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Structural identification and estimation of Rosuvastatin calcium related impurities in Rosuvastatin calcium tablet dosage form

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ABSTRACT

A precise, accurate, specific, linear, rugged and robust analytical method was developed and validated for estimation of process and degradant impurities of Rosuvastatin calcium (RSC) in Rosuvastatin calcium tablets. 150 mm length column, 4.6 mm diameter and 3.5μ particle size with C_{18} stationary phase and pH3.0 phosphate buffer as mobile phase. Column was maintained at 30 °C.All impurities are monitored at 248 nm.Impurities are separated in gradient elution mode. All degradant impurities of RSC (Anti-isomer, 5-ketoacid, lactone and meglumine adduct), process impurity (Imp-A) are well separated. Unknown impurity (Meglumine adduct) formed during stability studies was isolated using preparative HPLC and structure was characterized by NMR and Mass spectrometry (LC-MS and HRMS) studies. Method is capable of separating and estimating all the degradant and process impurities.

1. Introduction

Rosuvastatin calcium (RSC) is a synthetic lipid-lowering agent for oral administration. It inhibits HMG-CoA reductase, the rate-limiting enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol. The chemical name for rosuvastatin calcium is bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2 [methyl (methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. The empirical formula for rosuvastatin calcium is $(C_{22}H_{27}FN_3O_6S)_2Ca$ and the molecular weight is 1001.14. Rosuvastatin calcium drug substance is official in Ph. Eur monograph. Listed impurities in monograph are Anti-isomer (Imp-B), 5-keto acid (Imp-C), lactone (Imp-D) and Imp-A. Rosuvastatin calcium is available in 5, 10, 20 and 40 mg tablet dosage form. Stability studies play a crucial role in inspecting the quality of the drug product during its shelf life. Excipients used in formulating the drug product may react and form adduct with drug substance. Hence it is necessary to develop such a method which can detect and separate all the possible degradant and process impurities. Few methods are available for estimation of RSC in bulk and pharmaceutical dosage forms by HPLC [1–4], by HP-TLC [5], estimation of RSC in presence of degradation impurities [6,7]. Anti-isomer, 5-keto acid and lactone are the degradant impurities. Anti-isomer and lactone will be formed during acid degradation and 5-keto acid during oxidation. Impurity-A (Acetone adduct) is a process impurity formed during synthesis of Rosuvastatin calcium drug substance. Meglumine adduct is a degradant impurity and it will be formed during stability studies of Drug product at 40°C/75% RH condition. Meglumine is a base used as excipient to stabilize the formulation. Adduct impurity was synthesized by mixing RSC and Meglumine and keeping at 105 °C.Enriched sample was injected in preparative HPLC and collected the impurity. By using HRMS, 1H NMR and 13C NMR techniques, structure for impurity was identified. Structures, chemical names of RSC and impurities are tabulated in Table 1.

2. Instrumentation and reagents

Waters HPLC system (make: Waters) with Photo diode array detector (Model: 2996) was used. Mono basic phosphate was used in mobile phase preparation. Acetonitrile and methanol were used of gradient grade supplied by Merck. AR grade monobasic phosphate supplied by Merck was used in buffer preparation. Sonicator (model: powersonic420) was used in sample preparation. Centrifuge (make: Thermo scientific) was used to centrifuge the sample preparation. Rosuvastatin calcium and all impurities supplied by Biocon. Adduct impurity was synthesized by preparative HPLC.

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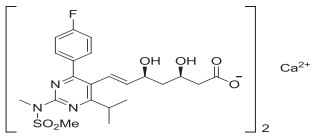
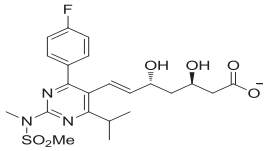
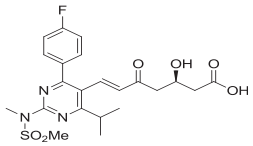
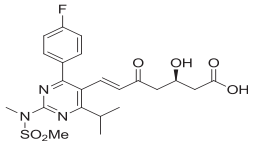
Table 1
Chemical names and structures for Rosuvastatin calcium and its related impurities.

S.no	Name	Chemical name	Structure
01	Rosuvastatin calcium	(E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl (methylsulphonyl)amine}pyrimidin-5-yl}-3,5-dihydroxyheptane-6-enoic acid.	
02	Imp-A	(3R,5S,6E)-7-[4-(4-Fluorophenyl)-2-[[(2-hydroxy-2-methylpropyl)sulfonyl](methyl)amino]-6-(1-methylethyl)-pyrimidinyl-5-yl]-3,5-dihydroxyhept-6-enoic acid.	
03	Anti-isomer	(3R,5R,6E)-7-[4-(4-Fluorophenyl)-6-isopropyl-2-(methanesulfonyl- methyl-amino)-pyrimidin-5-yl-3,5-dihydroxy-hept-6-enoic acid calcium	
04	5-Keto acid	Calcium ((3R,6*E*)-7 (4-(4-fluorophenyl)-6-isopropyl-2-(N mehylmethylsulfonamido)pyrimidin-5-yl)-3-hydroxy-5-oxohept-6-enoate)	
05	Lactone	*N*-{4-(4-Fluoro-phenyl)-5-[2-(4-hydroxy-6-oxo-tetrahydro-pyran-2-yl)-vinyl]-6-isopropyl-pyrimidin-2-yl}-*N*-methyl-methanesulfonamide	
06	Adduct	(3R,5S,*E*)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(N- methylmethylsulfonamido)pyrimidin-5-yl)-3,5-dihydroxy-N-methyl-N-((2S,3R,4R,5R)-2,3,4,5,6-pentahydroxyhexyl)hept-6-enamide	

3. Materials and solutions

3.1. Standard preparation (0.2% of test concentration)

Weighed accurately about100mgof Rosuvastatin calcium Working Standard into a 100 mL volumetric flask, added 75 mL of diluent, sonicated to dissolve the drug, and diluted to volume with diluent.10 mL of above solution transferred in to a 250 mL volumetric flask and diluted to volume with diluent. Pipetted 5 mL Rosuvastatin calcium standard stock solution in to 100 mL volumetric flask and diluted to volume with diluent.

3.2. Sample preparation

weighed and transferred 20tablets into a mortar and made a fine powder. Transferred sample equivalent to 100 mg of rosuvastatin calcium into 100 mL volumetric flask. Added 75 mL of diluent and sonicated for 30 min, at less than 25 °C.Diluted to volume with diluent and mixed. Centrifuged a portion of above solution with lid at 5000 rpm for 10 min.

3.3. Spiked sample preparation

Weighed and transferred 20tablets into a mortar and made a fine powder. Transferred sample equivalent to 100 mg of rosuvastatin calcium into 100 mL volumetric flask. Added required quantity of impurity stock solutions to sample solution to get 0.2% of test concentration for all impurities and 0.5% for 5-keto acid impurity. Added 75 mL of diluent and sonicated for 30 min, at less than 25 °C.Diluted to volume with diluent and mixed. Centrifuged a portion of above solution with lid at 5000 rpm for 10 min.

