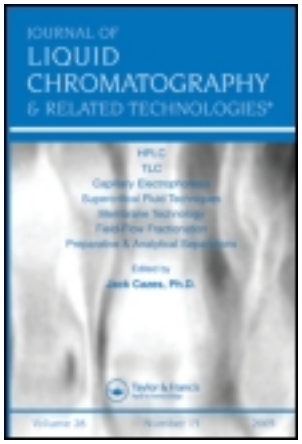

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SELECTIVE CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF ROSUVASTATIN CALCIUM IN THE PRESENCE OF ITS ACID DEGRADATION PRODUCTS

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□ *Two accurate and sensitive stability-indicating methods for the determination of rosuvastatin calcium in the presence of its acid degradation products are presented. The first method utilizes quantitative spectrodensitometric evaluation thin-layer chromatography (TLC) of rosuvastatin calcium in the presence of its acid degradation products, using ethyl acetate/methanol/ammonia (7:3:0.01, by volume) as a mobile phase. Chromatograms are scanned at 245 nm. This method analyzes rosuvastatin calcium in a concentration range of 0.6–3.4 μg/band with mean percentage recovery of 99.78 ± 1.42. The second method is a high-performance liquid chromatography (HPLC) method for the simultaneous determination of rosuvastatin calcium in the presence of its acid degradation products. The mobile phase consists of water/acetonitrile/methanol (40:40:20, by volume). The standard curve of rosuvastatin calcium shows a good linearity over a concentration range of 10–60 μg mL^{-1} with mean percentage recovery of 100.22 ± 0.86. These methods were successfully applied to the determination of rosuvastatin calcium in bulk powder, laboratory-prepared mixtures containing different percentages of the acid degradation products, and pharmaceutical dosage forms. The validity of results was assessed by applying standard addition technique. The results obtained were found to agree statistically with those obtained by a reported method, showing no significant difference with respect to accuracy and precision.*

Keywords degradation, densitometry, HPLC technique, rosuvastatin calcium, stability-indicating, TLC technique

INTRODUCTION

Rosuvastatin, bis((E)-7-(4-(4-flurophenyl)-6-1sopropyl-2-(methyl(methylsulfonyl) amino)pyrmidin-5yl)(3R,5S)-3,5-dihydroxyhept-6-enoic acid) calcium salt (Figure 1), is a highly effective 3-hydroxyl-3-methylglutaryl

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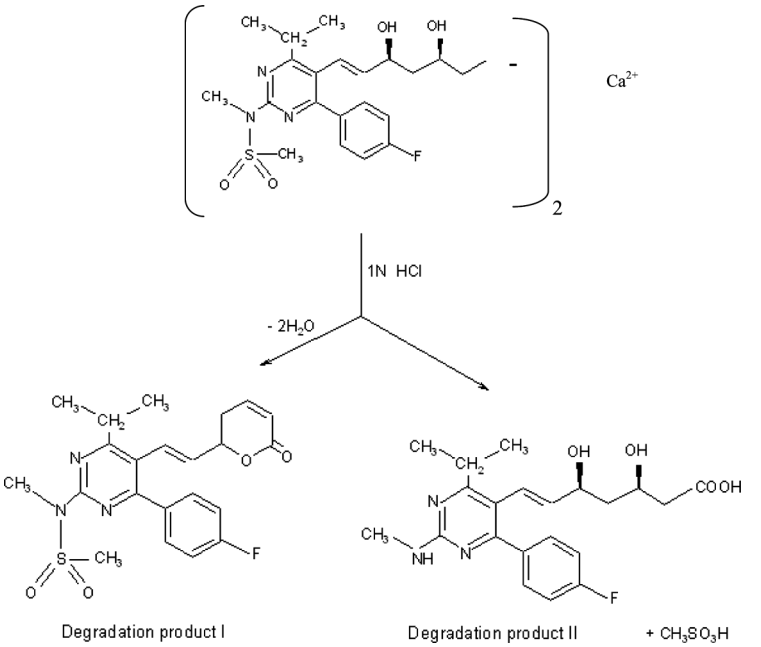


FIGURE 1 Suggested scheme for the acid degradation of rosuvastatin calcium.

coenzyme A (HMG-CoA) reductase inhibitor. It is widely used for the treatment of hyperlipidemia. In clinical trials, rosuvastatin achieved marked reductions in serum levels of low density lipoproteins (LDL) cholesterol, accompanied by modest increases in high density lipoproteins (HDL) cholesterol and reductions in triglycerides.[1–3]

It may also be used in patients with homozygous familial hypercholesterolemia. Rosuvastatin is given orally as the calcium salt, although the doses are expressed in terms of the base.

Despite the wide application of rosuvastatin in the treatment of hyperlipidemia, a literature survey reveals that only few methods have been reported for the determination of rosuvastatin calcium in pharmaceutical formulations and biological samples including high-performance liquid chromatography (HPLC),[4–8] spectrophotometry,[9] capillary electrophoresis,[10] and chemometry.[11]

An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradates.[12] None of these methods is concerned with the identification and elucidation of the structure of the resulting acid degradation products; thus, the objective of the present study was to develop simple and accurate stability-indicating methods for selective determination of rosuvastatin calcium in the presence of its acid degradation products with the application

to pharmaceutical dosage forms that could be applied for drug quality control.

