CHEMOMETRIC ASSISTED METHOD DEVELOPMENT AND VALIDATION OF ROSUVASTATIN USING RP-HPLC

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ABSTRACT:

The present work is based on the application of RP-HPLC determination of active substances of rosuvastatin by chemometric assisted method development and validation Rosuvastatin is an Anti-hyper lipidemic agent. Symmetric c_{18} column (150mm x 4.6 ,3.5µm) was used to perform the chromatographic separation. Mobile phase was used in the ratio of 40:60v/v 1ml TEA in 1000ml water and acetonitrile. The flow rate was set at 1ml/min at ambient temperature with detecting wave length at 247nm. Linearity was observed over the concentration range of 10-200 µg/ml for Rosuvastatin. Correlation coefficient was found to be 0.999 for Rosuvastatin drug. Percentage recovery values of pure drug were found to be 100.1% for Rosuvastatin Different stress condition such as acidic, basic, oxidative, temperature (45^0C) and hydrolysis degradation studies were performed for Rosuvastatin. HPLC method in combination with Chemometric calibration reduces errors in chromatographic analysis and satisfy the limits of ICH guidelines.

KEYWORDS: Chemometric method, Degradation studies, Rosuvastatin, RP-HPLC.

INTRODUCTION:

The science of analytical chemistry can be described in simplified terms as the process of obtaining knowledge of a sample by chemical analysis of some kind. The sample under investigation may consist of any solid, liquid or gaseous compound and the result of the analysis is data of some kind that is related to the initial question raised about the sample. From the data obtained in the analysis some knowledge about the sample can be extracted. This knowledge may be either qualitative or quantitative (or both). Examples of qualitative information are types of atoms, molecules, functional groups or some other qualitative measure, while the quantitative information provides numerical information such as the content of different compounds in the sample.

Rosuvastatin,chemically,(*E*,3*R*,5*S*)-7-[4-(4-fluorophenyl)-2-[methyl(methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid. Rosuvastatin belongs to class of drugs known as Anti-hyper lipedimic drugs. It is a competitive inhibitor of HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate limiting step in cholesterol biosynthesis. decreased hepatic cholesterol concentrations stimulate the up regulation of hepatic low density lipoprotein (LDL)receptors which increases hepatic up take of LDL. Rosuvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is decrease in plasma LDL and VLDL .in vitro and in vivo animal studies also demonstrate that Rosuvastatin exerts vasculo protective effects independent of its lipid lowering properties (1)

HPLC is one among the most useful tools, available for quantitative analysis. High Performance Liquid Chromatography is a special branch of column chromatography, in which the mobile phase ids force through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially (1,2).

METHODOLOGY

Reagents and chemicals used are water HPLC grade are in house production Acetonitrile, Formic acid, Tri ethyl amine, are HPLC grade it is a merck manufacturer

Instruments used p^H meter eutech manufacturer, weighing balance Denver manufacturer, ultra sonicator UCA 701 model unichrome manufacturer, HPLC LC model waters 2695-empower software, flow rate 1ml/min, pump Isocratic model are used (3).

CHEMOMETRICANALYSIS

In this chemometrics assisted HPLC study , PCA, PLS calibrations were used to analyse the drugs of Rosuvastatin and collagen type II at 221 nm by using PDA detector. The data obtained from analysed drugs were stored in computer having required software to perform chemometric analysis.

Acquisition software: In present study we are using following chemometric techniques.

- Principal component analysis (PCA)
- Partial least squares technique (PLS)

We are download the unscramble (camo software),it facilitates the PCA,PLS analysis more robust, accessible.

PLS Approach:

PLS calibration using the orthogonalized PLS algorithmin volves, simultaneously, independent and dependent variables on the data compression and decomposition operations. In the HPLC data analysis , HPLC-PLS calibration was obtained by decomposition of both the drugs of concentration, peak area matrix into latent variables.PLS calibration was obtained using the relationship between the decomposed peak area data and concentration set. The PLS linearity and accuracy numerical data of rosuvastatin was placed in table 1 & 2. The PLS linearity and accuracy spectral data of rosuvastatin was placed in figure 1 &2

PCA approach:

In PCA technique it gives relevant information from data set, and it can be used express the data on the basis of their similarity and differences. It is used to develop correlation structure between variables, and examine the changes. In PCA data transferred to describe the amount of same variability. In these HPLC data analysis the data of both drugs of Rosuvastatin peak area we get the Bio-plot (4). The PCA linearity and accuracy numerical data of rosuvastatin was placed in table 3&4. This PCA linearity and accuracy spectral data of Rosuvastatin was placed in figure 3&4

METHOD DEVELOPMENT:

Selection of the wavelength for Simultaneous Estimation

In setting up the conditions for development of the assay method, the choice of the detection wavelength was based on the scanned absorption spectrum for Rosuvastatin.

