Validated spectrophotometric method for simultaneous estimation of rosuvastatin calcium and aspirin in tablet dosage forms

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Abstract

The present work was aimed at method development and validation for simultaneous estimation of Rosuvastatin Calcium and Aspirin by UV-Spectrophotometric method in pharmaceutical dosage form. In UV method 0.1N NaoH as solvent and λmax of Rosuvastatin Calcium and Aspirin were found to be 232 nm and 222 nm respectively. Concentration ranges were found to be 4-20µg/mL for both drugs. The R^2 values were found to be 0.996 and 0.999 for Rosuvastatin Calcium and Aspirin respectively. The method was validated statistically and by recovery studies. Percentage Assay and Recovery were found to be 95-105% for Rosuvastatin Calcium and Aspirin. LOD and LOQ ranges were found to be 0.177 and 0.539µg/mL and 0.298 and 0.903µg/mL for Rosuvastatin Calcium and Aspirin respectively. This method was validated using ICH guidelines.

Key words: Aspirin, Rosuvastatin, UV-Spectrophotometric method, Simultaneous equation, Validation.

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Introduction

Rosuvastatin calcium, (E)-(3R,5S)-7-[4-(4-fluorophenyl)-6-isopropyl-2{methyl(methylsulphonyl amino)}pyrimidin-5-yl]-3,5-dihydroxyhepten-6-oic acid calcium, is a HMG Co-A Reductase inhibitor which is used in Hyperlipidemia1, 2. Literature survey revealed that various UV, HPLC and HPTLC methods reported for the estimation of Rosuvastatin calcium in pharmaceutical formulations[2-10]. Aspirin, 2-acetobenzoic acid, is a Non-steroidal anti-inflammatory, Antirheumatic, Antithrombotic which is used in pain; fever; inflammatory conditions; reduction of MI1. Literature survey revealed that there is titration, difference and HPLC methods are available for estimation of

Aspirin in pharmaceutical dosage form. Extensive literature survey reveals, only one UV method is available for simultaneous estimation of Rosuvastatin calcium and Aspirin in their combined dosage form. Aim of present work was to develop simple, precise, accurate and economical spectrophotometric methods for simultaneous determination of Rosuvastatin calcium and Aspirin in their combined dosage form. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines. **Literature survey[11]** revealed that there were few methods reported for the estimation of Rosuvastatin Calcium and Aspirin by RP-HPLC and UV Spectrophotometry in the combined dosage form. And also HPLC, Spectroscopic methods have been reported for the estimation of individual drugs and in combination with other drugs. Our study, attempts to develop a simple, precise, accurate, sensitive and economical method for the simultaneous estimation of Rosuvastatin Calcium and Aspirin by UV-Spectrophotometric method in pharmaceutical dosage form.

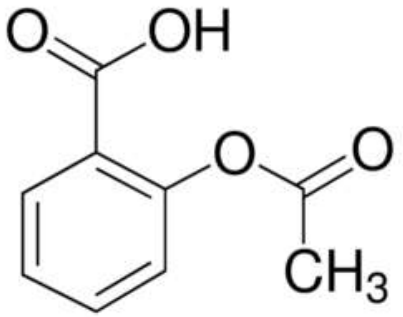

Fig. 1: Chemical Structure of Aspirin

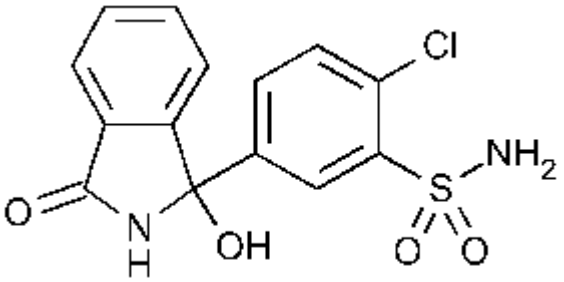

Fig. 2: Chemical Structure of Rosuvastatin

Experimental

Instrumentation: Double beam UV Visible - Spectrophotometer, Lab India (Brand), UV 3000$^+$ with a pair of 1cm matched quartz cells, all weighing was done on Shimadzu Electric balance, capacity (220gm), readability (0.001gm) and sonication was done in Digital Ultra Sonicator CITIZEN.

