six determinations of concentration (10µg/ml) of both the drugs were analyzed. The results are shown in Table 2

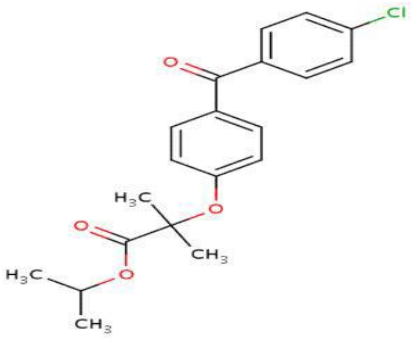
Accuracy

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to pre-analyzed tablet powder and percentage recoveries were calculated. The results are shown in Table 3.

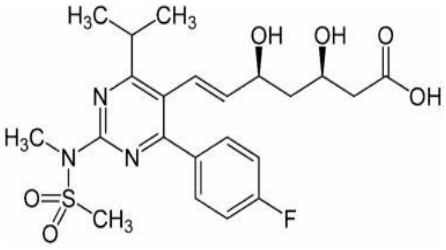
RESULTS AND DISCUSSION:-

For Method I under the established conditions, Rosuvastatin calcium and Clopidogrel bisulfate showed good correlation with Beer's law over the concentration range from 2-10 µg/ml and 5-25µg/ml excitation wavelengths 243nm and 230 nm with the regression equation [y = 0.036x + 0.015] & [y = 0.030x - 0.012] for RS & CP respectively. The correlation coefficient (r2) was found to be 0.99 for both the drugs. A relative standard deviation of 1.1 % and 1.01% was observed on analysis of six replicate samples at excitation wavelengths 243 nm and 230 nm respectively. The percent recovery studies revealed that the value lies between 99.99 % - 103 % and 99.00 % - 101 % at wavelengths 243nm and 230nm respectively. Results of recovery studies demonstrated that the proposed method was highly accurate. Both inter-day as well as intra-day precisions were carried out in different concentration of the solutions and the relative standard deviation (RSD) was found to be less than 2.0. Results obtained confirmed the ruggedness of the method. For Method II under the established conditions, Rosuvastatin calcium and Clopidogrel bisulfate showed good correlation with Beer's law over the concentration range from 2-10µg/ml at excitation wavelengths 227nm (iso-absorptive point) and 243nm with the regression equation
[y = 0.036x + 0.015] & [y = 0.030x - 0.012] for RS & CP respectively. The correlation coefficient (r2) was found to be 0.998 & 0.999 respectively. A relative standard deviation of 1.1 % and 1.01% was observed on analysis of six replicate samples at excitation wavelengths 227nm and 270nm respectively. The percent recovery studies revealed that the value lies between 99.79 % - 103 % and 99.48 % - 101% at wavelengths 227nm and 243nm respectively. Results of recovery studies demonstrated that the proposed method was highly accurate. Both inter-day as well as intra-day precisions were carried out in different concentration of the solutions and the relative standard deviation (RSD) was found to be less than 2.0.

Fig. 1 Chemical structures of the analytes (1a) CP & (1b) RS



(1a)



(1b)

Fig. 2 Standard spectra of Clopidogrel Bisulphate

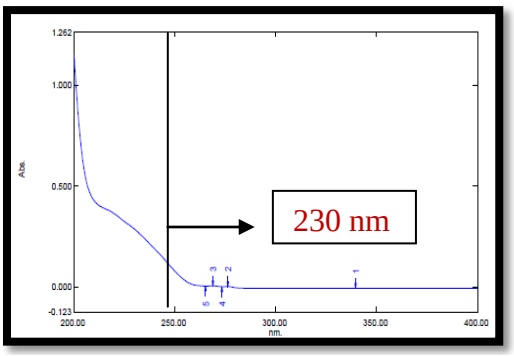


Fig 3 Standard curve of Clopidogrel Bisulphate

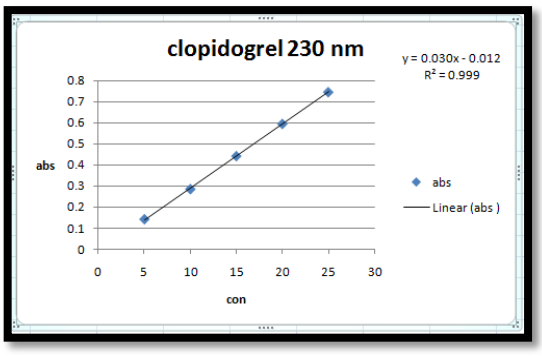


Fig. 4 Standard spectra of Rosuvastatin calcium.

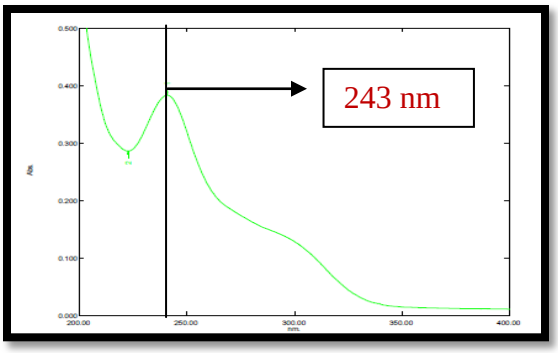


Fig 5 Standard curve of Rosuvastatin Calcium.