Accurately weigh 5mg of Rosuvastatin and transferred in to 10ml volumetric flasks, make up the flasks with acetonitrile & sonicate for 5 minutes then take 1ml of this solution was taken and made up to 10ml with acetonitrile with 1ml syringe then transfer this solution in to vial.

The prepared solution was loaded into the auto sampler and the system was set in order to take the auto injection in HPLC with PDA detector. The spectrum of rosuvastatin is shown in figure 5

Selection of chromatographic method

The choice of chromatographic method is based on the nature of sample, its molecular weight and solubility. As drugs are polar in nature, the reverse phase chromatographic technique was selected for the present work (5).

OPTIMIZED METHOD

Preparation of Mobile Phase:

1ml of TEA dissolved in 1lt of water, and acetonitrile in the ratio of 40:60 v/v was prepared.

Column : Symmetry C_{18} (150mmx4.6, 3.5µm)

Injection Volume: 10 µl

Wavelength : 247 nm

Flow rate : 1.0 ml/ min

Temperature : Ambient

Diluent : Same as mobile phase

Retention time of Rosuvastatin was about 2.353 respectively.

Preparation of standard solution:

Weigh accurately about5mg of Rosuvastatin working standard into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume with diluent.

Further dilute 5mL of the above solution to 50 mL with the diluent.

Preparation of Sample solution:

Weigh 1 tablet and crush to powder then take equivalent weight of sample into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 5 mL to 50 mL with the diluent. Filter through 0.45µ Nylon syringe filter.

Assay Procedure:
Separately injected 10µL of Standard solution five times and Sample solution in the chromatographic system. Recorded the chromatograms and measured the peak responses for Rosuvastatin (5,6,).The Chromatogram blank in assay is shown in figure 6 and chromatogram standard is shown in figure 7 also chromatogram sample is shown in figure 8

ASSAY CALCULATION:
% Assay of was carried out in tablet formulation with results were calculated by using the formula given below and reported

$$= \frac{\text{Test area x STD weight x Test dilution x Avg. Weight x Potency x 100}}{\text{STD area x test weight x STD dilution x label claim x 100}}$$

Assay Results:
Acceptance Criteria:
The % assay should be within range of 98-102%.

Observation: The % assay was found to be within the range (7).Assay observation of rosuvastatin is shown in Table 5

DEGRADATION STUDIES

1. Acid Degradation:
From the working standard solution 5ml was taken in 50ml volumetric flask, add 3 ml of 5N HCl was added, contents are mixed well kept aside for 1 hour. Then followed by neutralization with 3 ml of 5N NaOH and made up to volume with mobile phase. The solution was injected in HPLC system to obtain chromatograms (8).

2. Base Degradation:
From the working standard solution 5 ml was taken in 50ml volumetric flask, add 5 ml of 5N NaOH was added, contents are mixed well kept aside for 1 hour. Then followed by neutralization with 3 ml of 5N HCl and made up to volume with mobile phase. The solution was injected in HPLC system to obtain chromatograms (8).

3. Oxidation:
From the working standard solution 5 ml was taken in 50 ml volumetric flask, add 1 ml of 30% v/v H_2O_2 was added, contents are mixed well kept aside for 1 hour. Then make up to volume with mobile phase. The solution was injected in HPLC system to obtain chromatograms.

4. Temperature Stress Studies:
From the working standard solution 5 ml was taken in 50 ml volumetric flask. The solution was heated at 45ºC and cooled to room temperature. Then the solution is injected in HPLC system to obtain chromatograms (8,9).

5. Hydrolysis Degradation:
From the working standard solution 5 ml was taken in 50 ml volumetric flask, add 10ml of diluent added 20 ml of water to disperse and dissolve and heated at 70°C for 3 hours on a water bath. Remove the flask from the water bath, and allow the flask to cool at room temperature and diluted to volume with diluent and mixed. Then the solution is injected in HPLC system to obtain chromatograms (10).

