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STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF ROSUVASTATIN AND ITS RELATED SUBSTANCES IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The aim of the present work was to develop and validate a simple, efficient, economical method for the estimation of Rosuvastatin and its related substances in bulk and dosage forms by reverse phase high pressure liquid chromatography. Chromatography was performed on X-Terra -C18 (150 mm x 4.6 mm, 3.5µm) with mobile phase containing Mobile phase - A: pH 3.00 KH_2PO_4 buffer: Methanol (80:20 v/v) and Mobile phase - B: pH 3.00 KH_2PO_4 buffer: Methanol: Acetonitrile (25:15:60 v/v)at a flow rate of 1mL/min and eluents were monitored at 248nm. The retention times of Rosuvastatin,Anti-Isomer, 5-Oxo and Lactone impurities were 23.538min, 25.634,28.546 and 29.344min respectively and showed a good linearity in the concentration range of 0.3-7.5 µg/mL for Rosuvastatin, 0.2-7.5µg/mL for Anti-Isomer, 0.2-7.5µg/mL for 5-Oxo and 0.2-7.5µg/mL for Lactone impurities with a correlation coefficient of 0.9998, 0.9996, 0.9992 and 0.9999 respectively. The validation characteristics included specificity, linearity, and limit of detection, limit of quantification, precision, robustness and stability. Validation acceptance criteria were met in all cases. The percent recoveries ranged between 85-115%, RSD < 2%. The method could be successfully used for the analysis of Rosuvastatin and its related substances in bulk and dosage forms.

Keywords: Rosuvastatin (ROSV), Anti-Isomer, 5-Oxo, Lactone Impurities,X-Terra -C18,Method Validation, HPLC.

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INTRODUCTION

Rosuvastatin (ROSV) is a synthetic lipid-lowering agent. Chemically it is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid (Fig-1-5) and used for the treatment ofhyperlipidemia, itacts primarily in the liver. Decreased hepatic cholesterol concentrations stimulate the up regulation of hepatic low density lipoprotein

(LDL) receptors which increases hepatic uptake of LDL. ROSV also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL.Anti-isomer of ROSV, 5-oxo and lactone impurities are the major process-related impurities of ROSV.Literature survey has revealed that various methods were reported for estimation of ROSV those are Colorimetry, UV Spectrophotometry, HPLC, UPLC, LC/MS and HPTLC.

The objective of the proposed method is to develop simple and accurate method for the estimation of ROSV and its related substances in pharmaceutical dosage forms by HPLC. Hence, on the basis of literature survey it was thought to develop a precise, accurate, simple and reliable, less time consuming and less cost effective method for the estimation of related substances (1-4).