4. Methods

X Bridge 150 × 4.6 mm, 3.5μ HPLC column with C18 stationary phase was used to separate the entire impurities.20 mM monobasic phosphate buffer was and pH adjusted to 3.0 with dilute ortho phosphoric acid was used in mobile phase preparation. Mobile phase-A consists of buffer and Methanol in the ratio of 80:20. Mobile phase-B consists of buffer, acetonitrile and methanol in the ratio of 15:25:60. Column temperature was maintained at 30 °C.Sample temperature was maintained at 5 °C.All the impurities are monitored at 248 nm.Gradient elution mode was used to separate the impurities.20 μL sample was injected into HPLC system.

5. Results

Developed method is capable of separating all the degradant and process impurities of RSC. Method is validated as per ICH guidelines and found to be precise, specific, accurate, linear, robust and rugged. Limit of detection and Limit of quantification were established for all the impurities and RSC. %RSD for Imp-A, adduct, Anti isomer, 5-keto acid and lactone were found to be 0.6, 0.6, 0.6, 0.5 and 0.7 respectively. No placebo peaks are found at the retention times of Imp-A, adduct, Anti isomer, 5-keto acid and lactone impurities. Recovery values for all the impurities at each spike level were found to be between 85% and 115%.Method is found to be linear from LOQ level to 200% of test concentration for all the impurities. Correlation coefficient values are found to be more than 0.997. Reported the slope and intercept values LOQ and LOD values are established for impurities and RSC. LOQ values are found to be less than reporting threshold. Changes were done in mobile phase composition, pH variation, and column temperature and flow rate

Table 2
Comparison of related substance methods between drug substance and product.

Parameters	Drug substance			Drug product		
Column	Inertsil ODS 150 × 3.0 mm, C18,3 µ			X Bridge 150 × 4.6 mm, C18,3.5 µ		
Wave length	242 nm			248 nm		
Injection volume	10 µL			20 µL		
Column temperature	40 °C			30 °C		
Flow rate	0.75 mL/min			1.0 mL/min		
Mobile phase-A	0.1% TFA:ACN:Water (10:290:300)			Buffer:MeOH(8:2)		
Mobile phase-B	0.1% TFA:ACN:Water (10:750:240)			Buffer:MeOH:ACN (15:60:25)		
Gradient programme	Time	%A	%B	Time	%A	%B
	0	100	0	0	60	40
	30	100	0	20	50	50
	50	60	40	45	10	90
	60	0	100	55	10	90
	70	0	100	56	60	40
	73	100	0	60	60	40
	80	100	0			

to verify the robustness of the method. All system suitability parameters were evaluated and found to be within the acceptance criteria. Standard and solution stability was evaluated. Standard is found to be stable for 5days for bench top. Sample solution was found to be stable for 2 h s on bench top.

6. Discussion

6.1. Method development and optimization

Rosuvastatin Calcium Bulk drug substance was official in European pharmacopeia. Related substances method was given to estimate Lactone, Anti isomer and 5-keto analog in Rosuvastatin calcium drug substance. New analytical method was developed to estimate impurities in Rosuvastatin calcium Tablets. Comparison between drug substance and drug products were given in below Table 2. HPLC method was developed for separation of all impurities. Rosuvastatin calcium having a pKa values of 3.8, 4.9, 5.5 and 14.65. So acidic pH was chosen in the range of 3.0–4.0 for the initial Experiment. Acetic acid buffer was chosen for the initial Experiment. Sample spiked with all impurities. All impurities are well separated. But due to poor peak shape, resolution between RSC and Anti isomer was less. To improve peak shape phosphate buffer was used in mobile phase preparation. Trials were conducted with different columns to get good peak shape and optimum resolution between all the impurities. Development trials were mentioned in Table 3. Final method concluded based on resolution, peak shape and recovery of main peak and impurities. Chromatogram with final chromatographic conditions was figured in Fig. 1. Relative response factor for impurities was established using slope method. Forced degradation studies were conducted on the samples and injected into HPLC with developed method. Anti-isomer and lactone impurities are generated in acidic condition. Two unknown impurities are formed in photolytic degradation at 2.05 and 2.15RRT'S.In heated stressed condition one unknown impurity was generated at 0.68RRT.The ESI mass spectrum of Rosuvastatin 0.68RRT impurity was recorded on MDSciex 4000-q-Trap LCMSMS system. The ESI + ve ionization mass spectrum of Rosuvastatin 0.368RRT impurity displayed the protonated molecular ion at m/z = 659(Fig. 10). The mass data indicates formation of the condensed product of meglumine with Rosuvastatin. The HRMS

Table 3
Development trials.

Trial no	Mobile phase	Column	Observation
01	2.0 mL of Acetic acid to 1000 mL	Hypersil BDS C18,250 × 4.6 mm,5 µ	Rosuvastatin and Anti Isomer impurity were not well separated
02	2.0 mL of Acetic acid to 1000 mL	AAscentisExpressC18,150 × 4.6 mm,2.7 µ	Peak shape of Rosuvastatin need to be improved
03	2.0 mL of Acetic acid to 1000 mL	XX-Bridge C18,150 × 4.6 mm,3.5 µ	well separated with a resolution of 2.6.
04	10 mM of KH_2PO_4,pH to 3.0	XX-Bridge C18,150 × 4.6 mm,3.5 µ	All impurities are well separated

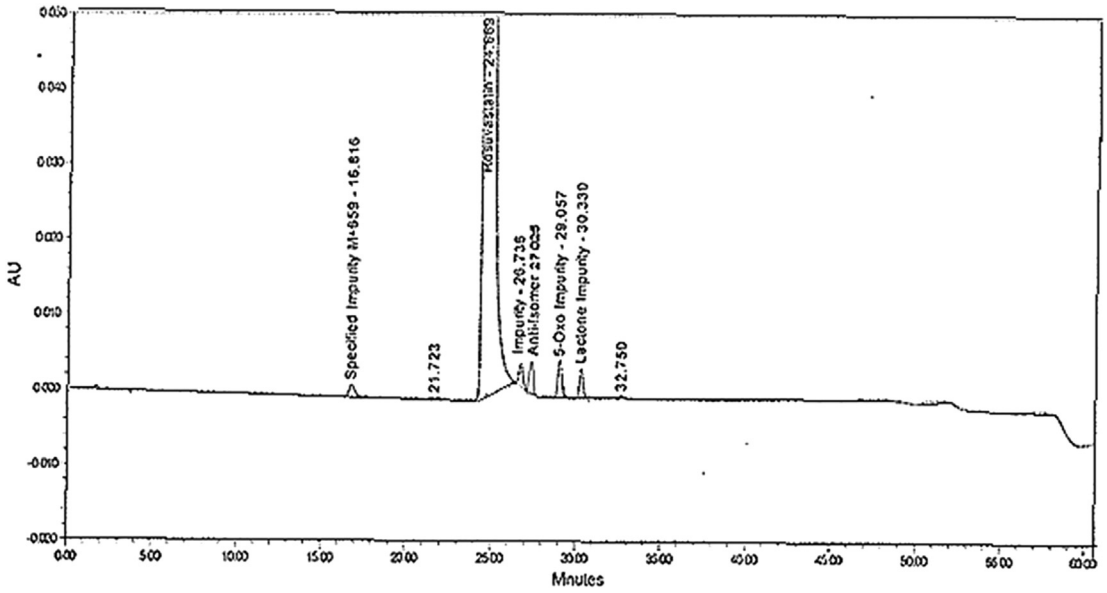


Fig. 1. Typical chromatogram for sample spiked with impurities.

