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A New Greening RP-HPLC Validated Method for Analysis of Rosuvastatin in Bulk Drug Sample and Its Marketed Formulation

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ABSTRACT

The aim of this study is to create and validate a modern green RP-HPLC system for measuring Rosuvastatin calcium (RSC) in a standard medication. The findings rule out using a 60:30:10 v/v mixture of ethanol, methanol, and ethyl actate at a rate of 1.0 ml/min to identify and separate RSC from its breakdown product. The detection was conducted at 240 nm using a Hypersil BDS 150mm4.6mm RP C18 column packed with a 5 m filter as the stationary step. Linearity, specificity, accuracy, precision, robustness, and device suitability were all tested on the established methodology. Analyzing RSC in marketed products demonstrated the applicability of the proposed procedure. The sum of RSC in commercially available tablets was discovered to be 98.88%. The HPLC-UV device that was created was able to accurately calculate the RSC peak as well as its breakdown properties, showing that the process is stable. The results showed that the evolved approach could be used to investigate RSC in common medications, prescription formulations, and drug release samples on a regular basis.

Keywords*: Rosuvastatin, RP-HPLC, Medications, Prescription Formulations.*

INTRODUCTION

The most commonly used analytical technique for pharmaceutical research is high-performance liquid chromatography (HPLC). Indeed, it is the most important technique used in the quality assurance of bulk drugs and pharmaceutical formulations (e.g., API analysis, impurity characterization, degradation product determination to test product stability, and enantiomeric purity determination), as well as the determination of drugs and metabolites in biological samples [1]. In RP-HPLC, mobile phases are usually mixtures of water, additives to change pH and ionic pressure, and an organic solvent. The two organic modifiers more often used by HPLC users in the RP are acetonitrile and MeOH. Unfortunately, both solvents are classified as dangerous owing to their harmful effects and the high priority put on the safe detoxification of their waste, notwithstanding the fact that MeOH is known to be more environmentally sustainable than ACN and should be used wherever possible [2-3]. The amount of waste produced by RP-HPLC studies must be taken into consideration. In reality, a continuous liquid chromatography with a traditional LC column (15–25 cm length, 4.6 mm i.d., filled with 5 m particles) and a mobile phase flow rate of 1 mL/min generates around 1.5 L of waste per day, or about 500 L of effluent per year [4]. Despite the fact that this amount of waste is insignificant in comparison to the waste created by major industrial production firms, several large pharmaceutical

companies use hundreds of liquid chromatographs in their research and development and quality control labs, resulting in thousands of litres of radioactive waste being collected every day. Furthermore, HPLC is being used more often as a result of technical advancements that enable for high-throughput analysis, which improves the amount of waste collected at the same time. In light of the health and environmental concerns posed by organic solvents widely used in RP-HPLC, the greening of RP-HPLC approaches has piqued the analytical community's attention, with the aim of finding innovative ways to substitute polluting analytical methods with safer ones. In the 2000s, green analytical chemistry (GAC) arose from green chemistry [5-6], and it has gained growing popularity and recognition among researchers. Its definition entails excluding or reducing toxic substances from analytical procedures in order to increase environmental and health friendliness while maintaining system efficiency [7]. At all stages of the study, from sample selection and preparation to isolation and final determination, chromatographic methods have the ability to be more environmentally friendly [8-10]. Several studies have been published in recent years on the application of GAC concepts to chromatography research in general, with some focusing on pharmaceutical analysis specifically [16–19]. Since eliminating the usage of organic solvents in RP-HPLC is impossible, the only approach to render this procedure more environmentally friendly is to substitute toxic solvents with more benign ones.

Ethanol (EtOH) is one of the most environmentally friendly organic solvents, making it ideal for green liquid chromatography [15]. EtOH is less volatile and has a lower vapour pressure than ACN and MeOH, resulting in less evaporation and, as a result, lower inhaled concentrations. EtOH is therefore more readily accessible and less costly (especially EtOH 96 percent) than ACN and MeOH, allowing it to be used in laboratories with restricted resources, such as those in developed countries [4]. For the study of drugs in pharmaceutical formulations as well as biological samples, green RP-HPLC approaches utilising ethanol-based mobile phases have been commonly published [20-21].