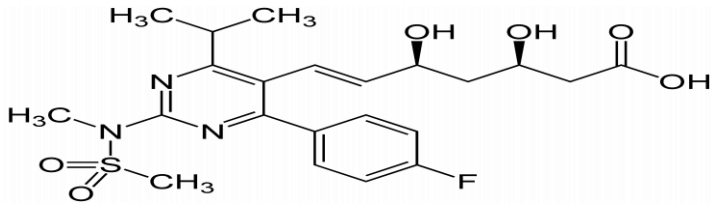

Fig-1 Structure of ROSV

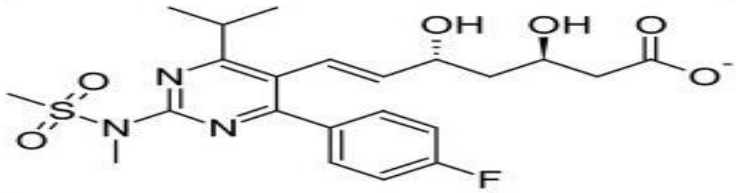

Fig-2 Structure of Anti-Isomer

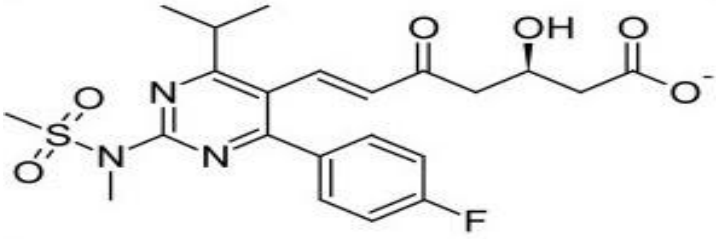

Fig-3 Structure of 5-Oxo

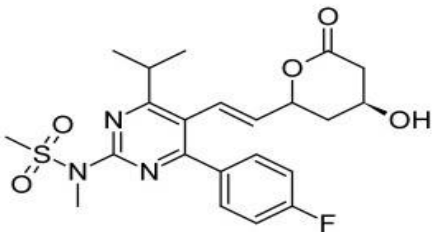

Fig-4 Structure of Lactone

EXPERIMENTAL SECTION

Material and methods

Chemicals

Reference standards of ROSV, Anti-Isomers, 5-Oxo and Lactone impurities were gift samples from Dr.Reddy's Laboratories Pvt.,. Ltd, India. Ortho Phosphoric Acid, Potassium Dihydrogen Phosphate, Acetonitrile, water and methanol were purchased from Rankem chemicals, Mumbai, India. All the solvents and reagents were of HPLC grade. ROSUVASTAT (manufactured by Dr.Reddy's Laboratories Pvt. Ltd, India) tablets containing RSV 5mg were commercially purchased.

Equipment

Agilent A 1200 model HPLC system provided with LC20ATPump, UV/Visible detector was used. Data acquisition was carried out using Empower-2software. The chromatographic analysis was performed on X-Terra C18 column(150 mm x 4.6 mm, 3.5µm).

Chromatographic Conditions

Mobile phase consisting of Mobile phase - A: pH 3.00 KH2PO4 buffer: Methanol (80:20 v/v) and Mobile phase - B: pH 3.00 KH2PO4 buffer: Methanol: Acetonitrile (25:15:60 v/v)was used in gradient mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 3 min before use. The flow rate was 1 mL/min and the injection volume was 10µL. UV/Visible detection was performed at 248nm and the separation was achieved at ambient temperature (5-8).

Preparation of Diluent

Mixture of water and Acetone (30:70 v/v) respectively.

Preparation of stock and standard solutions

The stock solutions of ROSV(mg/mL) were prepared by dissolving 10 mg of drugin 10 ml of diluent in volumetric flasks and volume was adjusted to the mark with the same. An appropriate volume of the stock

solution was then further diluted with diluent to get the required concentrations of standard solutions at a concentration range of 10µg/mL of ROSV (9, 10).

Preparation of test solution:

20 tablets are weighed then determined the average weight and then grinded into fine powder from that weight equivalent to 2985.02 mg of powder sample was weighed and transferred into 100 ml volumetric flask and then added 70 ml diluent, sonicate for 30min and centrifuge the portion of test solution at 5000 RPM for 10 min and adjusted the final volume with diluent use clear solution as test preparation (11, 12).

Preparation of placebo solution:

Weigh 2880.06 mg of placebo in to a 100 ml volumetric flask and then added 70 ml diluents mixed well and sonicate for 30 min in mechanical shaker and adjusted the final volume with diluent and mixed.

Preparation of system suitability solution

99.87 mg of ROSV working standard was weighed and transferred into 150ml flask then added 75 ml of diluent sonicate for dissolving. To the above solution added 5ml of above anti-isomer impurity stock solution and diluted to volume with diluents (13-16).

VALIDATION OF THE HPLC METHOD

The proposed method was validated as specified in USP.

System suitability

System suitability was carried out by Injecting system suitability solution six times into the HPLC system. The system suitability test parameters were noted and RSD was calculated and data was presented in Table-1.

Precision

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability was assessed by using a minimum of six determinations at 100% of the impurities test concentration. The % RSD of impurities were reported for precision. Less than 15% RSD for peak areas indicates the precision of the developed method and the data was presented in Table-2 (17-20).