Table 4
Raw materials.

S.no	Raw material	Input	Unit	Mol wt	Mole	Mole ratio
1	Rosuvastatin calcium	10.0	g	481.5	0.020	1.0
2	DCM	50.0	mL	–	–	–
3	Water	30.0	mL	–	–	–
4	Sodium sulphate	2.0	g	–	–	–
5	Meglumine	5.58	g	195.2	0.03	1.5
6	TBTU	9.63	g	321.1	0.03	1.5
7	DMF	15.0	mL	–	–	–

spectrum confirms the molecular formula as $C_{29}H_{44}N_4O_{10}FS$(-Fig. 11). This is in alignment with the condensed product. NMR analysis has been done in CD_3OD at 400 MHz for ^1H(Fig. 12) and 100 MHz for ^{13}C(Fig. 13) on Bruker Advance 400 MHz spectrometer. The chemical shift values are reported on δ scale in ppm with respect to TMS (0.00 ppm) and CD3OD (49.5 ppm) as internal standard respectively. In proton spectrum of impurity additional signals were observed at chemical shift δ ppm 3.0, 3.2, 3.5, 3.6 to 3.9 and 4.0 ppm when compared to RSC NMR spectrum. These were attributed to the additional one N-Methyl, four methane and two methylene groups of Meglumine. In ^{13}C NMR spectrum of RSC the signal corresponding to carboxylic acid was observed at 182.5 ppm.In impurity the signal has been shifted to 175 ppm.1H and 13C signals of the atoms involved in the structural change appear at two chemical shifts in impurity. This observation is attributed to the restricted rotation about the amide bond. The HMBC spectrum if impurity displayed the correlations of quaternary carbonyl carbon at 175 ppm with N-methyl protons at 3.0 and 3.2 ppm.This supports to the amide bond formation between Rosuvastatin carbonyl carbon at position 23 and Meglumine nitrogen at position 31. To confirm the adduct Rosuvastatin and Meglumine 1H NMR spectra's are done individually (Figs. 14 and 15). Based on the above spectrometric evidences the impurity structure has been confirmed. Meglumine is one of the excipient present in Rosuvastatin calcium tablets. To perform validation for impurity at 0.68RRT, it was synthesized using meglumine and Rosuvastatin calcium drug substance. Reagents used for the synthesis of adduct were tabulated in Table 4. Synthetic scheme was given in Fig. 2. BTU (2-(1H-Benzotriazole-1-yl)1,1,3,3 tetramethyluronium tetrafluoroborate) also known as Knorr's reagent was used for the formation of bond between carboxylic acid and secondary amine. Rosuvastatin calcium (10.0 g, 0.02 mol),Meglumine (5.58 g, 0.03 mol) and TBTU (9.63 g, 0.03 mol) were dissolved in dichloromethane 50 mL) and dimethylformamide(15.0 mL). The reaction was stirred at room temperature for overnight. The reaction mixture was diluted with water and thrice extracted with dichloromethane. The combined organic phases were dried over sodium sulphate and evaporated. The crude product was purified by column chromatography. Synthesized impurity structure was confirmed using the above mentioned techniques. Validation was performed with the synthesized impurity.

6.2. Method validation

6.2.1. System suitability

Standard solution was prepared using Rosuvastatin calcium standard at a concentration of 0.2% of target concentration as per method and injected six times into the HPLC system. The system suitability solution having the concentration of 0.006 mg/mL of Imp-A and 1 mg/mL of Rosuvastatin calcium was prepared and injected into HPLC. The system suitability parameters were evaluated. Results are summarized in Table 5.

6.2.2. Placebo and degradation products interference

Placebo solution was prepared by taking placebo equivalent to 100 mg or RSC into 100 mL flask. No placebo peaks are observed at the retention time. Forced degradation was performed on tablets formulation. Degradation was conducted by acid hydrolysis, base hydrolysis, peroxide oxidation, photo stability, Heat, humidity, and water stress conditions. All the samples were evaluated for peak purity of RSC and its impurities and found to be pure. Results are tabulated in Table 6 and 10. Chromatograms for degraded samples was presented in Fig. 3–9.

6.2.3. Precision

Precision of the test method was evaluated by injecting six

Table 5
System suitability parameters.

System suitability	Observed value	Acceptance criteria
Tailing factor for Rosuvastatin	1.1	Between 0.8 and 1.5
Theoretical plates for Rosuvastatin	31556	Not less than 2000
The resolution between Rosuvastatin and Imp-A peak	2.2	Not less than 1.5

Table 6
Placebo interference.

S.no	Peak found (Yes/No)				
	Imp-A	Adduct	Anti isomer	5-keto acid	Lactone
01	No	No	No	No	No

Table 7
Results for repeatability.

Sample no	% Impurity				
	Adduct	Imp-A	Anti-isomer	5-Keto acid	Lactone
1	0.203	0.203	0.215	0.500	0.230
2	0.203	0.204	0.216	0.500	0.226
3	0.203	0.203	0.213	0.498	0.227
4	0.206	0.200	0.216	0.505	0.229
5	0.205	0.198	0.213	0.501	0.227
6	0.204	0.199	0.215	0.499	0.226
Ave	0.204	0.201	0.215	0.501	0.228
%RSD	0.6	1.2	0.6	0.5	0.7

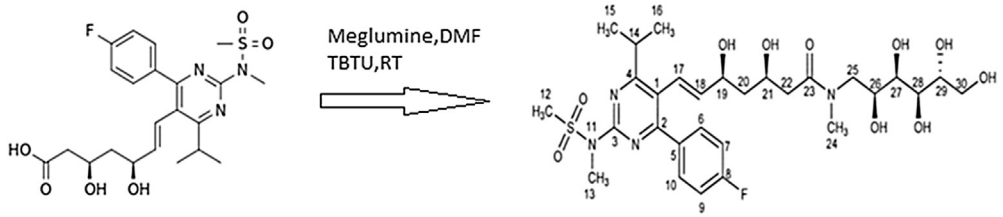


Fig. 2. Synthetic scheme.

Table 8
Results for accuracy.

Sample no	% Impurity				
	Adduct	Imp-A	Anti isomer	5-Keto acid	Lactone
50%-1	88.0	98.1	105.2	103.0	106.4
50%-2	89.6	97.1	105.2	102.2	105.6
50%-3	88.0	98.1	105.2	103.8	104.8
100%-1	88.8	104.8	104.6	105.4	105.0
100%-2	88.6	105.3	105.4	108.3	105.8
100%-3	88.8	104.8	104.8	106.2	105.6
150%-1	92.0	105.3	109.5	109.1	109.0
150%-2	92.0	106.0	109.7	109.4	108.4
150%-3	91.8	107.0	108.9	108.8	108.2
LOQ-1	107.1	104.8	106.2	103.4	108.7
LOQ-2	100.2	105.3	106.2	97.5	99.2
LOQ-3	103.6	104.8	109.5	91.6	113.4

sample, each prepared by spiking the test preparation with all the known impurities at target concentration of 0.2% of all impurities.(Anti isomer, Imp-A, lactone, Adduct) and 0.5% of 5-keto acid. The %RSD of the individual %RSC and impurities from six sample preparations were calculated and tabulated in Table 7.