Chemicals and Reagents: Rosuvastatin Calcium and Aspirin were reference samples were procured from cipla Pharmaceuticals, Mumbai. Marketed formulation Unistar 150 (Sun Pharmaceutical Ltd., H.P.), with label claim 10 mg RST, 150 mg asp and were purchased from local market. Sodium hydroxide of analytical grade and double distilled water were used throughout the analysis.

Preparation of 0.1N NaOH: 4gm. of NaOH was added to sufficient quantity of distilled water and finally volume was made up to 1000mL with distilled water to get the concentration of 0.1N NaOH.

Solubility Testing: As 0.2M H_2S0_4 was already reported as solvent, a trial was made to get an alternate solvent for estimation of Rosuvastatin Calcium and Aspirin by using 0.1N NaOH solution. It has been found that 0.1N NaOH is more suitable as solvent in terms of both solubility and stability.

Preparation of standard stock solutions: Standard stock solutions of Rosuvastatin Calcium and Aspirin were prepared by dissolving 100mg of each drug in 100mL of 0.1N NaOH individually to get the concentration of 1000 µg/mL.

Preparation of working standard solutions: 10mL of each drug solution was taken from the stock solution and diluted to 100mL with 0.1N NaOH in 100mL volumetric flask to get the concentration of 100 µg/mL individually.

Determination of λ max: The working standard dilutions of each drug were scanned from 200nm-400nmto determined the λmax individually. Rosuvastatin Calcium and Aspirin were showed maximum absorbance at 232 nm and 222nm respectively.

Validation Parameters

Linearity Range: Adequate dilutions were made from working standards to get the concentrations of 4-20 µg/mL for both Rosuvastatin Calcium and Aspirin using 0.1N NaOH. Absorbance of these solutions were determined at their corresponding λmax. The measured absorbance was plotted against concentrations.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The detection limit of an individual analytical procedure is the lowest of analyte in a sample which can be detected but not necessarily be quantitated as an exact value. The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It is used particularly for determination of impurities and/degradation studies. For these LOD and LOQ five sets of linear dilutions were prepared and standard graphs were drawn. From the standard graphs standard deviation of the intercept and mean of the slope were calculate, then LOD and LOQ values were calculated using the following formulas.

$$LOD = 3.3\ S_a/b$$

$$LOQ = 10\ S_a/b$$

S = Standard deviation of intercept

b = mean of slope of calibration curve

Precision Studies

System Precision: A fixed concentration of 12:1.5µg/mL of the each standard drug as mixture from the linearity range was checked for absorbance, then SD and percentage RSD values were calculated.

Method Precision: A fixed concentration in the ratio of 8:1µg/mL of the marketed formulation from the linearity range was checked for absorbance, then SD and percentage RSD values were calculated.

Intraday Precision: Dilutions of 4,8,12 µg/mL concentration mixtures were prepared from two drugs. Absorbance of all the dilutions was checked for every one hour for 5hours, then SD and percentage RSD values were calculated.

Inter day Precision: Dilutions of 4,8,12 µg/mL concentration mixtures were prepared from standard drugs. Absorbance of all the dilutions was checked for 5 days, then SD and percentage RSD values were calculated.

Assay: Accuracy weighed and powered 20 tablets of unistar-150, manufactured by Unichem Laboratories Himachal Pradesh. It contains 150 mg of Aspirin and 10 mg of Rosuvastatin. A weight equivalent to 50 mg Aspirin of the powdered tablet was taken and to this added 25mL of 0.1N NaOH in 50mL standard volumetric flask. Ultra sonication was down for 30min. and kept overnight for dissolving. Again ultrasonication was done filtered using Whattman filter paper grade 1, then volume was made up to the mark with 0.1N NaOH.

The stock solution prepared above contains both the drugs in the ratio of 1:8. 10 mL from the stock solution was taken in the 100mL volumetric flask and the volume was made up to the mark to get the concentration of 100 µg/mL of Aspirin and corresponding concentration of Rosuvastatin (working standard).

From working standard following dilutions were prepared for determination of the percentage purity of the marketed formulation.