Accuracy

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method recovery studies were performed by the test preparation with impurity stock solution addition method by spiking 50%, 100%, 150% of specification limit to the target concentration and these solutions were analyzed by developed method in triplicate. The % recovery and the RSD were calculated at each level of addition and the data was given in Table-3 (21, 22).

Limit of Quantification and Limit of Detection:

Determine the limit of Quantification and limit of Detection of ROSV and all known impurities based on signal to noise ratio method. For the limit of detection of ROSV and its impurities by identifying the concentration which gives a signal tonoise ratio about 3. Prepare six test solutions by spiking all the impurities, ROSV at about Limit of Quantification level in placebo equivalent to test concentration and inject into HPLC. If the drug product contains the known impurities, perform the study in the presence of placebo sample. . Prepare the test sample in triplicate by spiking all the impurities at about limit of quantification level on test preparation. Calculate the % RSD for all the impurities, ROSV from six preparations and data was given in Table-4 (23-26).

Linearity

To demonstrate the linearity of detector response for ROSV and its impurities prepare not less than six solutions with concentrations ranging from Limit of quantification level to 200 % of the target concentration at specification limit, inject in to the chromatographic system by following concentration in the test method. Inject the solutions in to the chromatographic system and the calibration curve was plotted using peak area ratio Vs concentration of the standard solution. From the calibration curve coefficient correlation was calculated and data was given in Table-5 (27).

Specificity

Specificity is to ensure that the signal measured comes from the substance of interest, and that there is no interference from excipient, degradation products and impurities.Prepare the placebo solution, known impurities solution and test solution individually in duplicate as per the test procedure. Inject into chromatographic system and check the interferences

due to placebo, impurities and degradation peaks at the retention time of ROSV and known impurities and data was given in Table 6 (28).

Robustness

Assay was recorded. The robustness is a measure of the capacity of a method to remain unaffected by small but deliberate changes in mobile phase composition (± 10% v/v), change in pH of buffer (± 0.2), change in flow rate (± 0.2 mL/min) and change in column oven temperature (± 5 °C) and the effect of wavelength was studied (29, 30).

RESULTS AND DISCUSSION

Various UPLC, UV, HPLC, methods were published for the estimation of ROSV and Related substance but only few methods were reported on theestimation of ROSV and Related substances with in bulk and dosage forms. Hence, the present investigation was aimed to develop a simple, economical RP-HPLC method for the determination of ROSV and its related substances in bulk, dosage forms.

Method Development

The main criterion for developing an RP-HPLC method for the determination of related substances in ROSV dosage form in a single run, with emphasis on the method being accurate, reproducible, robust, stability-indicating, linear, free of interference from other formulation excipients and convenient enough for routine use in quality control laboratories.A spiked solution of impurities (1 µg/mL), ROSV (10 µg/mL) and placebo peaks were subjected to separation by RP-HPLC. Initially, the separation of all peaks was studied using pH 3.00 KH_2PO_4 buffer as mobile phase A and Acetonitrile and KH2PO4 buffer (ratio 80:20)as mobile phase B on an Inertsil ODS 3V-C18 (150 mm x 4.6 mm, 5 µm) with an isocratic program. The 2.0 mL/min flow rate was selected to achieve the separation of peaks. The column oven temperature was maintained at 25°C. These conditions resulted in separation of the ROSV peak with less intensity and poor resolution between ROSV and Anti-Isomer impurity. It is not incorporated withreference method. Based on obtained results, the isocratic program was replaced with the gradient program in an effort to achieve high resolution between the known impurities

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters viz., capacity factor, tailing factor, no. of theoretical plates and % and all degradant peaks. With the X-Terra -C18 (150 mm x 4.6 mm, 3.5 µm) column, different combinations of mobile phase A and B were studied to optimize the method, including any observations noted. From the mobile phase selection study, the optimized HPLC parameters were as follows: flow rate, 1.0 mL/min; column oven temperature, 30°C (±2), injection volume, 10 µL; and a gradient program with mobile phase A and B. Based on the UV spectrum of the compound, 248 nm was found to be appropriate for the determination of ROSV impurities in pharmaceutical formulations. ROSV and all impurities are well resolved with respect to each other in a reasonable time of 60 min. No chromatographic interference due to the blank (diluent) and other excipients (placebo) at the retention time of ROSV and all impurities were observed.