6.2.4. Accuracy

A study of accuracy was conducted by spiking the impurities at different spike levels. Three samples were prepared by spiking the test preparation with 0.25% (2.5 ppm) of all impurities. Six samples were prepared by spiking the test preparation with all impurities at 0.5% (5 ppm) concentration. Six more samples were prepared by spiking the test at 0.75% (7.5 ppm). The individual % recoveries were calculated and tabulated in Table 8.

6.2.5. Limit of detection and quantification

Determined limit of detection and quantification based on S/N method. S/N ratio was derived about 3.0 for limit of detection and 10.0 for limit of quantification. Six test solutions were prepared having impurities at limit of quantification level and injected. %RSD and %recovery of impurities at LOQ was calculated and tabulated in Table 11.

Table 9
Results for linearity of detector response.

Imp-A		Adduct		Anti isomer		5-Keto acid		Lactone	
0.48	8089	0.29	4534	0.27	6150	0.34	5048	0.21	5120
1.01	18000	0.97	16788	0.98	22134	0.97	14906	1.01	25080
1.47	25909	1.93	32425	2.94	65802	2.90	42128	3.02	74199
2.02	37169	2.90	47254	4.90	108304	4.83	68884	5.04	123755
3.02	56515	3.86	62750	5.88	128343	5.80	82736	6.05	147922
4.03	75627	4.83	83763	7.36	162172	7.25	105808	7.56	184912
Correlation	0.9999	Correlation	0.9984	Correlation	0.9999	Correlation	0.9998	Correlation	1.0000
Slope	19119.155	Slope	16975.38	Slope	21913.77	Slope	14368.61	Slope	24450.54
Intercept	−1447.068	Intercept	−563.69	Intercept	553.4708	Intercept	338.6483	Intercept	233.5764
%Bias	−3.9	%Bias	−1.74	%Bias	0.51	%Bias	0.49	%Bias	0.19

Table 10
Table of results of forced degradation of Rosuvastatin calcium Tablets.

S.no	Stress condition	% Degradation	Purity angle	Purity threshold
1	Refluxed with 0.1 N HCl for 2 h s at 60 °C	6.0395	0.064	0.268
2	Refluxed with 5 N NaOH for 12 h s at 60 °C	0.2448	0.112	0.271
3	Refluxed with 10% peroxide for 30 min at 60 °C	1.4468	0.093	0.292
4	Exposed to 200 W h/m^2 and 1.2million lux hour in photostability chamber for 16 h	11.9969	0.060	0.260
5	Heat at 105 °C for 6 h s	0.4595	0.070	0.277
6	Humidity at 90% RH for 7 day at 25 °C	0.2509	0.065	0.273
7	Refluxed with water for 30 min at 60 °C	0.2858	0.059	0.277

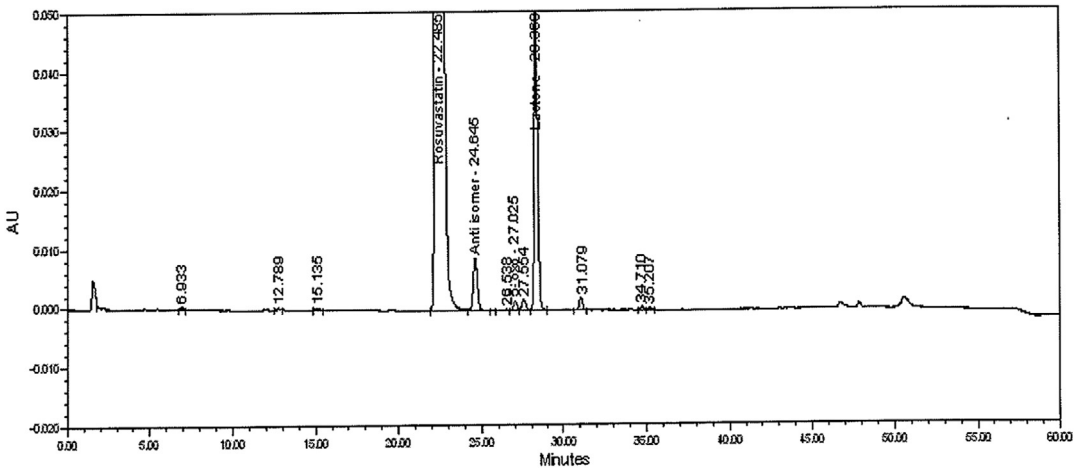


Fig. 3. Typical chromatogram of acid degraded sample.

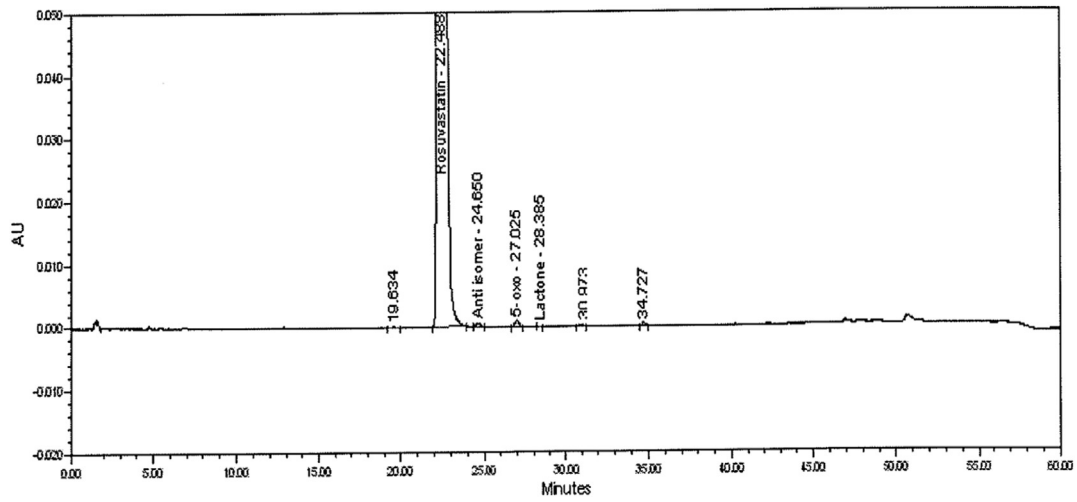


Fig. 4. Typical chromatogram of base degraded sample.