8:1 µg/mL: 0.8mL of working standard was taken and diluted to 10mL to get concentration of 8 µg/mL of Aspirin and 1 µg/mL of Rosuvastatin.
12:1.5 µg/mL: 1.2mL of working standard was taken and diluted to 10mL to get concentration of 12 µg/mL of Aspirin and 1.5µg/mL of Rosuvastatin.
16:2 µg/mL: 1.6mL of working standard was taken and diluted to 10mL to get concentration of 16µg/mL of Aspirin and 2µg/mL of Rosuvastatin.

Amount of Rosuvastatin Calcium and Aspirin present in the marketed formulation was determined by using the following simultaneous equations.

$$C_T = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$$

$$C_R = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$$

Where C_T = concentration of Aspirin

C_R = concentration of Rosuvastatin

A_1, A_2 = Absorbance of drug at the selected two wavelengths

ax_1, ax_2 = Absorptivity values of Aspirin

ay_1, ay_2 = Absorptivity of Rosuvastatin

Amount found = Concentration in mg. × Dilution factor × Average weight

% Purity = Amount found / Labeled claim × 100

Recovery Studies (Accuracy): To ensure the reliability (accuracy) of the method recovery studies were carried out by mixing standard quantity of drug with the pre analyzed sample formulation and the contents were reanalyzed by the proposed method. To perform the recovery studies 3 dilutions were prepared using both standard drug and marketed formulation. The dilutions prepared were having concentrations of the marketed formulation in the ratio of 4:0.5 (RSC: ASP) was kept fixed and the standard drugs mixture in the ratio of 8:1(RSC:ASP) was added in 50%, 100%, 150% respectively.

First dilution (6:0.75) : To 4:0.5(RSC:ASP) µg/mL of marketed formulation 2:0.25(RSC:ASP) µg/mL standard drugs mixture was added in the 10mL volumetric flask to get the final concentration of 6:0.75 µg/mL and its absorbance value was observed.

Second dilution (8:1) : To 4:0.5(RSC:ASP) µg/mL of marketed formulation 4:0.5 (RSC:ASP) µg/mL standard drugs mixture was added in the 10mL volumetric flask to get the final concentration of 8:1 µg/mL and its absorbance value was observed.

Third dilution (10:1.25) : To 4:0.5(RSC:ASP) µg/mL of marketed formulation 6:0.75 µg/mL standard drugs mixture was added in the 10mL volumetric flask to get the concentration of 10:1.25 µg/mL and its absorbance value was observed.

Results and Discussion

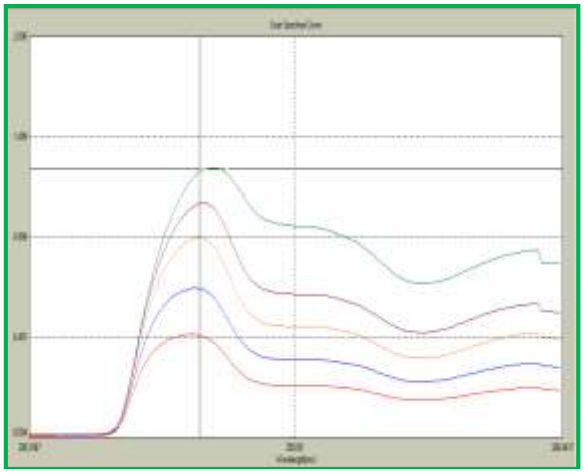

Fig. 3: UV Spectrum of RSC for Linearity

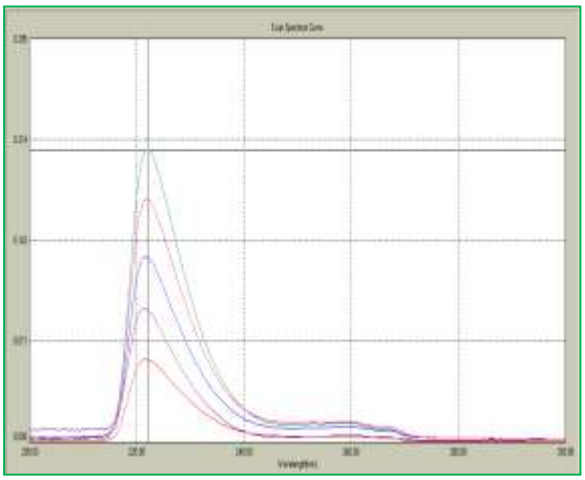

Fig. 4: UV Spectrum of ASP for Linearity

Table 1: Absorbance values of Aspirin with 0.1N NaOH

S. No.	Concentration (µg/mL)	Absorbance	
		at 232nm	At 222nm
1.	4	0.511	0.365
2.	8	0.742	0.453
3.	12	0.994	0.532
4.	16	1.168	0.607
5.	20	1.373	0.670

