

Quantitative Analysis of Rosuvastatin Calcium in Bulk and Solid Pharmaceutical Dosage Forms Using Green and Rapid Fourier-Transform Infrared Spectroscopic Method

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Singh *et al.*: Fourier-Transform Infrared Spectroscopic method for Rosuvastatin Calcium

A fast, inexpensive and direct quantitative Fourier-Transform Infrared Spectroscopic method was established for rosuvastatin calcium for analysis in bulk and solid pharmaceutical dosage forms. Content of rosuvastatin calcium was directly analysed without any solvent extraction by this method. Absorption mode was used on KBr pallets for acquisition of Fourier-Transform Infrared spectra. The method was validated against prescribed parameters as per International Council for Harmonisation guidelines. Range of linearity range was found to be 5-20% w/w in KBr pellet method. Proposed method was successfully applied for the determination of rosuvastatin in solid pharmaceutical dosage forms (tablets). The results suggest that the method is precise, accurate and reproducible. Due to its simplicity, cost effectiveness and less time taking, this method can be employed for routine estimation of rosuvastatin calcium in solid dosage forms in pharmaceutical industry.

Key words: Fourier-Transform infrared spectroscopy; rosuvastatin; pharmaceutical analysis; quantitative analysis; green analysis

Rosuvastatin calcium (fig. 1) is a new generation β -Hydroxy β -methylglutaryl-CoA (HMG-CoA) reductase inhibitor and the most potent of the currently available statins. It is mostly prescribed to reduce the elevated levels of cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides and to increase the levels of high-density lipoprotein cholesterol (HDL-C), apolipoprotein B: A1 ratio in the patients suffering from mixed dyslipidaemia, primary hypercholesterolemia, and homozygous familial hypercholesterolemia. It is also prescribed for acute coronary syndromes. It has low extrahepatic tissue penetration as well as lower potential for CYP3A4. Adequate clinical trial evidences are available that confirms the LDL-C lowering efficacy and safety of high-dose rosuvastatin calcium in acute coronary syndromes^[1-4]. Rosuvastatin calcium is mostly prescribed drug in obesity management thus manufactured by all leading pharmaceutical firms across the globe. Its sale is estimated to elevate further due to increasing trend of obesity in peoples (<https://www.transparencymarketresearch.com>).

Increased consumption directly elevate the demand which end up in increased production. Meantime quality of the marketed formulations during manufacturing process totally depends upon the reliability and effectiveness of analytical methods that are able to generate consistent and interpretable data^[5,6]. Various methods have been developed so far by the analysts across the globe for the assessment of rosuvastatin calcium in individual or combined dosage form based on various analytical techniques. Those established method includes spectrophotometric^[7-16], High Performance Liquid Chromatography (HPLC)^[17-50], UltraPerformanceLiquidChromatography (UPLC)^[51,52], electrode^[53], High Performance Thin Layer Chromatography (HPTLC)^[54,55], Capillary zone

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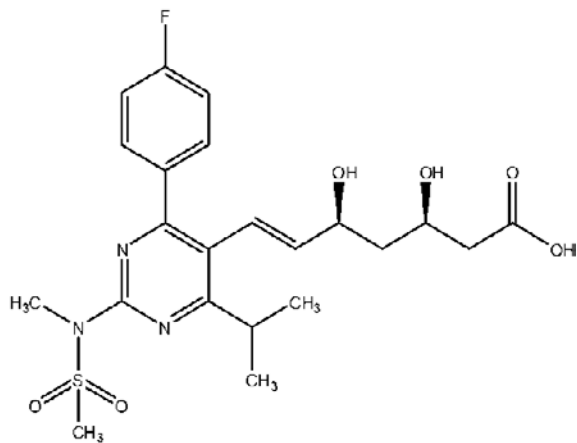


Fig. 1: Chemical structure of rosuvastatin calcium.

electrophoresis^[56], Voltammetry: hanging mercury drop electrode^[57], Charge-transfer complex^[58]. No doubt these methods are able to deliver desired analytical accuracy but came with added disadvantages too. Along with high cost, complicated procedures and long time consuming analysis, major emerging issue is the impact of these analytical methods on environment. All these established methods consume large volume of organic solvents (someone include hazardous solvents) that are toxic to the workers as well as environment that is forcing the pharmaceutical and chemical firms to look after the ways to reduce the generation of toxic waste^[59]. Therefore, it is a global emergency to develop green methods of analysis especially for the analytical operations carried out in pharmaceutical and chemical industries.