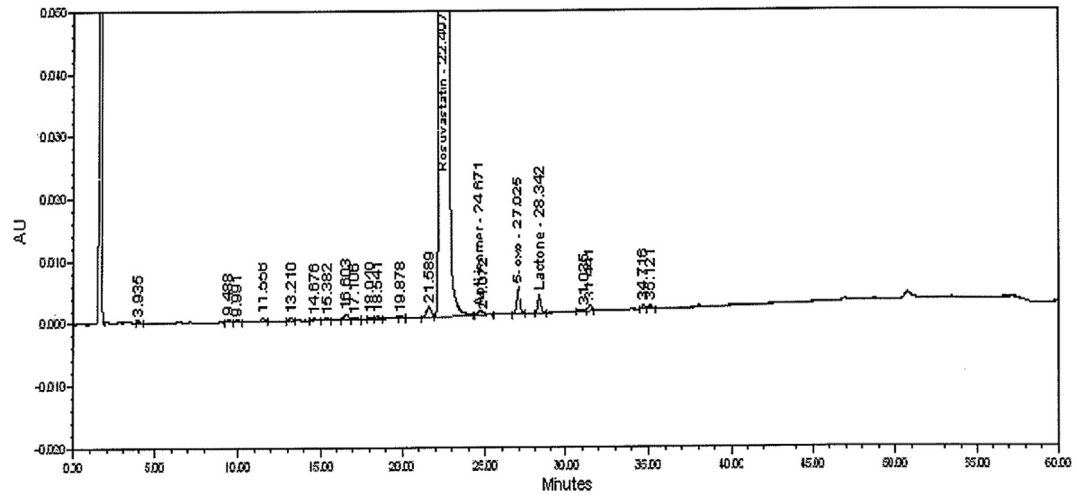


Fig. 5. Typical chromatogram of peroxide degraded sample.

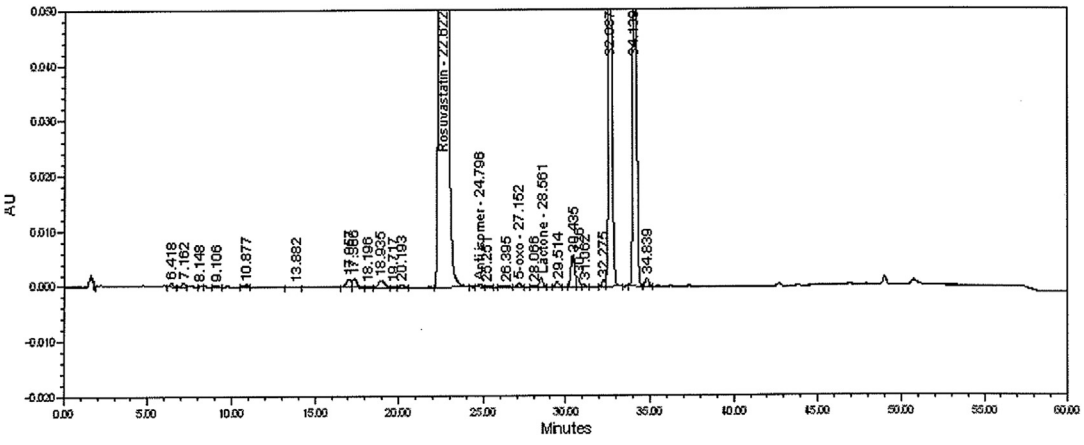


Fig. 6. Typical chromatogram of photolytic degraded sample.

6.2.6. Linearity of detector response

A linearity study was performed from LOQ level concentration to 150% of target concentration for all impurities. Calculated correlation coefficient, slope and intercept for concentration versus response and results are tabulated in Table 9.

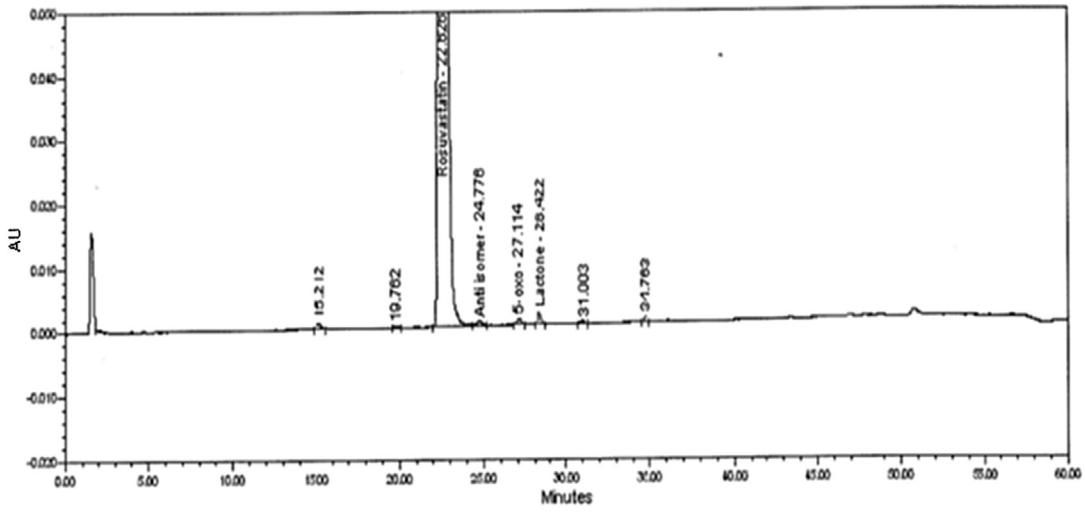


Fig. 7. Typical chromatogram of thermal degraded sample.

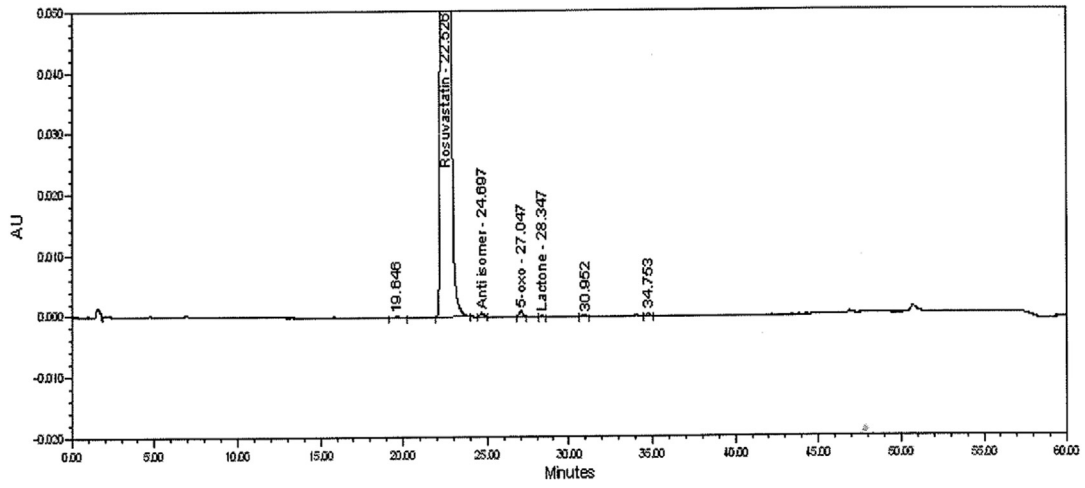


Fig. 8. Typical chromatogram of humidity degraded sample.