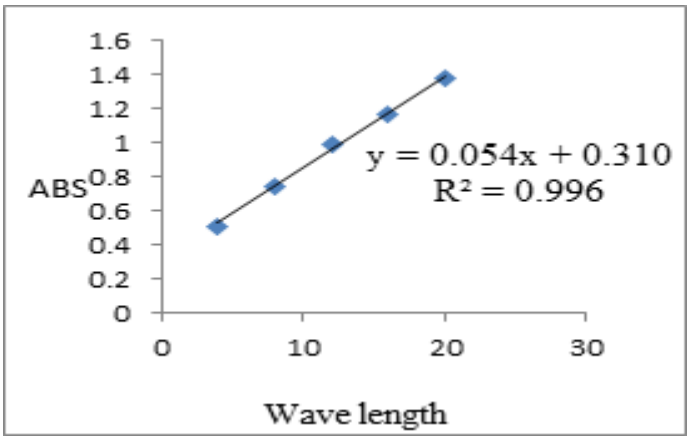

Fig. 5: Standard curve of RSC at 232nm

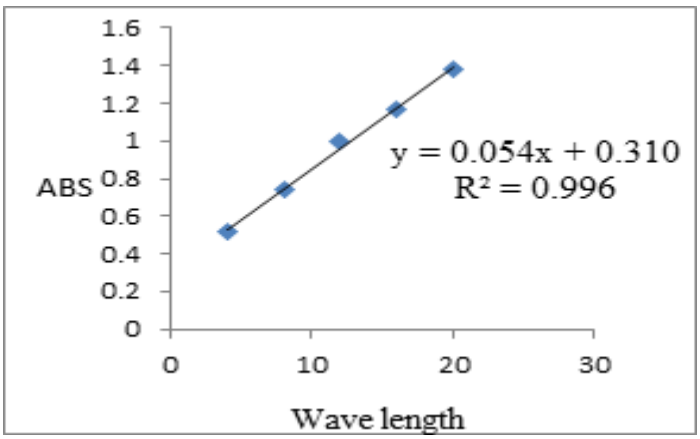

Fig. 6: Standard curve of RSC at 222nm

Table 2: Absorbance values of Rosuvastatin with 0.1N NaOH

S. No.	Concentration(µg/mL)	Absorbance	
		at 222nm	At 232nm
1.	4	0.058	0.023
2.	8	0.094	0.035
3.	12	0.131	0.050
4.	16	0.172	0.064
5.	20	0.206	0.079