Among the spectroscopic techniques, Infrared (IR) spectroscopy has its distinguished place because of analytical ability without utilization of organic solvents. IR radiation region exist between microwave and visible region which can be efficiently used for the analysis of compounds sensitive between 4000 and 400 cm^{-1} .

Fourier-transform infrared (FT-IR) spectroscopy is gaining much popularity in pharmaceutical industry as quality control tool due to its rapid and non-destructive nature, simple sample preparation methods, less or no solvent consumption in qualitative as well as quantitative analysis of raw materials and finished drug products. FT-IR spectroscopy is an optimum choice for minimizing the environmental hazards due to industrial chemical waste as it need very less or no solvent for analysis^[60-68]. The structure of rosuvastatin calcium contains various functional groups major one includes O-H stretching of carboxylic acid, O-H stretching (intermolecular bonded), S=O stretching. The band appearing near 810 and 1197 cm^{-1} were selected for quantification

as at that band interference from excipient's in pharmaceutical formulation is minimal.

Objective of the present investigation was to developing an inexpensive, rapid and environmental friendly method for quantitative analysis of rosuvastatin calcium in bulk as well as solid dosage forms using FT-IR spectroscopy.

MATERIALS AND METHODS

Analytical grade rosuvastatin calcium kindly gifted by Lupin Research Park was employed in the present study. IR grade KBr was purchased from Sigma Aldrich for standard as well as sample pellets. Some solid tablet dosage forms of rosuvastatin calcium (i.e. Akroz, Arvast, Bestor, Crestor, Novostat and razel) were procured from local market of India. All IR spectra were recorded on IR Affinity-1 spectrometer of SHIMADZU with KBr accessory and DLATGS detector. The instrument was operated with IRsolution 1.50.

FT-IR calibrations:

For calibration purpose, a set of 4 rosuvastatin calcium standards ranging from 5 to 20 mg in KBr were prepared and to get uniform pellet 100 mg was weighed each time. To get homogenous pellets as well as reproducible results identical pressure was applied each time. Then standards spectra of different concentrations of rosuvastatin calcium in mentioned range were analysed and calibration curve was plotted to get straight line equation. Area under the peak for all standard spectra was computed by selecting specific region for quantification of rosuvastatin calcium in bulk as well as solid dosage forms.

Sample Preparation:

This method needs a simple sample preparation prior to FT-IR spectrum acquisition. This one step method involves grinding of the accurately weighed bulk or solid pharmaceutical samples as fine powder in agate mortar that results decrease in particle size for pellet.

Preparation of KBr pellets (with as well as without sample):

KBr pellets of 100 mg were prepared by taking specific amount of rosuvastatin calcium and oven-dried KBr to ensure no residual moisture content. The mixture was condensed in 13 mm die at a pressure of 7 tons for 5 min. Similar procedure was applied for the pharmaceutical samples also. 2 mg of sample was taken in 98 mg KBr

for sample pellet. Prepared pellets were scanned in Mid-Infrared region for recording FT-IR spectra.

FT-IR Parameters for spectral acquisition:

The resolution of the instrument was optimized at 2 cm⁻¹ by average 20 scans in Mid IR region of 4000-400 cm⁻¹. As per Beer's law the concentration and absorbance is proportional to each other, therefore absorbance mode was selected for quantification of rosuvastatin. Fresh background spectrum was recorded from KBr pellet before each FT-IR run for sample as well as standard. All spectra were recorded in KBr pellet form using same optimized analytical conditions in absorption mode (Table 1).

Method validation^[69]:

Linearity: Linearity of proposed method was established by analysing 4 separate standard rosuvastatin calcium concentrations (5-20% w/w). To get required data least square regression analysis was performed.