RESULTS AND DISCUSSION:

Method Development and Validation for simultaneous estimation of Rosuvastatin by RP-HPLC

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a simple, rapid, precise, accurate and sensitive HPLC method for the simultaneous estimation of Rosuvastatin in bulk and its pharmaceutical dosage form.

The developed method for simultaneous estimation of Rosuvastatin was carried out X- bridge phenyl (250mmx 4.6mm, 5µ) in an isocratic mode, using mobile phase composition of 0.1% formic acid and acetonitrile in the ratio (50:50 v/v) with a flow rate 1.0 ml/min. The effluents were monitored at 290 nm.

From the results % assay value of Rosuvastatin was found to be 100.1%.

Linearity was observed over the concentration range of 10-200 µg/ml for Rosuvastatin. Correlation coefficient was found to be 0.999 for Rosuvastatin drug which indicates that the concentration had given good linearity.

The %RSD values of Rosuvastatin for System precision and Method precision was found to 0.157 & 0.162 respectively. As the results are within acceptance limit of less than 2%, indicates that the proposed method has good reproducibility. The results are good for both method precision and system precision.

From the results shown in accuracy, it was found that the mean percentage recovery values of pure drug were found to be 100.1% for Rosuvastatin and as these results are within the acceptance limit of 98%-102% which indicates that the method was accurate.

The robustness of the developed method was evaluated by changing the flow rate and mobile phase composition. All the parameters were within the limits and all variable conditions, which indicate that the method was robust.

The ruggedness of the proposed method was analyzed by two different analysts. The %RSD was found within the limits i.e., should not be more than 2.0. Hence the proposed method has good repeatability. Validation parameters of rosuvastatin was shown in Table 6

STABILITY:

The solution stability of rosuvastatin in diluents were determined by storing sample solution in a tightly capped volumetric flask at room temperature for 24 hr. The amount of Albuterol sulphate, Theophylline and Bromhexine Hcl were measured at different time intervals like 6,12,18 and 24 hrs and results obtained were compared with rosuvastatin freshly prepared solution. The solution stability of Rosuvastatin is shown in Table 7

CONCLUSION:

In the present investigation Chemometric analytical methods has been used for the estimation of potent drugs Rosuvastatin. The results of forced degradation studies for Rosuvastatin the purity threshold is more than 70%,it will not satisfy the criteria of ICH guideline. While performing analysis of Rosuvastatin we exclude the conditions which effect the purity. This study contains the evaluation of HPLC data for Chemometric techniques of PCA, PLS. HPLC-Chemometric approaches have many advantages ,*e.g.*, robust and reliable results, a decreased the injection size due to the use of a multi chromatographic data procedure ,and noise elimination due to mainly the mechanisms of the PLS and PCA approaches.

We believe that use of the HPLC method in combination with chemometric calibrations is a viable new alternative determination method that eliminates or reduces in convenient errorsin the chromatographic analysis. HPLC-chemometric calibration techniques can be successfully applied to the routine and quality control analysis of subject drugs and other drugs in sample analysis.

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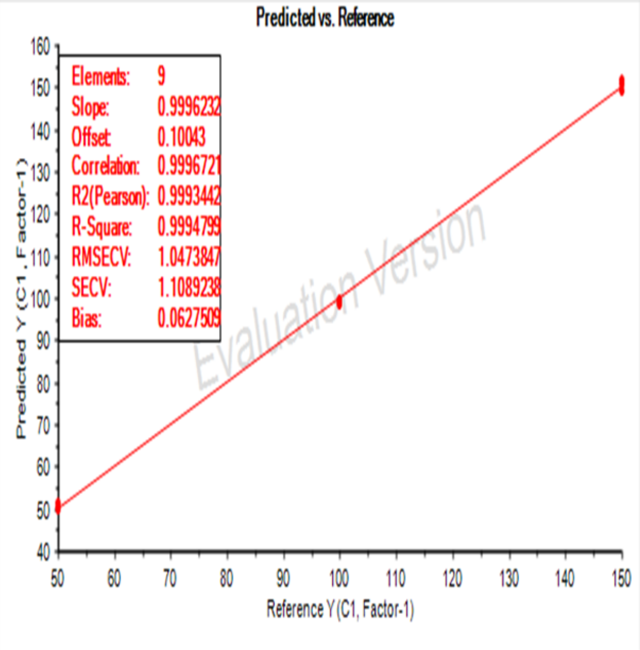
TABLE 1 PLS Linearity numerical data of Rosuvastatin:

S.NO	Y REFERENCE	Y PREDICTED
		TCC
	1	2
1	0	-0.00528371334
2	0.25	0.284716487
3	0.5	0.498388737
4	0.75	0.728064537
5	1	0.962942243
6	1.25	1.28400016
7	1.5	1.50864947

TABLE 2 PLS Accuracy numerical data of Rosuvastatin

S.NO	Y REFERENCE	Y PREDICTED TCC
	1	2
1	50	49.6940
2	50	50.4248
3	50	50.9950
4	100	98.7081
5	100	99.4286
6	100	99.7523
7	150	150.4779
8	150	149.2234
9	150	151.2958



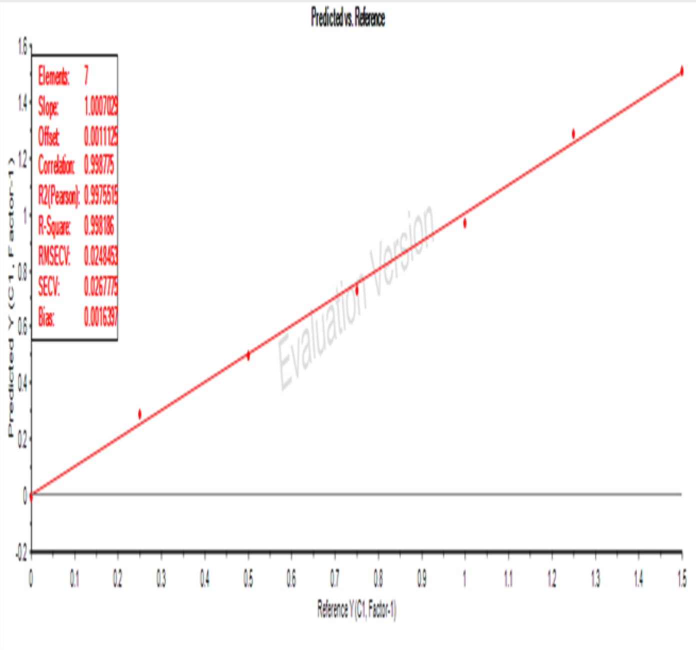


FIG 1: PLS of accuracy spectral data of Rosuvastatin

FIG 2: PLS of Linearity spectral data of Rosuvastatin

Table:3 PCA Accuracy Numerical Data of Rosuvastatin

PC-1	PC-2
-1502217	-1502217
-1480396	-1480396
-1463368	-1463368
-38578	-38578
-17062	-17062
-7396	-7396
1507351	1507351
1469890	1469890
1531776	1531776
-1502217	-1502217

Table:4 PCA Linear Numerical Data of Rosuvastatin

PC-1	PC-2
-2250615.5	-2250615.5
-1419222.5	-1419222.5
-751354.5	-751354.5
-56196.5	-56196.5
655486.5	655486.5
1565965.5	1565965.5
2255937.5	2255937.5