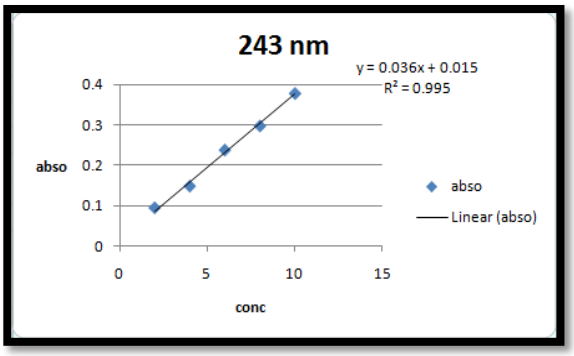


Fig. 6 Standard spectra at Iso-absorptive point.

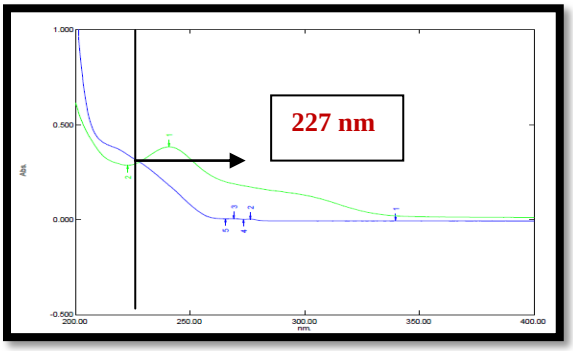


Fig 7 Standard curve of Rosuvastatin calcium.

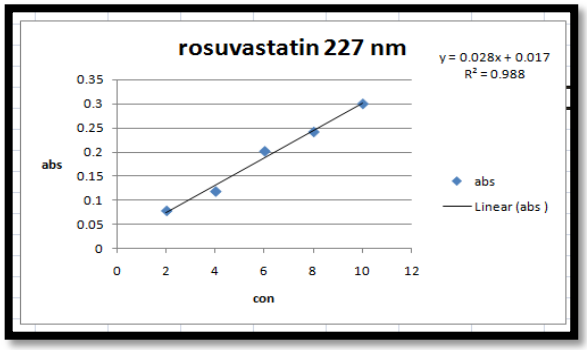


Fig 8. Overlay Spectra of mixture.

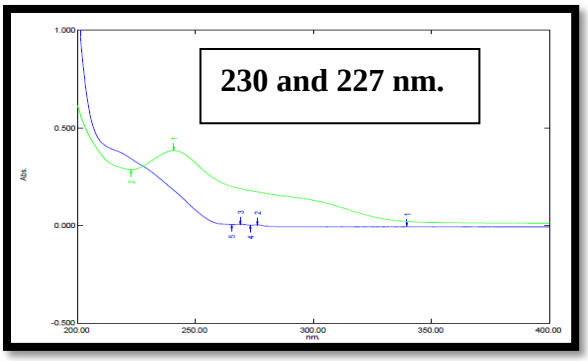


Fig 9. Overlay Spectra of both drug

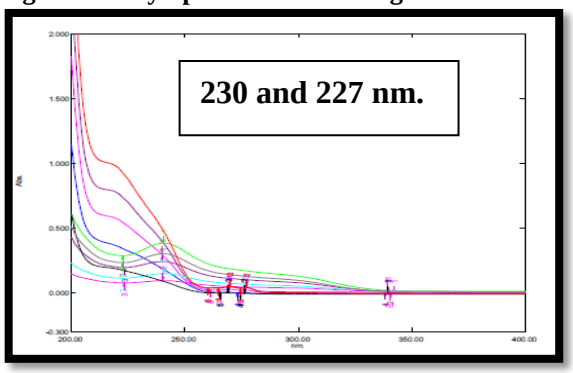


Table 1. Result of Analysis of CP & RS in tablet formulation

	Mg/tablet				% lable claim	
Method	**CP**	**RS**	**CP**	**RS**	**CP**	**RS**
	Label claim	**Label claim**	**Obtain**	**Obtain**		
Method I (SE)	75	5	75.039	5.001	100.412	100.02

MethodII (AR)	75	5	75.046	5.032	100.06	100.64

* Average of six Estimation, SE- Simultaneous Estimation, AR- Absorbance Ratio.

Table 2 Recovery Studies:-

Parameter	**Simultaneous Estimation Method**		**Absorbance Ratio Method**	
	CP	RS	CP	RS
Linearity Range (µg/ml)	5-25	2-10	5-25	2-10
Intercept	0.0124	0.0171	0.0124	0.0171
Slope	0.0304	0.030	0.0304	0.030
Correlation Coefficiant Precision (%RSD)	0.997	0.998	0.997	0.988
Intraday Precision %RSD	0.043	0.003	0.044	0.033
Interday Precision %RSD	0.044	0.003	0.044	0.033

Table 3 Recovery Studies:

Name of Drug	**Recovery Level**	**Method 1**		**Method 2**	
		% Recovery *	RSD	% Recovery *	RSD
CP	80	100.66	1.1	103	1.2
	100	100.11	0.93	100.6	1.1
	120	100.86	1	100.1	0.93
RS	80	103	1	99.89	1.3
	100	100.66	1	100.7	1.1
	120	99.96	1.3	102	1

* Average of three Estimation

CONCLUSION:-

Proposed UV methods are specific, accurate and precise for the simultaneous determination of Rosuvastatin calcium and Clopidogrel bisulphate from pharmaceutical dosage form. The described methods are suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

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