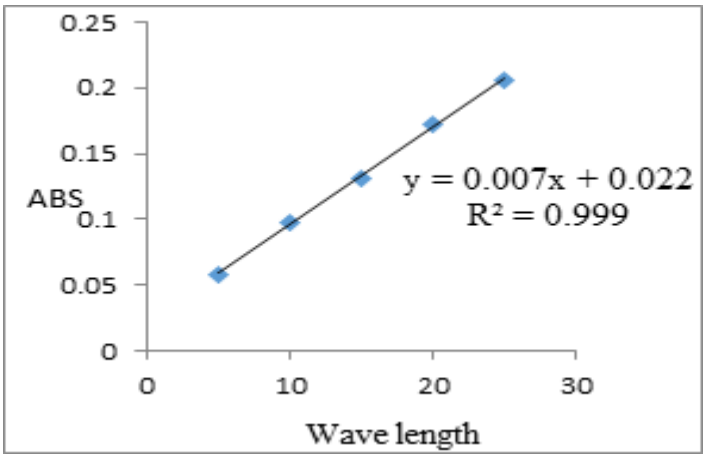

Fig. 7: Standard curve of ASP at 222nm

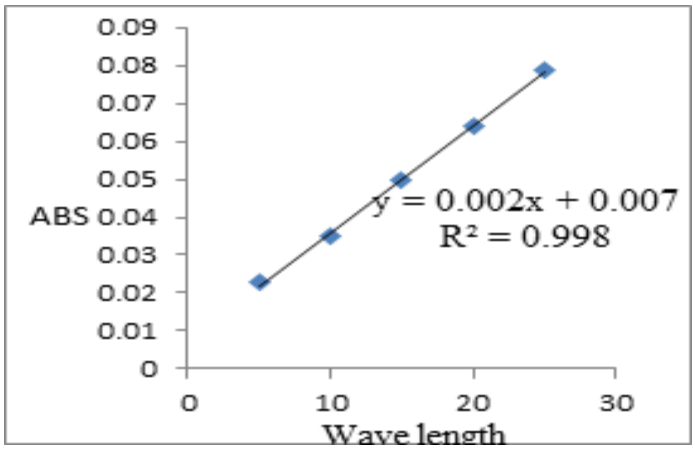

Fig. 8: Standard curve of ASP at 232nm

Table 3: Absorptivity values of Rosuvastatin Calcium and Aspirin

S. No.	Conc.(µg/mL)	Absorptivity of RSC		Absorptivity of ASP	
		232nm	222nm	232nm	222nm
1.	4	12.775×10^{-2}	9.125×10^{-2}	0.575×10^{-2}	1.45×10^{-2}
2.	8	9.2×10^{-2}	5.665×10^{-2}	0.4375×10^{-2}	1.175×10^{-2}
3.	12	8.2×10^{-2}	4.43333×10^{-2}	0.4166×10^{-2}	1.0916×10^{-2}
4.	16	7.3×10^{-2}	3.79375×10^{-2}	0.4×10^{-2}	1.075×10^{-2}
5.	20	6.8×10^{-2}	3.35×10^{-2}	0.39×10^{-2}	1.03×10^{-2}
	Avg.	8.899×10^{-2}	5.27291×10^{-2}	0.44483×10^{-2}	1.1643×10^{-2}

Table 4: LOD and LOQ values for Aspirin at 232nm

S. No.	Conc. (µg/mL)	Absorbance at 232nm				
		Set 1	Set 2	Set 3	Set 4	Set 5
1	4	0.511	0.519	0.521	0.506	0.515
2	8	0.742	0.749	0.752	0.756	0.732
3	12	0.994	0.959	0.0.963	0.967	0.999
4	16	1.168	1.171	1.175	1.156	1.165
5	20	1.391	1.410	1.376	1.371	1.388
Intercept		0.305	0.300	0.317	0.312	0.306
S.D. of Intercept 0.0029						
Avg. of Slope 0.0538						
LOD 0.177µg/ml LOQ 0.539 µg/ml						

Table 5: LOD and LOQ values for Rosuvastatin at 222nm

S. No.	Conc.(µg/mL)	Absorbance at 222nm				
		Set 1	Set 2	Set 3	Set 4	Set 5
1	4	0.058	0.062	0.057	0.058	0.063
2	8	0.094	0.099	0.093	0.099	0.095
3	12	0.131	0.139	0.132	0.132	0.135
4	16	0.172	0.182	0.171	0.181	0.175
5	20	0.206	0.212	0.205	0.210	0.215
Intercept		0.02	0.023	0.020	0.02	0.019
S.D. of Intercept 0.0006324						
Avg. of Slope 0.007						
LOD 0.298µg/ml LOQ 0.903µg/ml						

Table 6: Absorbance values for the System Precision

S. No.	Conc. (µg/mL)	Absorbance of RSC and ASP	
		232nm	222nm
1.	12:1.5	1.008	0.708
2.	12:1.5	1.010	0.695
3.	12:1.5	1.001	0.714
4.	12:1.5	1.012	0.691
5.	12:1.5	1.005	0.710

Table 7: Statistical Report of System Precision for RSC and ASP

S. No.	Parameter	RSC and ASP	
		232nm	222nm
1.	Mean	1.0072	0.7036
2.	Standard deviation	0.0019235	0.0044721
3.	Percentage Relative Standard deviation	0.19097	0.635