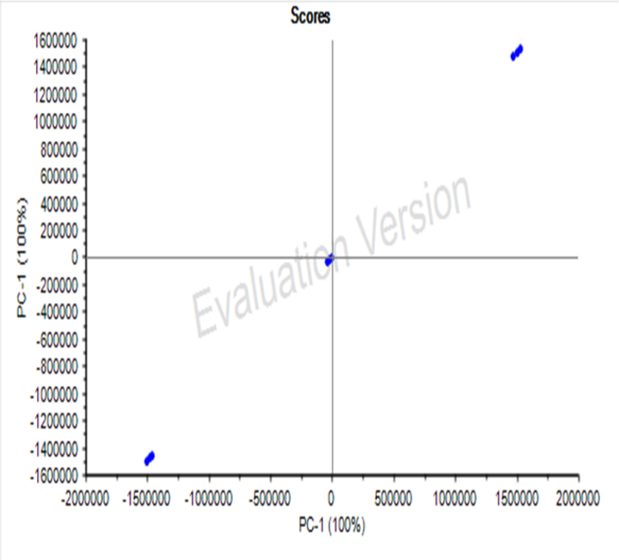


Fig:3 PCA Accuracy Spectral Data of Rosuvastatin

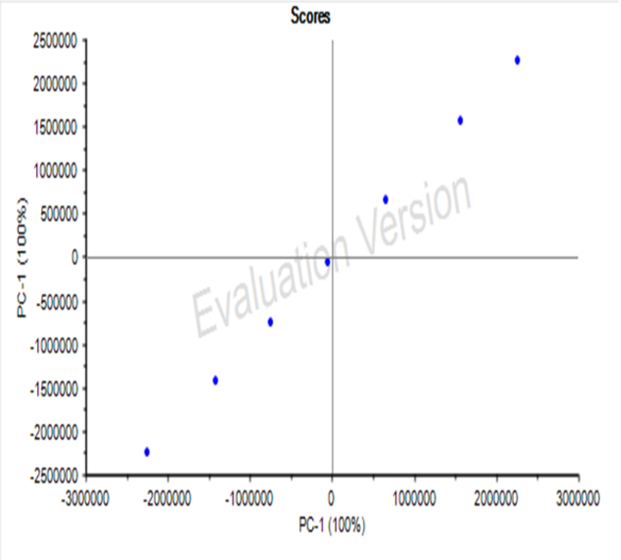


fig:4 PCA Lineraity Spectral Data of Rosuvastatin

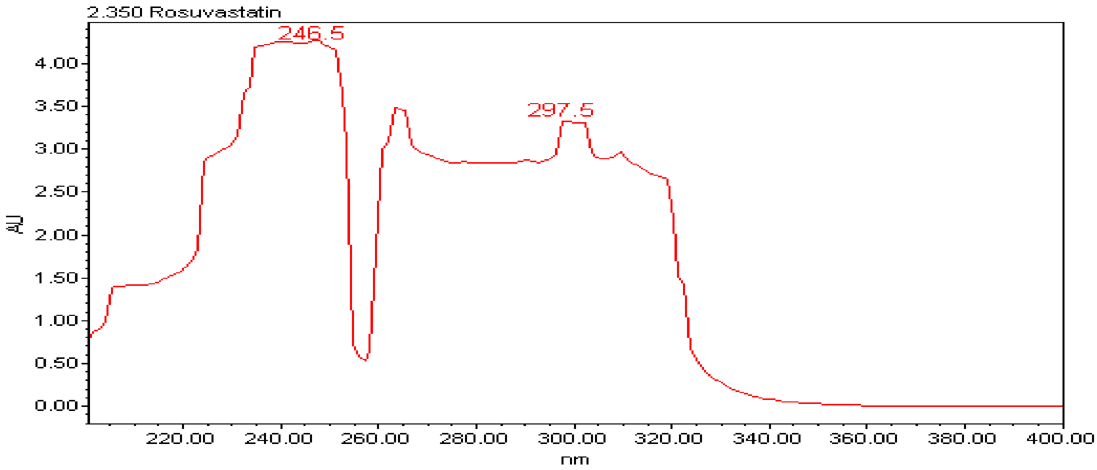


Figure: 5 Spectrum of Rosuvastatin

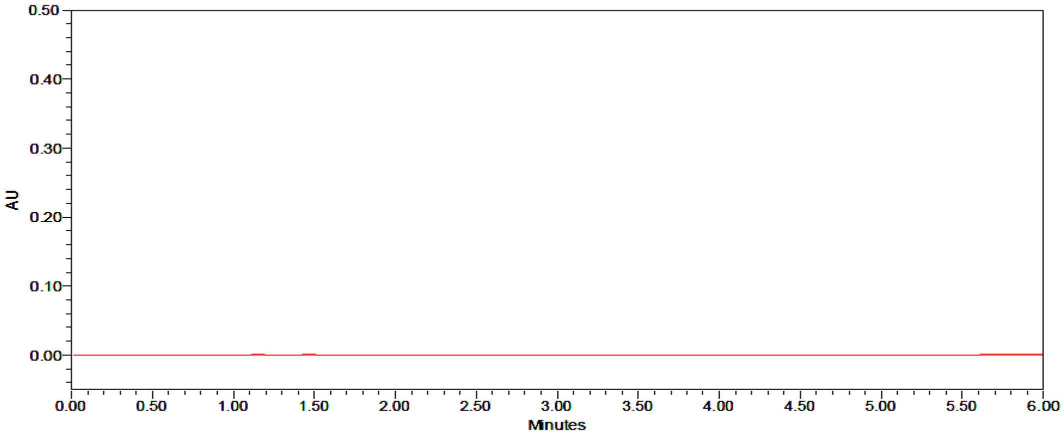


Figure:6 Chromatogram of Blank

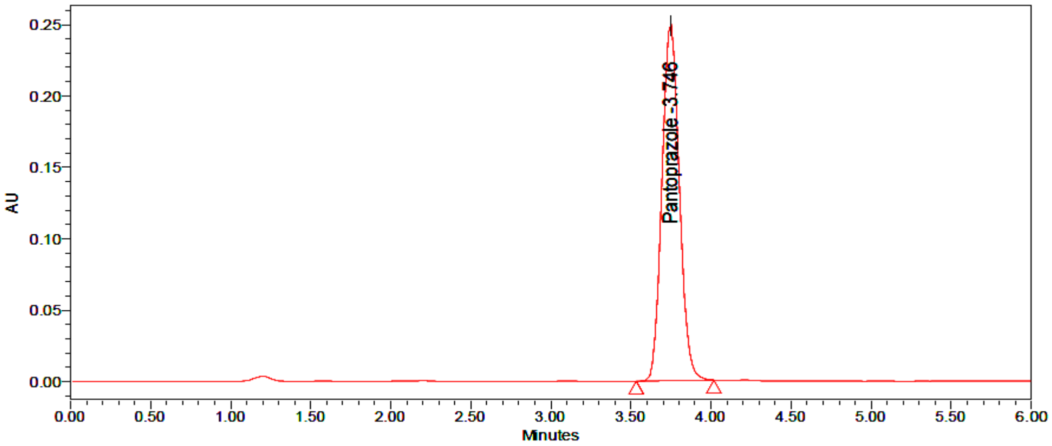


Figure:7 Chromatogram of Standard

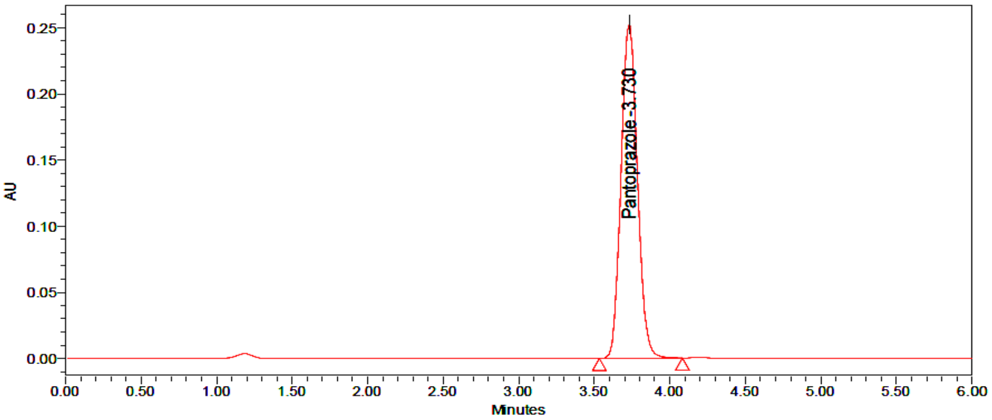


Figure:8 Chromatogram of sample

Drug	**Label Claim for sample taken (mg)**	**Sample weight (mg)**	**% of Assay**
Rosuvastatin	100	172	100.1

Table:5 Assay observations of Rosuvastatin

PARAMETER	ACCEPTANCE CRITERIA	ROSUVASTATIN
Linearity Range Correlation Coefficient	Correlation coefficient r 2 > 0.999	r 2 = 0.99928
System Precision	RSD < 2%	%RSD = 0.631
Intermediate Precision	RSD < 2%	%RSD = 0.092
Method precision	RSD < 2%	%RSD = 0.09
Accuracy	Recovery 98- 102% (individual)	% recovery(50%)= 99.9 % recovery(100%)=99.8 recovery(150%)=101.2
Robustness RSD < 2%	RSD NMT 2% in modified condition Flow minus Flow plus Organic plus Organic minus	Complies %RSD= 0.96 %RSD= 0.499 %RSD=0.75 %RSD=0.096

Table 6:. Validation parameters for Rosuvastatin

S No.	Stability (hrs)	Rt(min)	Peak area	USP Plate count	USP Tailing	% Assay	% Deviation
1	INITIAL	2.341	2923165	2278	1.16	100	0.00
2	6 HRS	2.347	2917246	2285	1.20	99.8	-0.20
3	12 HRS	2.351	2903568	2280	1.09	99.4	-0.60
4	18HRS	2.342	2896310	2277	1.11	99	-1.00
5	24 HRS	2.348	2886443	2289	1.17	98.7	-1.30

Table:7 Solution stability of Rosuvastatin