Accuracy: Recovery studies were performed to evaluate the accuracy of proposed method. For recovery studies, different concentration levels of rosuvastatin calcium, that were prepared independently and analysed (n=9). Accuracy was determined by using percentage relative standard deviation (%RSD) and from mean percentage recovery. To establish additional data for the same standard addition method was performed. In this, different concentrations of standard rosuvastatin calcium (8, 10, 12 mg) were added to known pre-analysed solid formulation sample and total concentration was determined using proposed method. Percentage recovery or added standard was calculated as: % Recovery=(Ct-Ci)/Ca

Where,

Ct = Total rosuvastatin calcium concentration measured after standard addition

Ci = Concentration of rosuvastatin calcium in formulation

Ca = Concentration of rosuvastatin calcium added to formulation

TABLE 1: OPTIMIZED FT-IR CONDITIONS

Measurement mode	Absorption
Apodization	Happ-Genzel
Frequency range	4000-400 cm ⁻¹
Number of scans	20
Resolution	2.0 cm ⁻¹

Precision:

Repeatability was determined by analysing different concentrations (n=9) of rosuvastatin calcium. Inter-day and Intra-day variations were performed to determine precision of proposed method. Different concentrations of drug in triplicates was prepared on 3 different times in single day and intra-day variation was analysed. Similarly inter-day variation was studied (n=27), %RSD was assessed.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ was determined by using calibration of standard. LOD and LOQ were assessed from 3.3σ/S and 10 σ/S. Where, σ is standard deviation and S is slope of y-intercept of regression equation.

Robustness:

Robustness of proposed method was established by analysing slight change in rosuvastatin calcium-KBr weight by ± 0.2 mg and assessed as relative standard deviation.

RESULT AND DISCUSSION

This novel method for analysis of rosuvastatin calcium in pharmaceutical formulations with single step sample preparation using FT-IR spectroscopy has wide range of advantages over previously available lengthy, costly as well as time consuming methods. In this method, extraction process is completely eliminated that make this method rapid, inexpensive and green. That will not only reduce the cost and time of analysis but also eliminate the potential risk of toxic chemicals to manpower as well as environment.

Most commonly used excipients for the solid pharmaceutical dosage forms are magnesium stearate, talcum powder, lactose, starch and avicel. They are added in various ratios that vary from product to product. From comparison of both (fig. 2 and fig. 3), it is clear that standard and excipients + standard spectra easily stretch over the surface of one another. Therefore there is no any significant interference caused by the matrix components which allows that method to practice without any extraction.

FT-IR spectra of pure rosuvastatin calcium were used for the calibration purpose. Spectra contain several prominent bands; instead of selecting single band, different regions were used for making calibration curves (5-20 % w/w) from peak intensity and peak

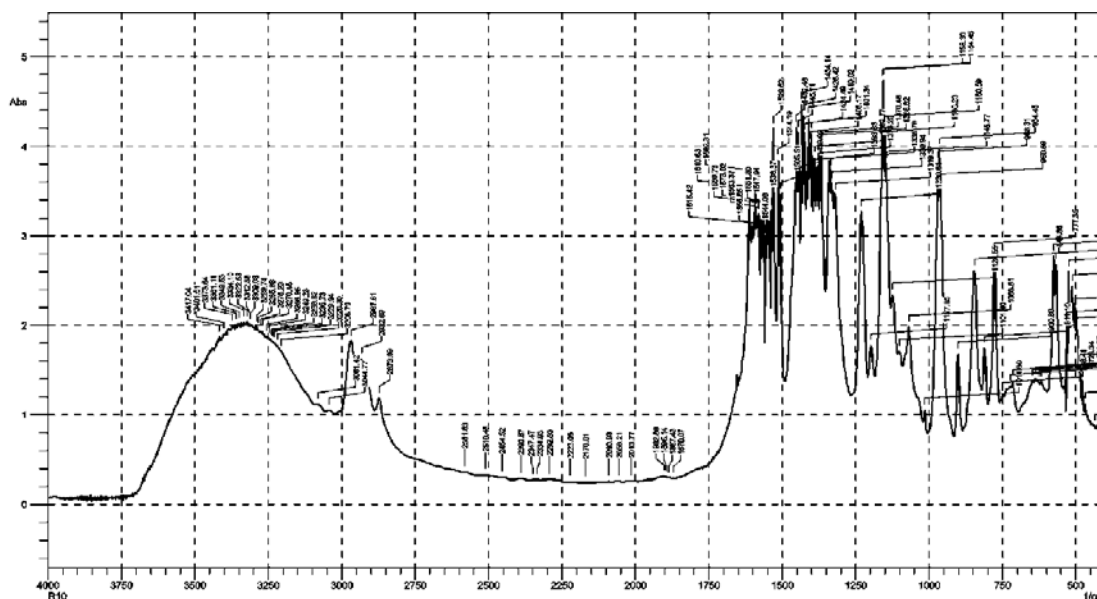


Fig. 2: FT-IR spectrum of standard rosuvastatin calcium.