Method Precision

Table 8: Absorbance values for Method Precision

S. No.	Conc. (µg/mL)	Absorbance of RSC and ASP	
		232nm	222nm
1.	8:1	0.701	0.480
2.	8:1	0.714	0.489
3.	8:1	0.694	0.485
4.	8:1	0.725	0.475
5.	8:1	0.705	0.487

Table 9: Statistical Report of Method Precision for RSC and ASP

S. No.	Parameter	RSC and ASP	
		232nm	222nm
1.	Mean	0.708	0.4832
2.	Standard deviation	0.0052535	0.0025298
3.	Percentage Relative Standard deviation	0.74201	0.523

Table 10: Intraday Precision values for RSC and ASP at 232nm

S. No.	Conc.(µg/mL)	ABS at 232nm				
		S1	S2	S3	S4	S5
1.	4:4	0.397	0.391	0.393	0.389	0.385
2.	8:8	0.724	0.729	0.725	0.719	0.726
3.	12:12	0.976	0.971	0.985	0.980	0.975

Table 11: Intraday Precision values for RSC and ASP at 222nm

S. No.	Conc.(µg/mL)	ABS at 222nm				
		S1	S2	S3	S4	S5
1.	4:4	0.372	0.379	0.365	0.375	0.369
2.	8:8	0.596	0.599	0.585	0.582	0.591
3.	12:12	0.717	0.720	0.715	0.725	0.721

Table 12: Intraday Precision

S. No.	Mean		Standard deviation		%RSD	
	232nm	222nm	232nm	222nm	232nm	222nm
1.	0.391	0.372	0.002	0.0024083	0.5115	0.6473
2.	0.7246	0.5906	0.0016124	0.0031937	0.222	0.540
3.	0.9774	0.7196	0.0023664	0.0017029	0.24211	0.23664

Table 13: Inter day Precision

S. No.	Conc.(µg/mL)	ABS at 232nm				
		S1	S2	S3	S4	S5
1.	4:4	0.481	0.477	0.485	0.489	0.479
2.	8:8	0.751	0.759	0.745	0.741	0.755
3.	12:12	0.984	0.989	0.979	0.986	0.980

Table 14: Inter day Precision

S. No.	Conc.(µg/mL)	ABS at 222nm				
		S1	S2	S3	S4	S5
1.	4:4	0.405	0.409	0.403	0.413	0.415
2.	8:8	0.580	0.589	0.575	0.583	0.577
3.	12:12	0.700	0.708	0.712	0.701	0.710

Table 15: Statistical Report of Inter day Precision for RSC and ASP

S. No.	Mean		Standard deviation		%RSD	
	232nm	222nm	232nm	222nm	232nm	222nm
1.	0.4822	0.409	0.0021447	0.0022803	0.444	0.5575
2.	0.7502	0.5808	0.0032557	0.0024494	0.43397	0.4217
3.	0.9836	0.7062	0.0018439	0.0024083	0.18746	0.3410

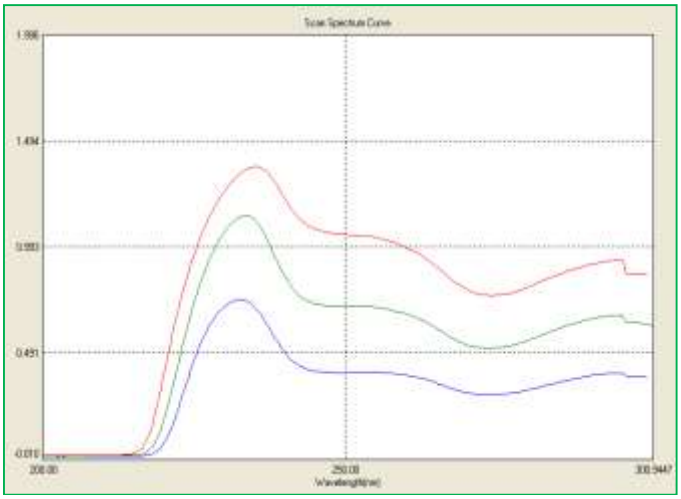

Fig. 9: Asaay Spectrum of Rosuvastatin Calcium and Aspirin

Table 16: Assay Results for the Marketed Formulation

S. No.	Conc. (µg/mL) RSC :ASP	ABS at (nm)		Amount estimated(µg/mL)		% Purity(w/w)	
		232	222	232	222	232	222
1.	8:1	0.742	0.449	8.285	1.040	103.5	104
2.	12:1.5	1.125	0.680	12.5	1.4935	104.5	99.5
3.	16:2	1.365	0.827	15.235	2.02	95.22	101.3