The chemical name for rosuvastatin calcium (RS) is (3R,5S,6E)-7-(4-fluoro phenyl)-6(1-methylethyl) pyrimidin-5-yl-2-[methyl (methylsulfonyl) amino] -3,5-dihydroxyheptanoic acid Calcium chloride of -6-enoic acid (2:1) (Figure 1). It's a synthetic, orally active, and successful inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase that lowers LDL cholesterol in an important and particular way in vitro and in vivo [22-24]. Osteoporosis, benign prostatic hyperplasia, and Alzheimer's disease are also treated with it [25].

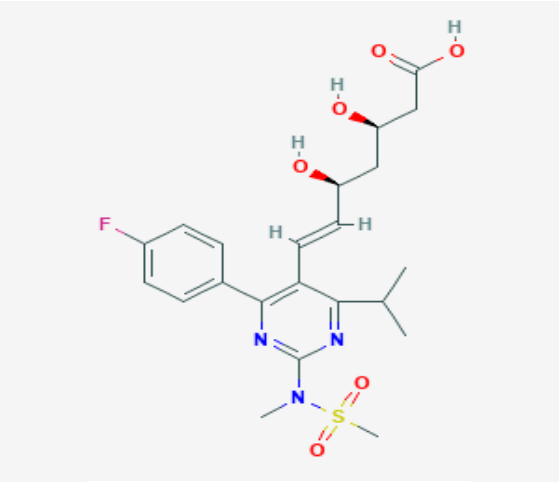

Fig 1. Structure of Rosuvastatin

There have been many assays for quantifying rosuvastatin in bulk products, marketed formulas, and biological matrices such as rat plasma, rabbit plasma, human plasma, serum, and so on. Chemometric analysis of rosuvastatin in branded

formulations has been performed [26–28]. RS research of biological samples [29–31], pharmaceuticals [32–34], and both biological samples and pharmaceuticals [35–36] has previously been successfully performed using a high-performance liquid chromatography (HPLC) device with ultraviolet (UV) detection. Capillary zone electrophoresis [37] and micellar electrokinetic chromatography [38] were used to evaluate ROC in commercial goods. Pharmacokinetics in volunteers [39-40] and rats [41] have been explored in just a few HPLC study reports. Because of its crystalline composition, RS has a low aqueous solubility and is extensively metabolised by the liver through oxidation, lactonization, and glucuronidation, its oral bioavailability is only 20%. Biliary secretion, in addition to overt secretion from the blood to the intestine, is used to remove by-products or metabolites [42–43].

As a result, increasing the solubility of RS and bypassing its hepatic metabolism is a good way to improve its therapeutic performance. Lipid-based drug delivery systems are gaining popularity in the oral administration of poorly bioavailable medications as a way to avoid drug passage into the hepatic portal vein and, therefore, hepatic deterioration [44]. As a result, a lipid vesicular style drug delivery mechanism is one of the methods used in the current research to have a larger interfacial area for drug partitioning between oil and GI fluid. The developed method was used to determine RS in a bulk sample using green solvents.

Ethyl acetate is a green solvent that can be used to substitute ACN and MeOH in mobile phases [45]. Despite these limitations, Haq et al. [46] established and validated an environmentally friendly RP-HPLC approach for the study of indomethacin in bulk products, nanoemulsions, and different pharmaceutical formulations using a mobile step of 100 percent ethyl acetate.

MATERIALS AND METHODS

Chemicals and Reagents

Century Pharmaceuticals provided us with rosuvastatin (99.9% pure) (Varodara, Gujrat, India). Finar Ltd provided HPLC quality ethyl acetate, methanol, and ethanol (Ahmedabad, Gujrat, India). The reagents and chemicals used for the remainder of the experiment were of analytical reagent consistency. RSC 10 mg commercial tablets (Rozucyn-10, Servocare Lifesciences Pvt. Ltd., Haryana, India) were collected from the Delhi, India, domestic industry.

Instrumentation and Chromatographic Conditions

With some changes, the green HPLC system described by Nazrul Haq (2018) was used to analyse Rosuvastatin [47]. The WATERS HPLC 2965 device with Auto Injector and PDA Detector (Waters Corporation, Milford, MA, USA) was used. The stationary process was a 150 mm 4.6 mm RP C18 column filled with 5 m filler from the Hypersil BDS C18 column (ThermoFisher Scientific, Waltham, MA, USA). The mobile process was made up of 60:30:10 v/v ethanol, methanol, and ethyl acetate. The mobile phase was rendered to flow at a rate of 1.0 ml/min, and detection was done at 240 nm.