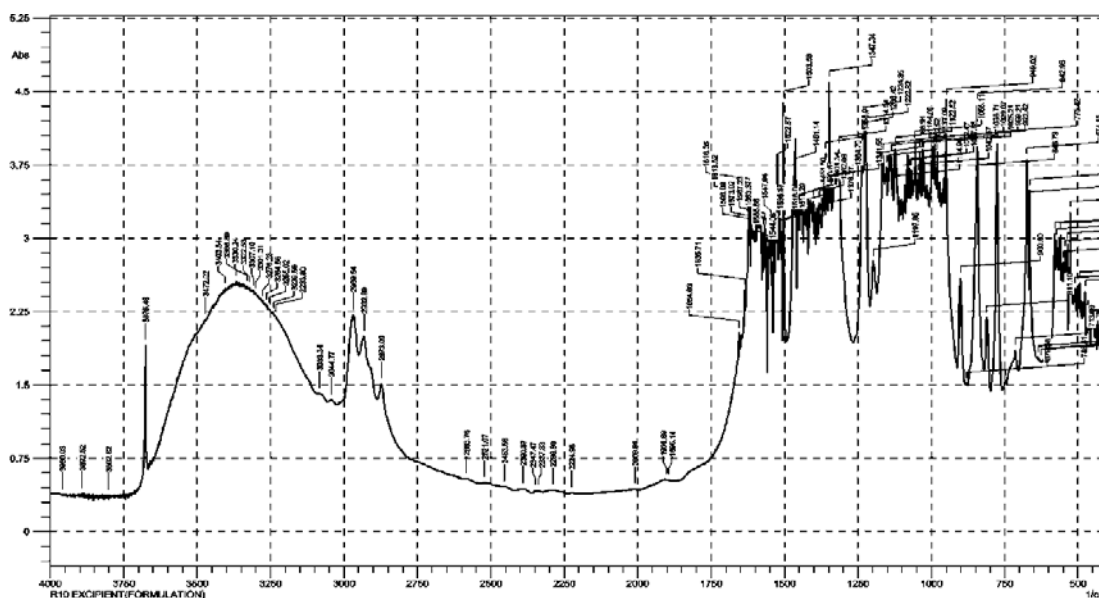


Fig. 3: FT-IR spectrum of mixture of rosuvastatin calcium and excipients.

area for more accurate results. Most linear and suitable regions ($810\text{-}800$ and $1210\text{-}1180\text{ cm}^{-1}$) were selected for the analytical purpose (Table 2). Beer's law was obeyed between 5 -20 % w/w (fig. 4 and fig. 5).

Accuracy ranges from 8 - 12 % w/w. Mean % recovery values were near to 100 % with low standard deviation values (S.D. < 2.0). The reliability of method was established from recovery studies by using standard addition method (Table 2). The mean percentage recovery for $810\text{-}800\text{ cm}^{-1}$ region was 99.47, 100.23 and 100.16 while for $1210\text{-}1180\text{ cm}^{-1}$ region was 99.28, 100.59 and 100.22, respectively.

Precision was evaluated by performing repeatability and intermediate precision data types. Results obtained

from repeatability studies indicated the precision of proposed method under similar conditions over short period of time. In intermediate precision results % RSD values were not more than 2.0 % in all cases. That indicates the precision of proposed method within laboratories (Table 3).

LOD as well as LOQ were also calculated and were found to be 0.044 and 0.134 % w/w for $810\text{-}800\text{ cm}^{-1}$ region while 0.0084 and 0.069 % w/w for $1210\text{-}1180\text{ cm}^{-1}$ region respectively. Suitability of the method was confirmed by analysing rosuvastatin calcium in its marketed solid dosage formulations. Assay values indicated insignificant interference of excipients matrix in analysis (Table 4).