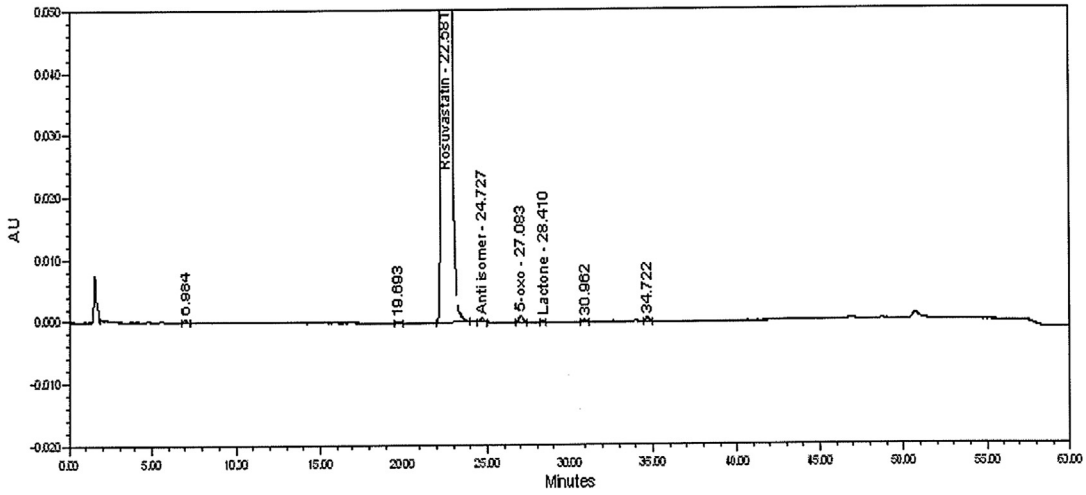


Fig. 9. Typical chromatogram of water degraded sample.

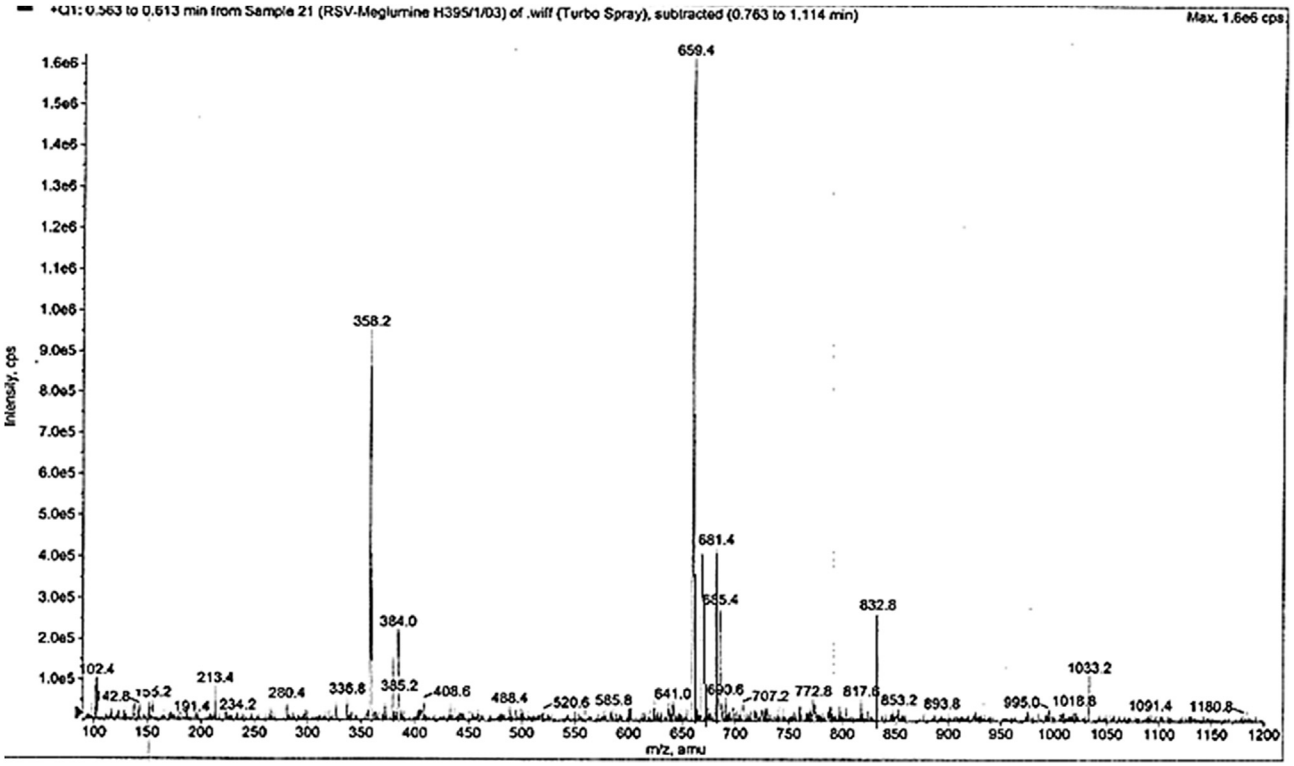


Fig. 10. ESI (+ve) mass spectrum for adduct impurity.

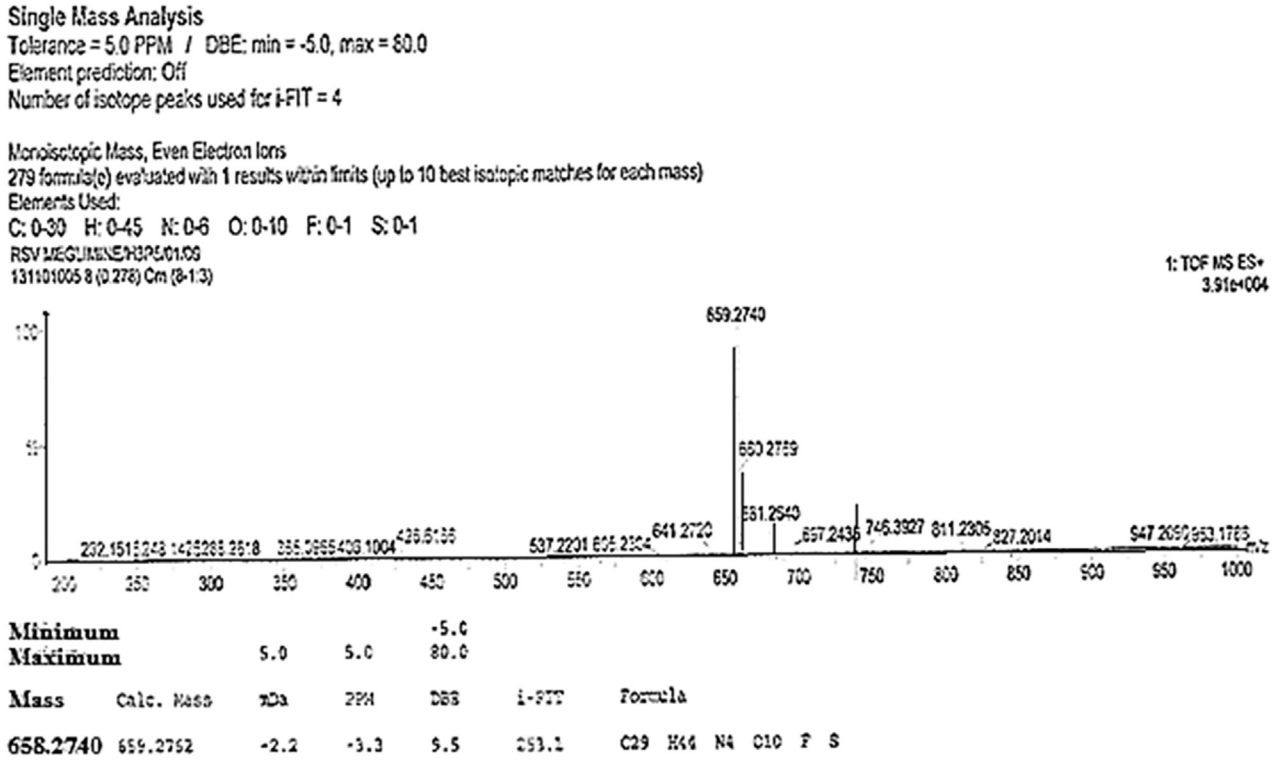


Fig. 11. HRMS spectrum for adduct impurity.