Method validation

The method has been validated as per USP for following parameters.

System suitability

The percentage relative standard deviation (RSD) of area from six replicate injections was below 5.0 % (diluted standard solution, 5µg/mL). Low values of RSD for replicate injections

Indicate that the system is precise. The results of other system suitability parameters such as peak tailing and theoretical plates are presented in Table below. As seen from this data, the acceptable system suitability parameters would be as follows: the tailing factor ROSV is not more than 1.5, the theoretical plates are not less than 2000, the resolution between ROSV and anti-Isomer peak is not less than 2.0% and the relative standard deviation of replicate injections is not more than 10.0 %.

Precision

Precision studies were carried out in terms of repeatability. Intermediate precision of standard application was assessed by using six replicates of test solutions and the data was given in Table 2. The RSD was found to be below 15 for peak areas, this shows

the closeness of the data values to each other, indicating the precision of the method.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. The amount recovered (for LOQ, 50, 100 and 200% level) was within ±10 % of amount added; for the LOQ level, the amount recovered was within 85.0 to 115%, indicating that the method is accurate and that there is no interference due to other excipients present in the injection. The results of the recovery assay are shown in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The concentration (in µg/mL) with a signal to noise ratio (S/N) of at least 9.0 was taken as the LOQ, which meets the criteria defined as per USP. The LOQ for the ROSV, Anti isomer, 5-oxo and lactone peaks was found to be within the limits. The precision and accuracy was also established at the quantification level. The % RSD of the peak area was well within the acceptance limit of <15.0%, and accuracy between 85.0 to 115.0% respectively. The determined limit of qualification, S/N ration, precision at LOQ and accuracy at LOQ values for ROSV, Anti isomer, 5-Oxo and lactone are presented in Table 4.

Linearity

Linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.To demonstrate the linearity of detector response for ROSV and its impurities prepare not less than six solutions with concentrations ranging from Limit of quantification level to 200% of the target concentration at specification limit, inject in to the chromatographic system by following concentration in the test method. The regression statistics are shown in Table-5, with the linearity curve for ROSV, Anti isomer, 5-Oxo and Lactone represented in Fig-5-8.

Specificity

The specificity of the method was established by injecting the solutions of diluent, placebo and forced degradation studies were performed to demonstrate the selectivity and stability indicating capability of the proposed method. Figure shows that there is no inference of at retention time of ROSV and all know impurities from the blank and other excipients. Significant degradation was not observed when ROSV was subjected to oxidation, base, thermal and hydrolytic, whereas significant degradation was observed when the ROSV was subjected to acid hydrolysis (0.1N HCl, 60°C, 2 h) and UVconditions, leading to the formation of Anti isomer and unknown impurities. The acid hydrolysis product (ROSV anti isomer) and ROSV are well separated fromeach other, as seen in Fig-9. The peak attributed to ROSV was investigated for spectral purity in the chromatogram of all exposed samples and was found to be spectrally pure. The purity and related substances of ROSV were unaffected by the presence of other excipients and thus the stability-indicating power of this method is confirmed. The results of the forced degradation study are presented in Table-6.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. No significant effect was observed on system suitability parameters such as RSD, tailing factor, or the theoretical plates of ROSVwhen small but deliberate changes were made to chromatographic conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

Table-1 Results of system suitability

System suitability parameters	Observed value	Acceptance limit
Tailing Factor for standard preparation	1.0	Between 0.8 to1.5
Theoretical Plates for ROSV Standard solution	26184	NLT 2000
The Resolution between ROSV and Anti-Isomer peak in SST solution	4.0	NLT 2.0%
The %RSD for ROSV from Six replicate injections of standard solution	1.5	NMT 10.0%