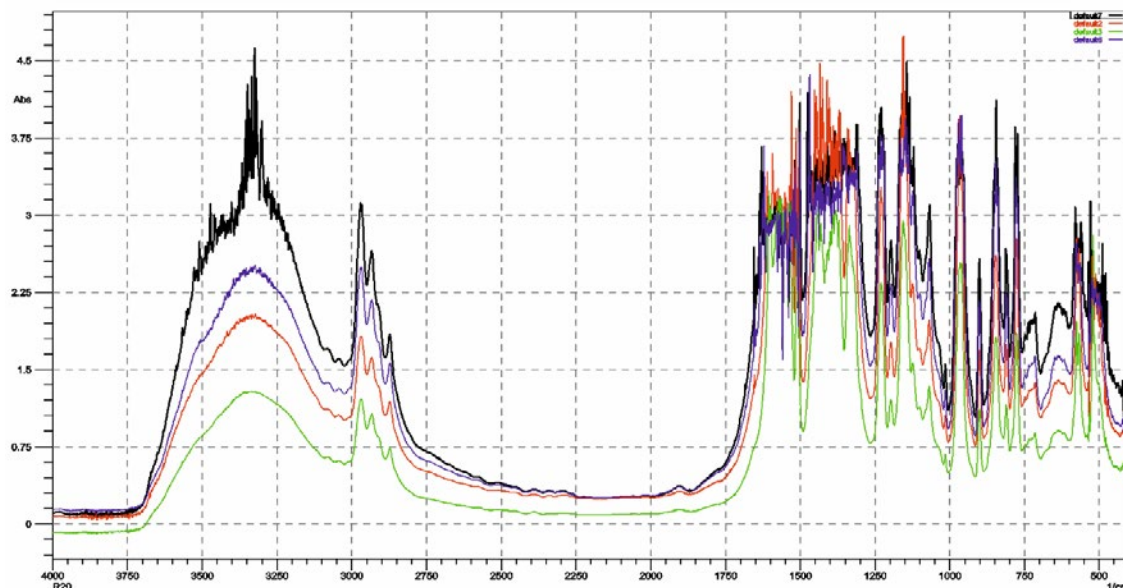


Fig. 4: Overlain FT-IR spectrum of different concentrations of rosuvastatin calcium (4000-400 cm^{-1}).

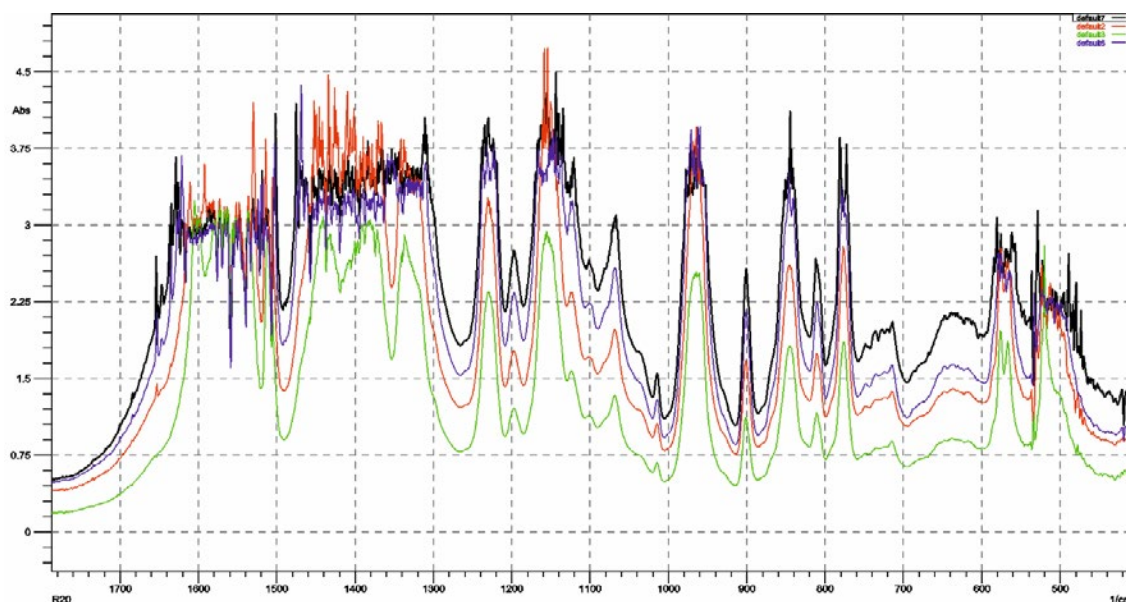


Fig. 5: Overlain FT-IR spectrum of different concentrations of rosuvastatin calcium (1800-400 cm^{-1}).