A Waters Autosampler was used to insert 10 l samples into the device (Waters Corporation, Milford, MA, USA). Waters Corporation, Milford, MA, USA, using a Waters HPLC 2956 system isocratic (Waters, USA) pump, an Autosampler (Waters Corporation, Milford, MA, USA), quaternary LC-10AVP pumps (Waters Corporation, Milford, MA, USA), a programmable UV-visible variable-wavelength detector (Waters Corporation, Milford, MA, USA), a BDS C18 column

oven (Waters Corporation, Milford (Waters Corporation, Milford, MA, USA).

Table 1. Chromatographic Conditions

S.No.	Parameters	
1	Mobile Phase	Ethanol:Methanol:Ethyl Acetate (60:30:10 v/v)
2	Flow rate	1.0 ml/ min
3	Column	Hypersil BDS C18 column150mm x 4.6 mm, 5⌈.
4	Detection wavelength	240 nm
5	Column Temperature	30 ± 1°C
6	Injection volume	10 µl
7	Run time	20 min
8	Retention time	2.655

Preparation of Stock solutions of Rosuvastatin (RSC)

Standard stock solutions (working dilutions) of RS in the required range were prepared by suitably combining the necessary aliquots with the eluent to obtain the desired concentration from the stock solution (200 g/ml).

Method Development and Optimization

Different compositions and ratios were tried for the construction of the mobile process, including OPA: Acetonitrile (50:50), KH2PO4: ACN (65:35), KH2PO4: ACN (60: 40), and flow rate (1.0ml/min). The unusual peak form or a lower number of USP plates were received, however.

We have experimented with scanning wavelengths (200-400nm). Thermo Science BDS C18 150mm x 4.6mm, particle size 5m, flow rate 1.0ml/min obtained the strongest results. At 240nm, detection was monitored. Ethanol: methanol: ethyl acetate (60:30:10v/v) made up the mobile step.

System Suitability Parameters

Establishing device suitability is a crucial step in deciding whether a chromatographic method is suitable for the test at hand. The machine suitability parameters were calculated by preparing regular Rosuvastatin solutions and injecting the solutions six times to calculate peak tailing, resolve, and USP plate count.

Linearity

As an analyte is administered at different concentration ranges, it produces a linear reaction through the method's spectrum, with a correlation coefficient of around 1. Various dilutions ranging from 5g/ml to 30g/ml were prepared.

Specificity

By injecting sample solution with added excipients under optimal chromatographic conditions to show isolation of RSC from excipients, specificity was tested for the intervention of excipients in the analysis of sample solution. There is no interference of the excipient peak with the peak of ROS, meaning that the approach has a strong precision.

Precision

Various concentrations were prepared to see how closely the data values for several measures under the same analytical conditions agreed. The samples' inter-day and intra-day accuracy was tested on varying drug concentrations on separate days.

Accuracy

Accuracy is a measure of how close an experimental value is to the true value and may be assessed by three samples at three concentrations each covering the specified range. The results are calculated as a 0

recovery and must be within certain pre-set acceptance criteria.

Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature were made but there were no recognized change in the result and were within range as per ICH Guide lines. Robustness conditions like Flow minus, Flow plus, mobile phase minus, mobile phase plus, temperature was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Robustness for all drugs was determined.

LOD Sample Preparation

About 0.25ml of standard stock solution (Rosuvastatin) was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution, 0.1ml solution was transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ Sample Preparation

About 0.25ml of standard stock solution (Rosuvastatin) was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution, 0.3ml solution was transferred to 10ml volumetric flasks and made up with the same diluent.

Forced Degradation Studies

Forced degradation studies may be used to further strengthen the specificity of an analytical method. During these studies samples of placebo, API and finished product were subjected to various stressed conditions to determine the degradative pathways of the product. These conditions may consist of thermal, oxidative, light, ultraviolet, humidity, acid hydrolysis, and basic hydrolysis. [48]

Solution Stability

The stability studies were carried out in mobile phase after 24hrs at ambient temperature using the mentioned chromatographic conditions.

APPLICATION OF PROPOSED METHOD

Marketed Product Analysis

The powdered condition of commercially available formulations in the form of tablets was well compressed. A 50 ml volumetric flask was filled with accurately measured fractions of the powder equal to 5 mg, 25 mg, and 50 mg of rosuvastatin. Methanol (approximately 30 mL) was applied to the flask, which was then sonicated for 3 minutes in an ultrasonic wash. After sonication, about 20 ml of methanol was added to make 50 ml, and the mixture was sonicated for 5 minutes. The solution was then filtered through a 0.45 m nylon filter, and the filtrate was collected after discarding the first few ml. Five millilitres of this filtrate were transferred to a 50-millilitre volumetric flask, diluted to volume with diluent, and thoroughly mixed.