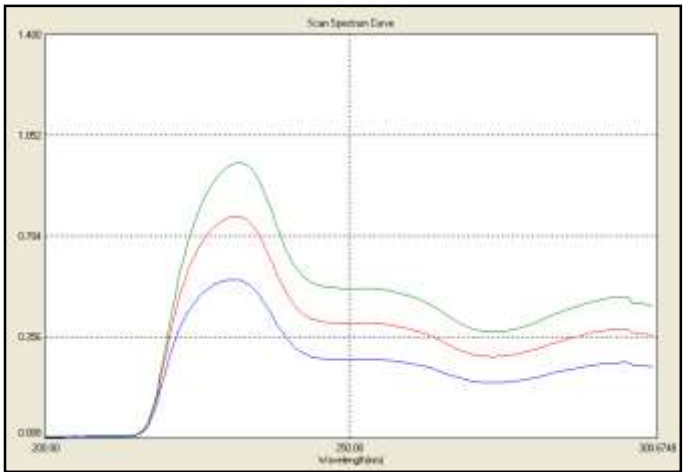

Fig. 10: Accuracy Spectrum of Rosuvastatin Calcium and Aspirin

Table 17: Accuracy Results for Aspirin

S. No.	Amount of RSC in mrkd. Form. (µg/mL)	Amount of STD RSC added(µg/mL)	Total amount of RSC (µg/mL)	ABS	Total amount of RSC found (µg/mL)	%Recovery
1	4	2(50%)	6	0.557	6.219	105
2	4	4(100%)	8	0.741	8.27	105
3	4	6(150%)	10	0.882	9.846	96.1

Table 18: Accuracy Results for Rosuvastatin

S. No.	Amount of ASP in mrkd. Form. (µg/mL)	Amount of STD ASP added(µg/mL)	Total amount of ASP (µg/mL)	ABS	Total amount of ASP found (µg/mL)	%Recovery
1	0.5	0.25(50%)	0.75	0.337	0.775	105
2	0.5	0.5(100%)	1	0.448	0.995	99.12
3	0.5	0.75(150%)	1.25	0.534	1.268	103

Table 19: Validation results for UV Method (Rosuvastatin Calcium and Aspirin)

S. No.	PaASPeters		Aspirin	Rosuvastatin	Acceptance Criteria
1.	Linearity		R^2= 0.996	R^2 = 0.999	Correlation coefficient (R^2 = 0.996-0.999)
2.	Precision	System	%RSD = 0.19097	%RSD = 0.635	RSD<2%
		Method	%RSD = 0.74201	%RSD = 0.523	
		Intra day	%RSD = 0.222-0.5115	%RSD = 0.23664-0.6473	
		Inter day	%RSD = 0.1874-0.444	%RSD = 0.341-0.557	
3.	Assay		95.22-103.5%	99.5-104	95-105%
4.	Accuracy		96.1-105%	99.12-105%	
5.	LOD		0.177µg/mL	0.2981µg/mL	-
6.	LOQ		0.539µg/mL	0.9034µg/mL	-

Conclusion

Literature survey indicates that the methods for the determination of RSC and ASP (UV) were less sensitive and costlier. So the present work aimed for the development of sensitive, economical and simpler methods for the RSC and ASP by UV in pharmaceutical dosage form. The UV Spectrophotometric method, with 0.1N NaOH was proved to be simple, Precise, Acccurate and Sensitive from the results of validation and it is suitable method for the simultaneous estimation of RSC and ASP in the pharmaceutical dosage form. Finally, it can be concluded that the method for quantitation of RSC and ASP (UV), in their pharmaceutical dosage form can be applied for the routine analysis because of simplicity, accuracy and Preciseness.

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