6.2.7. Ruggedness

Bench top stability of Mobile phase, standard and sample solutions: Stability of standard and sample solutions on bench top was established over a period of 2days.Mobile phase and standard preparations are found to be stable up to 2days on bench top. Sample solution is found to be stable up to 2 h.

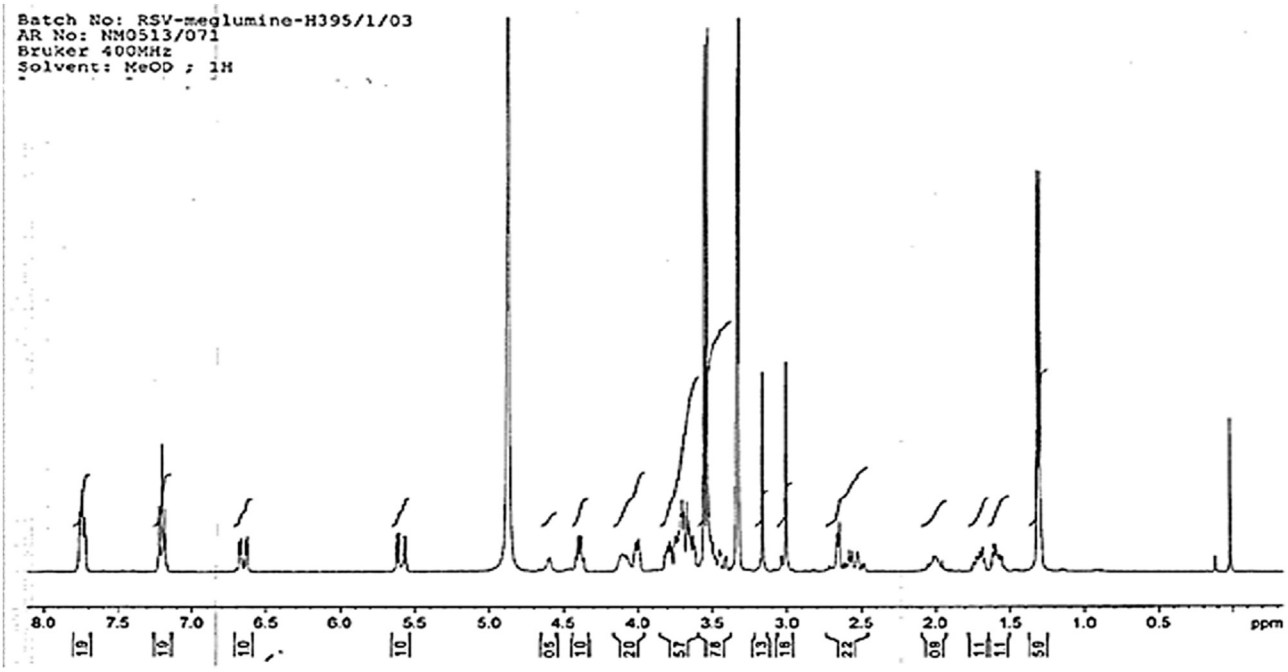


Fig. 12. ^{1}H NMR spectrum for impurity.

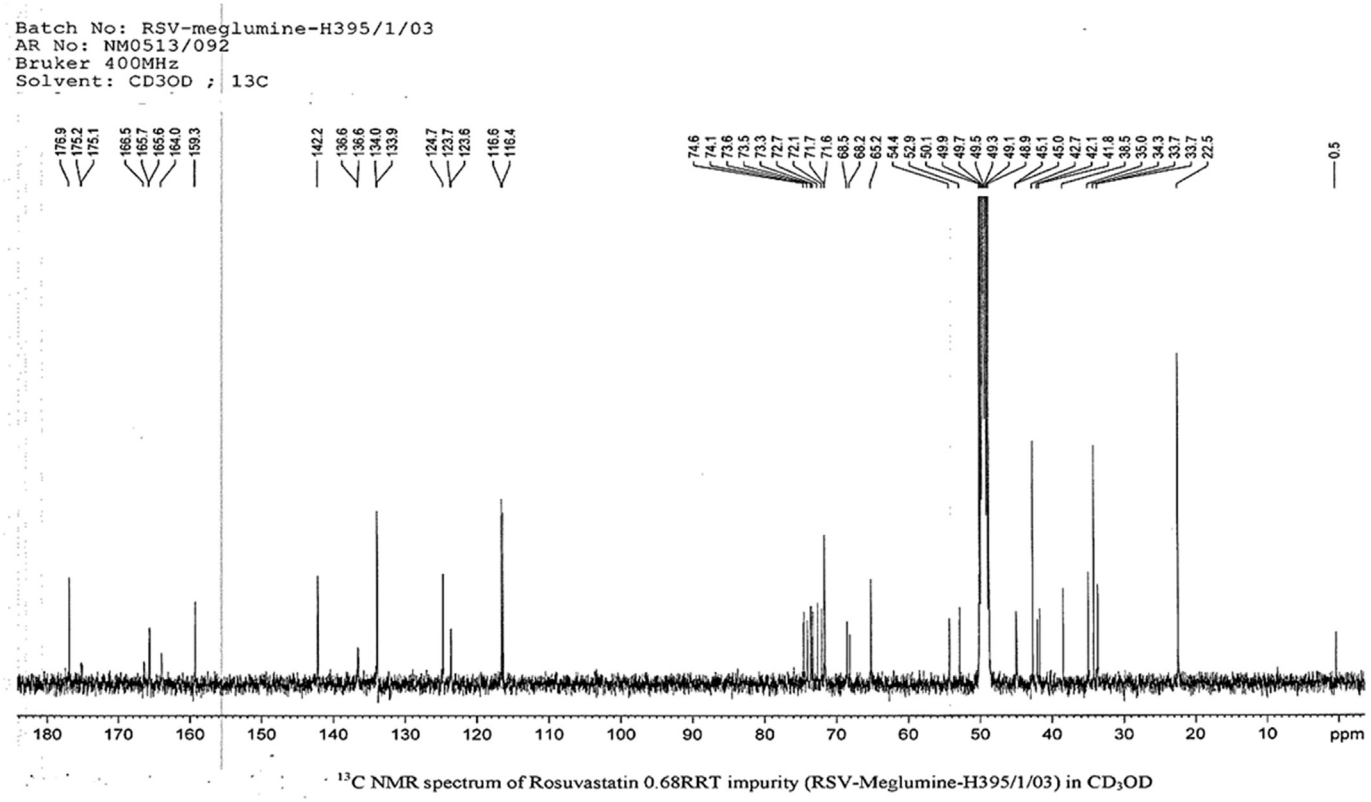


Fig. 13. ^{13}C- NMR spectrum for impurity.