Table-2 Results for Precision and Intermediate Precession of all Impurities

Substances	Precision		Intermediate precision	
	% impurity#	% RSD*	% impurity#	% RSD*
Anti-Isomer	0.568	0.9	0.570	0.3
5-Oxo	0.541	1.6	0.547	0.9
Lactone	0.531	2.6	0.558	0.3
#... Average of six determinations; *… Determined on six values				

Table-3 Accuracy Results

Substance		At 50%	At 100%	At 150%
ROSV	#Mean Accuracy	101.3	97.3	98.6
Anti-isomer	#Mean Accuracy	105.2	104.9	108.3
5-Oxo	#Mean Accuracy	103.0	106.6	108.5
Lactone	#Mean Accuracy	105.6	105.4	108.2
#... Mean of three determinations				

Table-4 Results of LOQ

Substance	LOQ (µg/mL)	S/N Ratio	Precession (% RSD*)	Accuracy (% recovery*)
ROSV	0.30	10.4	3.5	104.5
Anit-Isomer	0.27	10.0	3.9	104.9
5-Oxo	0.34	10.3	7	99.9
Lactone	0.21	10.2	6.4	105.5
*… Determined on six values				

Table-5 Results for Linearity of Detector Response

substance	Linearity range (µg/mL)	Correlation Coefficient (R^2)
ROSV	0.30 to 7.54	0.9998
Anti-Isomer	0.21 to 7.56	0.9996
5-Oxo	0.21 to 7.56	0.9992
Lactone	0.21 to 7.56	0.9999

Table-6 Summary of forced Degradation results

Degradation	Conditions	Peak Purity (ROSV)	Observation
Acid Degradation	0.1N HCL 2hrs at 60^{o}C	Pass	Significant degradation
Base Degradation	5N NaoH 12hrs at 60^{o}C	Pass	No significant degradation
peroxide Degradation	10% peroxide 30min at 60^{o}C	Pass	No significant degradation
Thermal Degradation	6hrs at 105^{o}C	Pass	No significant degradation
Water Degradation	30min at 60^{o}C	Pass	No significant degradation