TABLE 2: SELECTION OF FT-IR REGION FOR APPLICATION OF SIMPLE BEER'S LAW CALIBRATION AND PERCENTAGE RECOVERY

Region Selected (cm^{-1})	Measurement mode	Regression line	Correlation Coefficient	Recovery (%)		
				8% (w/w)	10% (w/w)	12% (w/w)
800-810	Peak Intensity	$0.0956x + 0.700$	0.997	100.64	99.87	99.76
	Peak Area	$1.669x + 11.135$	0.9988	99.47	100.23	100.16
920-880	Peak Intensity	$0.0968x + 0.675$	0.9958	99.10	101.3	101.6
	Peak Area	$1.18144x + 10.72$	0.9978	100.89	101.12	99.37
1080-1090	Peak Intensity	$0.1173x + 0.7825$	0.9976	99.34	99.12	99.03
	Peak Area	$4.9086x + 41.13$	0.9953	99.22	99.36	99.97
1210-1180	Peak Intensity	$0.1038x + 0.715$	0.9958	99.89	99.57	100.57
	Peak Area	$2.1607x + 14.164$	0.9987	99.28	100.59	100.22
3000-2900	Peak Intensity	$0.1514x + 0.1398$	0.9962	100.24	101.2	101.7
	Peak Area	$4.6958x + 36.98$	0.9954	100.97	99.05	99.38

TABLE 3: INTERMEDIATE PRECISION DATA

Concentration (% w/w)	Peak area			
	800-810 cm ⁻¹		1210-1180 cm ⁻¹	
	Day 1	Day 2	Day 1	Day 2
10	29.2435	29.2431	35.4077	35.4012
10	29.2441	29.2433	35.4099	35.4125
10	29.2427	29.2436	35.3982	35.4215
10	29.2442	29.2439	35.4122	35.4018
10	29.2437	29.2440	35.4015	35.4072
10	29.2439	29.2432	35.4103	35.4123
	Mean peak area	29.2436	Mean peak area	35.4066
	S.D	0.0005	S.D	0.0055
Intraday Precision (n=6)	%R.S.D	0.0018	%R.S.D	0.015
	Mean peak area	29.2435	Mean peak area	35.4094
	S.D	0.0003	S.D	0.0076
Interday Precision (n=6)	%R.S.D	0.0012	%R.S.D	0.021

TABLE 4: DETERMINATION OF ROSUVASTATIN IN SOLID DOSAGE PHARMACEUTICAL FORMULATIONS (TABLETS)

Commercial product	800-810 cm ⁻¹		1210-1180 cm ⁻¹	
	Amount found (n=5)	% Amount found	Amount found (n=5)	% Amount found
Akroz tablets (10 mg)	10.09 ± 0.11	100.9 ± 0.022	10.11 ± 0.11	101.1 ± 0.022
Arvast tablets(20 mg)	20.12 ± 0.24	100.6 ± 0.015	20.24 ± 0.24	101.2 ± 0.015
Bestor tablets(10 mg)	10.03 ± 0.09	100.3 ± 0.038	9.97 ± 0.09	99.7 ± 0.038
Crestor tablets(10 mg)	10.26 ± 0.15	102.6 ± 0.024	10.16 ± 0.15	101.6 ± 0.024
Novostat tablets(10 mg)	10.17 ± 0.17	101.7 ± 0.026	10.05 ± 0.17	100.5 ± 0.026
Rzel tablets(10 mg)	10.19 ± 0.06	101.9 ± 0.017	10.01 ± 0.06	100.1 ± 0.017

The present study represents the development of green, sensitive and rapid method for accurate quantification of rosuvastatin calcium in pure drug as well as solid pharmaceutical dosage formulations i.e. tablets using FT-IR spectroscopy. This method performed better as compare to previously reported methods. This method has made analysis simple by eliminating hazardous and complex step of extraction. This method has potential to replace the existing laborious, non-ecofriendly as well as costly methods for the analysis of rosuvastatin calcium in pharmaceutical as well as chemical industry.

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