6.2.8. Robustness

Effect of variation in flow rate: Robustness for flow rate was evaluated by varying the flow rate from 0.8 mL/min to 1.2 mL/min.Standard, spiked sample and system suitability solutions were injected. System suitability parameters and RRT'S for known impurities were calculated and tabulated in Table 12

Effect of variation in column temperature: Robustness for column temperature was evaluated by varying the column temperature from 25 °C to 35 °C. Standard, spiked sample and system suitability solutions were injected. System suitability parameters

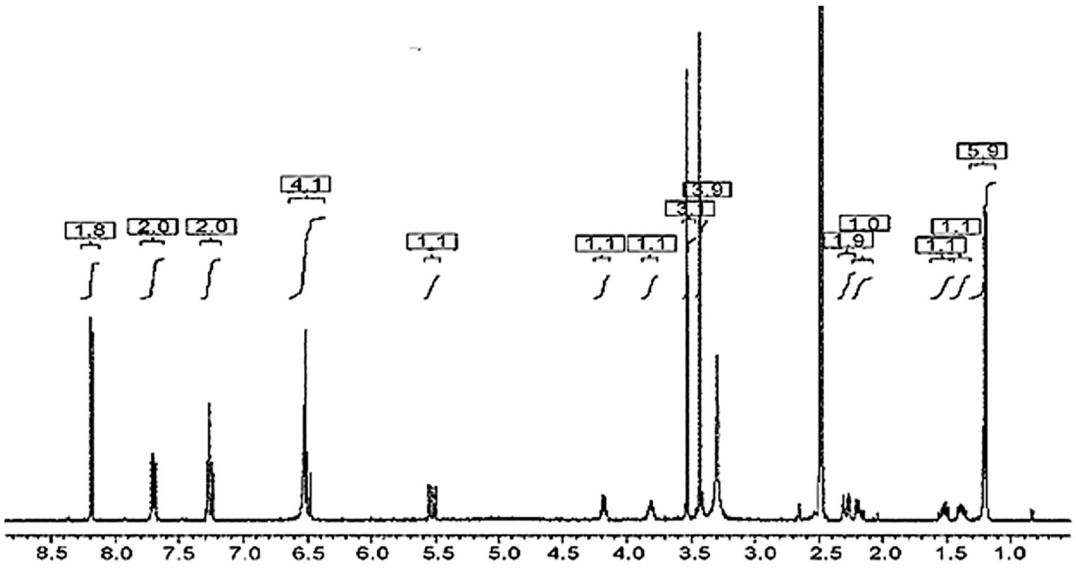


Fig. 14. [1]H NMR spectrum for Rosuvastatin.

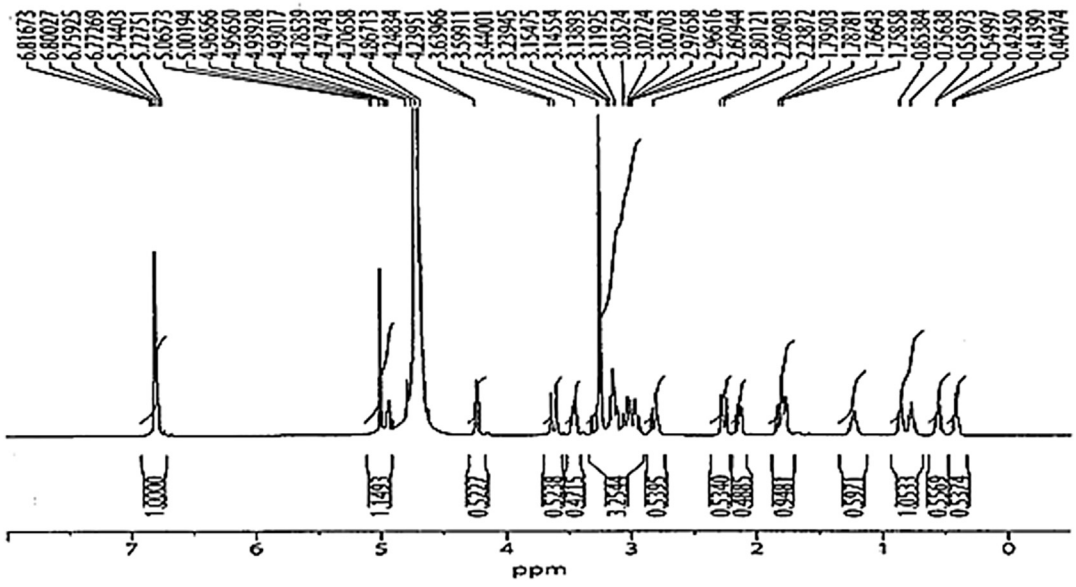


Fig. 15. [1]H NMR spectrum for Meglumine.

Table 11
Precision at limit of quantification.

S.no	% Impurity				
	Imp-A	Adduct	Anti isomer	5-Keto acid	Lactone
1	0.054	0.030	0.027	0.037	0.021
2	0.055	0.029	0.029	0.033	0.023
3	0.053	0.030	0.027	0.034	0.025
4	0.054	0.030	0.029	0.038	0.022
5	0.053	0.029	0.029	0.031	0.024
6	0.060	0.029	0.027	0.033	0.022
Ave	0.054	0.030	0.028	0.034	0.023
%RSD	2.2	1.9	3.9	7.7	6.4

Table 12
Robustness.

Parameter	RRT'S of impurities				
	Imp-A	Adduct	Anti isomer	5-Keto acid	Lactone
(−) Flow	1.03	0.72	1.06	1.13	1.18
(+) Flow	1.07	0.67	1.10	1.21	1.27
(−) Temperature	1.04	0.70	1.07	1.15	1.19
(+) Temperature	1.05	0.68	1.09	1.19	1.25
(−) MeOH	1.04	0.69	1.08	1.16	1.21
(+) MeOH	1.06	0.67	1.10	1.22	1.29
(−) ACN	1.05	0.68	1.09	1.18	1.23
(+) ACN	1.06	0.68	1.11	1.23	1.30
(−) pH	1.06	0.66	1.11	1.24	1.30
(+) pH	1.04	0.69	1.07	1.16	1.21

and RRT'S for known impurities were calculated and tabulated in Table 12.

Effect of variation in pH **of mobile phase**: Robustness for pH of mobile phase was evaluated by varying the pH of mobile phase from 2.8 to 3.2. Standard, spiked sample and system suitability solutions were injected. System suitability parameters and RRT'S

for known impurities were calculated and tabulated in Table 12.

Effect of organic phase variation in mobile phase composition: Robustness for organic phase variation in mobile phase composition was evaluated by varying the %organic phase in mobile phase from 90% to 110%. Standard, spiked sample and system suitability solutions were injected. System suitability parameters and RRT'S for known impurities were calculated and tabulated in Table 12.

7. Conclusion

The developed method is stability indicating. Hence it can be used in estimating the process and degradant impurities of rosuvastatin calcium.

Acknowledgments

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