RESULTS AND DISCUSSION

Validation of Developed Methods for the Estimation of Rosuvastatin calcium (RSC)

Following drug selection, the medications were dissolved in a suitable diluent to provide a transparent solution. Various combinations of buffers and organic solvents were used to maximise the mobile process. The pka importance of selected drugs was also taken into account when determining the pH of the buffer. The 240 nm wavelength was selected as the detection wavelength for Rosuvastatin calcium. Using a Hypersil BDS C18 150mm x 4.6mm, 5(. column at 30°C and ethanol: methanol: ethyl acetate (60:30:10, v/v) as mobile phase (flow rate-1.0 ml/min.), a strong peak shape with less

tailing factor was observed. Rosuvastatin Calcium was found to have retention of 2.677.

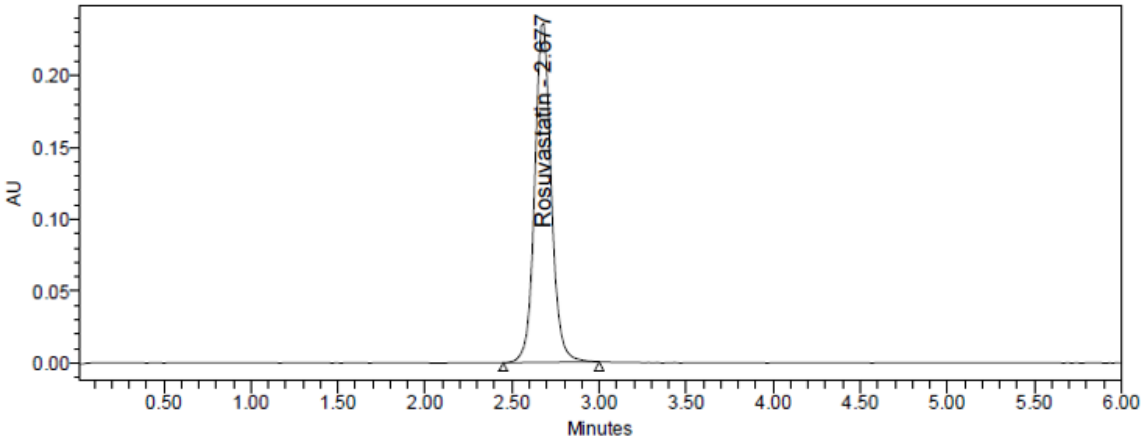

Fig 2. Standard Chromatogram of Rosuvastatin calcium (Optimized Conditions)

SYSTEM SUITABILITY

Six repeat injections of a 100 percent target solution of Rosuvastatin were used to assess system suitability. The number of theoretical plates, area, and peak tailing were all determined, and all of the parameters were found to be within the limits.

Table 2. System Suitability Parameters for Rosuvastatin Calcium (RSC):

Parameter	Result
Retention time	2.677 min
Theoretical Plates	4425
Tailing factor	1.03

The %RSD was found to be 0.8% which was less than 2.0%.

Linearity

The concentration of the drug versus the corresponding peak areas at 240nm were obtained using calibration curves. With a correlation coefficient of 0.998, the calibration curve was found to be linear.

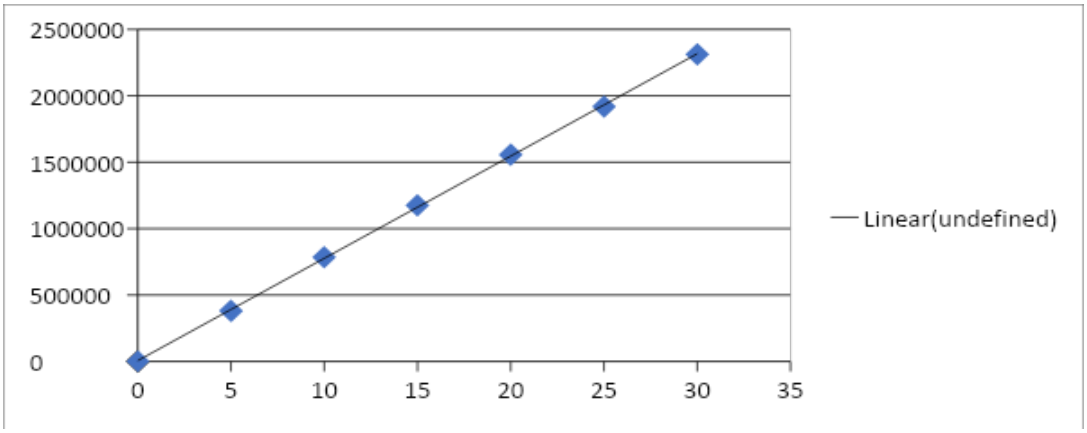

Fig 3. Linearity Plot of Rosuvastatin Calcium (RSC)