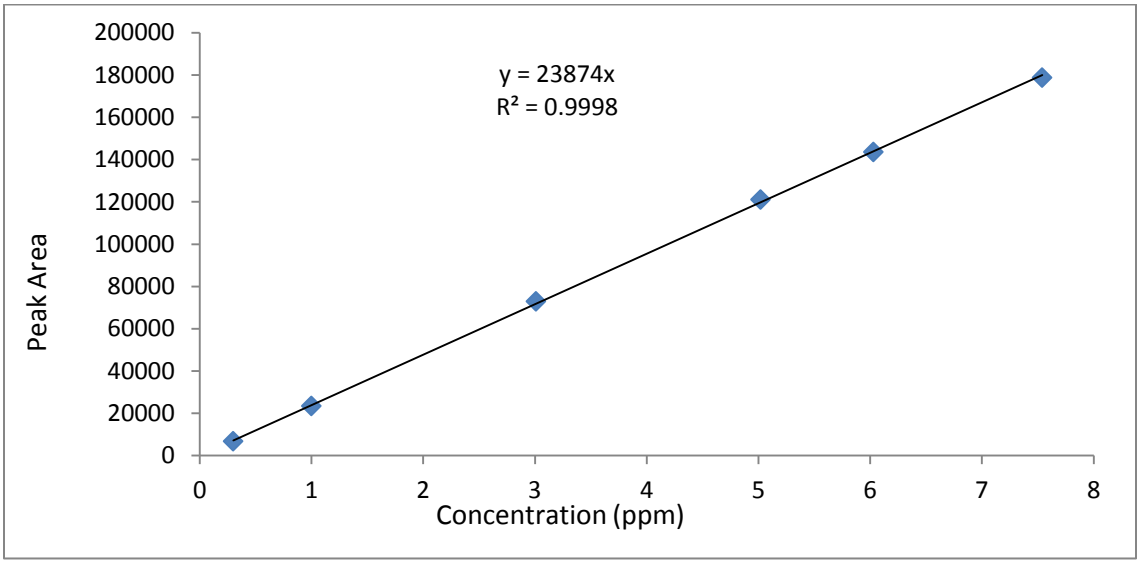

Fig-5 Linearity of ROSV

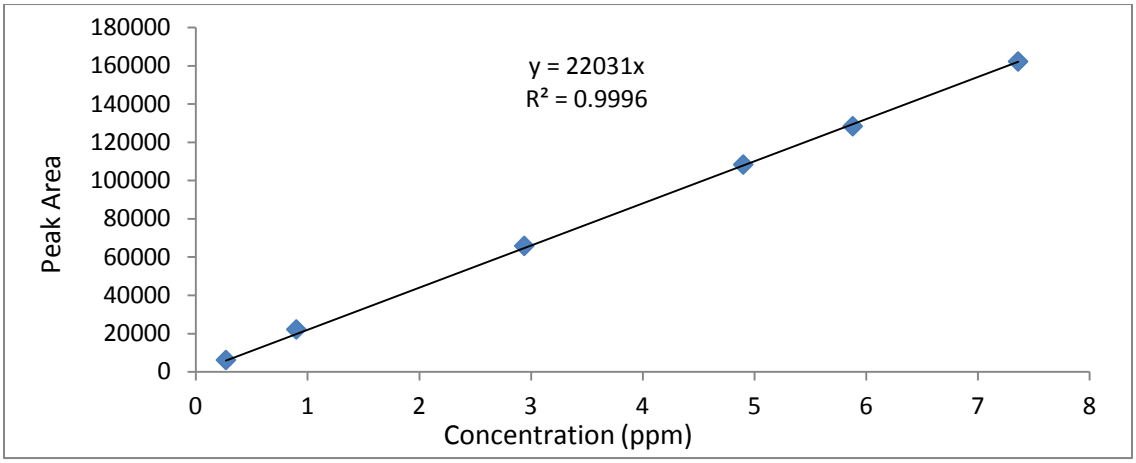

Fig-6 Linearity of ROSV Anti-isomer

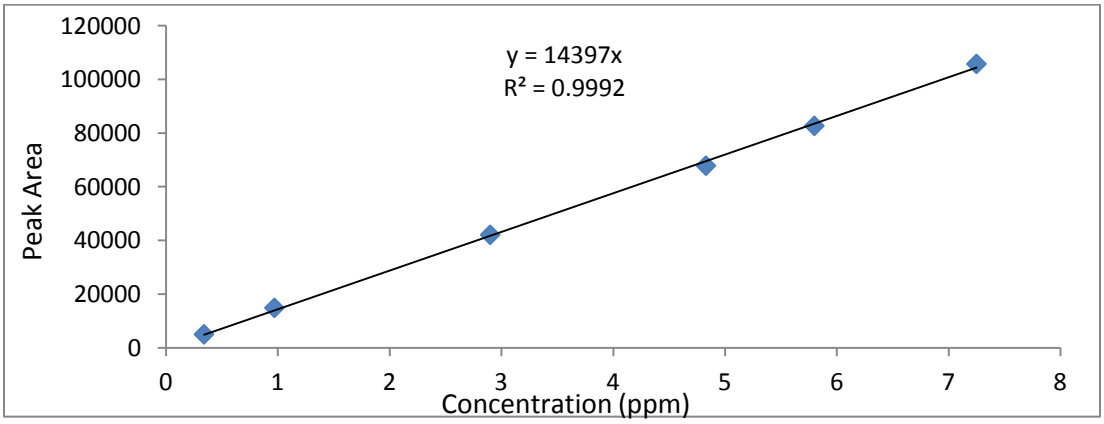

Fig-7 Linearity of 5-Oxo

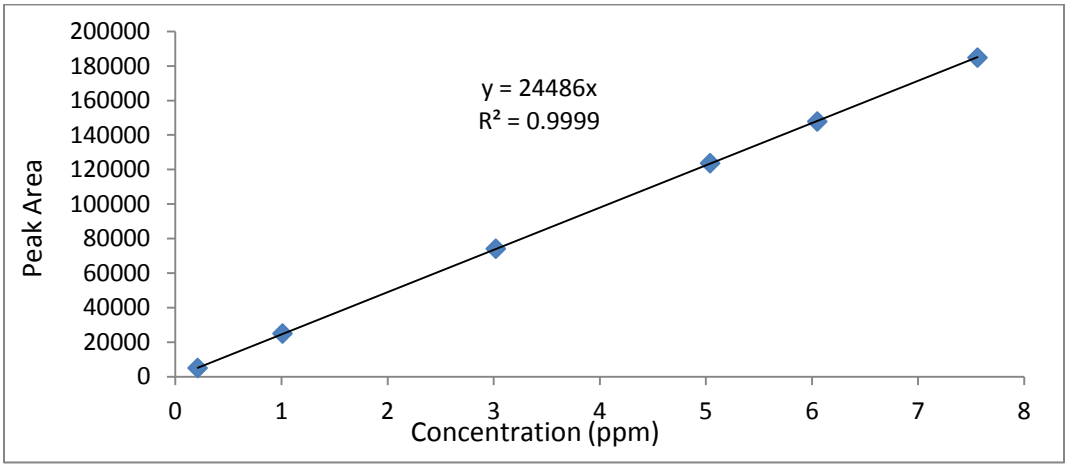

Fig-8 Linearity of Lactone

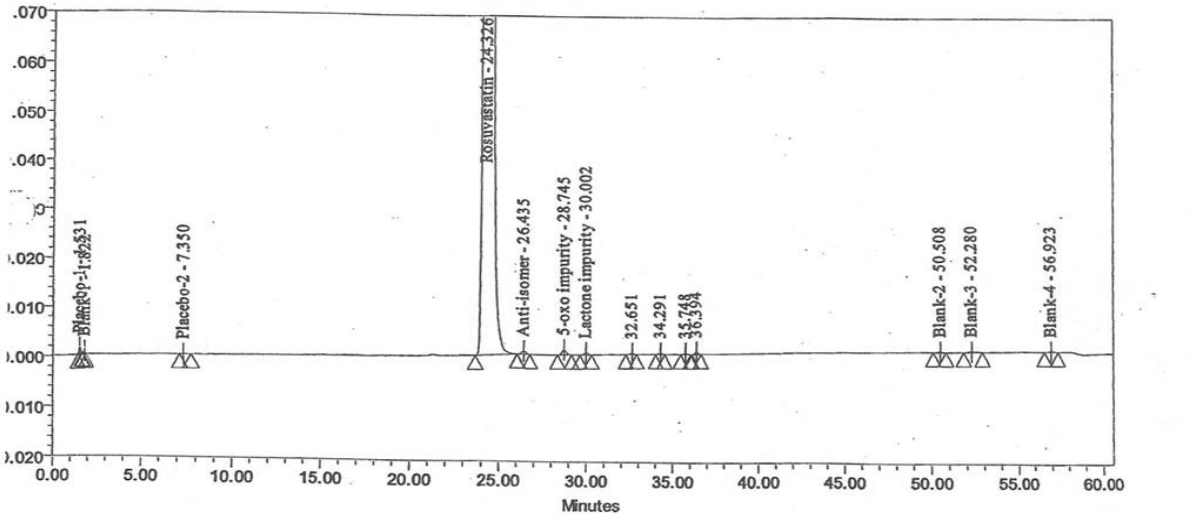

Fig-9 Acid degradation chromatogram of Reference

CONCLUSION

A new RP-HPLC method was successfully developed for the estimation of related substances in ROSV tablets. The developed chromatographic (RP-HPLC) method for ROSV and their related substances is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis in research institution, quality control department in industries, approved testing laboratories, biopharmaceutical studies, and clinical pharmacokinetic studies and for determination of impurities in formulated products. From the overall results obtained it was concluded that the developed method was more accurate, precise, specific and robust with ±2º C in temperature, ±0.2 mL/min in flow rate, ±10% variation in organic phase.

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