Specificity

The mobile process, placebo, sample solution, unspiked, and spiked samples

were all used in the specificity experiment. There was no intervention at the RSC retention time, according to the findings. The following Figure depicts a representative Placebo chromatogram.

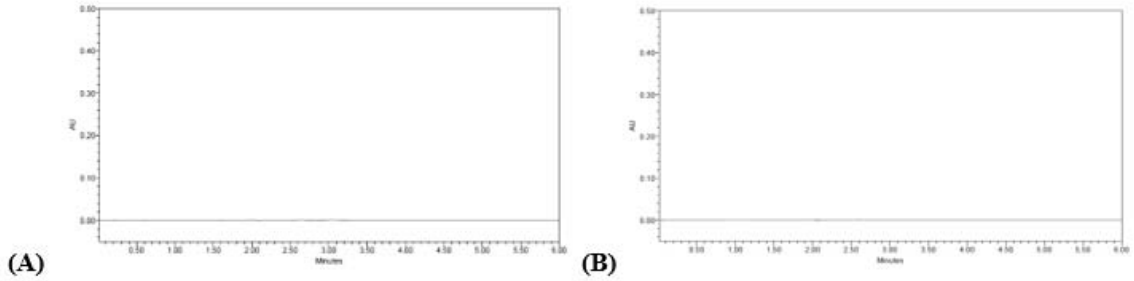

Fig. 4. (A) Blank Chromatogram and (B) Placebo Chromatogram

Precision

The developed method's precision was evaluated for intraday (Precision) and interday (Precision) (intermediate precision). For Rosuvastatin, the percent RSD obtained was 0.85 and 1.17 for intraday and interday, respectively.

Table 3 Intraday and Inter-Day Precision Data of Rosuvastatin Calcium (RSC)

S. No.	Intraday Precision data	Inter-day Precision data
	Peak Area	Peak Area
1	1472563	1476011
2	1490305	1500746
3	1500778	1528749
4	1501065	1495210
5	1503565	1511504
6	1506830	1506209
AVG	1495851	1503072
STDEV	12685.4	17548.6
%RSD	0.85	1.17

Accuracy

To the sample solutions of the selected drugs, known quantities of reference solution for the selected drug, *i.e.* Rosuvastatin, equal to 50 percent, 100 percent, and 150 percent of the label claim, were added. Rosuvastatin had a percent mean recovery of 100.31, suggesting that the procedure was correct.

Table 4. Accuracy Data of Rosuvastatin Calcium (RSC)

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	9.840765	98.41	100.03%
	50	10.04586	100.46	
	50	9.970044	99.70	
100%	100	19.98577	99.93	

	100	20.18813	100.94	
	100	20.10957	100.55	
150%	150	29.80545	99.35	
	150	30.40379	101.35	
	150	29.87854	99.60	

Robustness

Slight differences in the flow rate (flow minus & flow plus), column temperature (Temperature minus & Temperature Plus), and mobile phase concentration were used to test robustness (Mobile phase minus & Mobile phase plus). The above conditions' percent RSD was determined. The method's robustness was demonstrated by the lack of a substantial impact with the above adjustments.

Table 5 Robustness data of Rosuvastatin Calcium (RSC)

Parameter	%RSD
Flow rate (-) 0.9ml/min	0.8
Flow rate (+) 1.1ml/min	0.6
Mobile phase (-) 20B:80A	0.5
Mobile phase (+) 25B:75A	0.1
Temperature (-) 25°C	0.9
Temperature (+) 35°C	0.3

LOD and LOQ Data

The linearity curve method was used to assess the LOD and LOQ of Rosuvastatin calcium in this study. RSC has been found to have LOD and LOQ values of 0.11g/ml and 0.34g/ml, respectively.

Frced Degradation Studies

During these experiments, samples were subjected to a variety of intense conditions in order to show that the analytical method could detect sample degradation. Both samples were injected and tested for peak purity using a photo-diode array (PDA) detector. The analyst will see the angle of inflection of the peak in contrast to the underlying baseline using the purity analysis with PDA detection. The purity angle will be greater than the threshold angle if an unknown or associated compound peak co-eluted with the active peak of interest. The assay of the injected samples was measured, and all of them passed the degradation limits.

Table 6. Degradation Data of Rosuvastatin Calcium (RSC)

S.No.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.40	0.172	0.306
2	Alkali	5.12	0.256	0.321
3	Oxidation	3.58	0.323	0.376
4	Thermal	2.74	0.313	0.350
5	UV	1.07	0.328	0.379
6	Water	0.74	0.315	0.392

Solution Stability

The findings showed that Rosuvastatin calcium (RSC) was stable in mobile phase for at least 24 hours, indicating the validity of the proposed procedure's study.

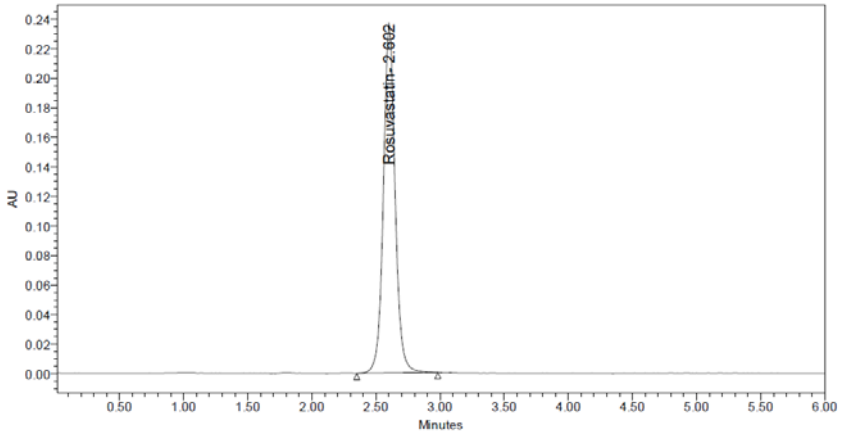

Fig. 4. Solution stability graph of Rosuvastatin at 0^oC

The %RSD was found to be 0.3% which was less than 2.0%.

Assay Result of Tablet formulation

Table 1.7 Assay Results of Rosuvastatin Calcium (RSC) marketed tablet (Rozucyn-10)

Tablet	Label Claim (mg)	Assay (% label claim)	%RSD
Rozucyn-10	10	99.99	0.25

DISCUSSION

When acetonitrile was combined with buffer at different ratios during the chromatographic process development stage, chromatograms with irregular peak shapes and/or fewer plates were observed. A mixture of ethanol and ethyl acetate was tested as an alternative eluent to get a sharp peak response with good sensitivity while avoiding the use of toxic chemicals. From the various ethanol and ethyl acetate formulations studied, a ratio of ethanol, ethyl acetate, and fewer methanols was chosen in the ratio of 60:30:10 v/v to minimise the viscosity of the mobile process. With a retention time of 2.677 minutes, the mobile step thus chosen was an environmentally friendly and fast method for RSC assay. In the concentration range of 5-30g/ml, linearity was observed. The concentration over peak area regression equation was discovered to be 77012x + 5842.9 (r2=0.998). The number of theoretical plates was discovered to be 4425, indicating that the column is performing well. The tailing factor was found to be 1.03, indicating that the peak is in good shape. The method's limit of detection and limit of quantification were found to be 0.11g/ml and 0.34g/ml, respectively, suggesting its sensitivity. The proposed approach was found to be highly reliable, with a mean percentage recovery of 100.03 percent. The percent RSD value of 2% for both process and interday precision showed that the method was very precise. Forced degradation tests revealed no major effects, indicating the method's robustness. Excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC process, as no interfering peaks were found in the chromatogram during the run time.

CONCLUSION

The proposed green RP-HPLC method for estimating Rosuvastatin calcium (RSC) in bulk and formulation was simple, accurate, precise, specific, stability indicating, and cost-effective. The proposed method is ideal for simultaneous determination of pharmaceutical formulations with virtually no intervention from common additives found in pharmaceutical formulations, according to the results of the study of pharmaceutical formulations. The process was beneficial because it used no solvent. As a result, the proposed green HPLC approach can be used to analyse Rosuvastatin calcium in formulation on a regular basis. Furthermore, the proposed approach can be used to analyse a number of samples with high precision and accuracy.

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