EXPERIMENTAL

Apparatus

Experiment was performed using the following: precoated thin-layer chromatographic (TLC) plates, silica gel 60 F_{254} (20 cm × 20 cm, 0.25 mm), E. Merck (Darmstadt, Germany); Camag TLC scanner 3 S/N 130319 with win-CATS software; Camag Linomat 5 autosampler (Muttenz, Switzerland); Camag microsyringe (100 µL).

A liquid chromatograph consisted of an isocratic pump (LC-10 AD, Shimadzu, Kyoto, Japan), an ultraviolet visible wavelength detector (SPD-10A, Shimadzu), and a Rheodyne injector (Model 7725 I, Rohnert Park, CA, USA) equipped with 20 µL injector loop, with stationary phase C_{18}: a 250 mm × 4.6 mm, i.d. 5 µm analytical column, Waters (Milford, MA, USA).

IR spectrophotometer, Shimadzu 435 (Kyoto, Japan), sampling was undertaken as potassium bromide discs. Ultra performance liquid chromatograph–mass spectrophotometer (UPLC MS–MS), Acquity TQ, Waters (Milford, MA, USA), was used for mass spectrophotometric analysis.

Materials and Reagents

Samples

Pure Sample. Rosuvastatin calcium was kindly supplied by Chemipharm Parmaceutical Industry, (6 October city, Egypt), and its purity was found to be 99.75 ± 1.057 by a reported spectrophotometric method.[13]

Pharmaceutical Dosage Forms. The following available pharmaceutical preparations were analyzed: (1) Rosuvast tablets, labeled to contain 10 mg/tablet rosuvastatin calcium, batch number 100333A, manufactured by Chemipharm Parmaceutical Industry, (6 October, Egypt); (2) Sovikan tablets, labeled to contain 10, 20 mg per tablet rosuvastatin calcium batch numbers, 003 and 001, respectively, manufactured by Hikma Pharma, (6 October, Egypt).

All chemicals and reagents were of pure spectroscopic analytical grade: 1 N HCL, ethyl acetate, concentrated ammonia (specific gravity 0.91) (Adwic, El-Nasr Pharmaceutical Chemicals. Co. Cairo, Egypt). Deionised water, acetonitrile, and methanol (E. Merck, Darmstadt, Germany) were of HPLC grade.

Standard Solutions. The following solutions were prepared: (1) Stock standard solution of Rosuvastatin calcium or its acid degradates (1 mg mL^{-1}) in methanol, for spectrodensitometric method; and (2) Rosuvastatin calcium working standard solution or its acid degradates (100 μg mL^{-1}) in methanol for HPLC method.

Procedures

Preparation of Acid Degradation Products of Rosuvastatin Calcium

Fifty milligram of rosuvastatin calcium was weighed in a conical flask, dissolved in 20 mL methanol, 25 mL 1 N HCl was added, and the solution was subjected to reflux at 100°C for 3 hr. A colored precipitate was formed, filtered, and washed with water and then dissolved in methanol. The degraded solutions were applied as bands onto several preparative TLC plates. The plates were developed using, ethyl acetate/methanol/ammonia (7:3:0.01, by volume) as a developing solvent[11] in chromatographic tank previously saturated for 30 min with the developing solvents and then dried in air.

The bands were visualized under UV light at 254 nm. Then the bands were scraped and the silica was suspended in the least amount of methanol and filtered, and then the filtrate was left to dry at room temperature (25°C) to obtain the degradation products. The structure of the isolated degradation products was elucidated by carrying out IR and mass spectroscopy.

TLC Spectrodensitometric Method

Linearity. Aliquots equivalent to 0.6, 1, 1.4, ..., 3.4 μL of rosuvastatin calcium standard stock solution (1 mg mL^{-1}) were spotted using Camag Linomat autosampler with microsyringe (100 μL). Bands were spaced 1.5 cm apart from each other and 2 cm from the bottom edge of the plate. The plate was developed in a chromatographic tank previously saturated for at least 1 hr with the developing mobile phase, ethyl acetate/methanol/ammonia (7:3:0.01, by volume), by ascending mode. The plate was removed and dried in air, and the bands were visualized under UV lamp at 254 nm and scanned at 245 nm. The calibration curve was plotted between the recorded area under the peak and the corresponding concentration, from which the regression equation was calculated.

Assay of Laboratory-Prepared Mixtures. Aliquots equivalent to 2.8–0.6 mg were accurately transferred from rosuvastatin calcium stock standard solution (1 mg mL^{-1}) into a series of 10 mL volumetric flasks. To the previous solutions, complementary aliquots equivalent to 0.6–2.8 mg of

rosuvastatin calcium acid degradation products stock standard solution ($1\,mg\,mL^{-1}$) were added. The volumes were completed to volume with methanol and mixed thoroughly. Then we proceeded as mentioned under linearity and the concentrations were calculated from the corresponding regression equation.

HPLC Method

Linearity. Accurately measured volumes of rosuvastatin calcium working standard solution ($100\,\mu g\,mL^{-1}$) were transferred into 10 mL measuring flasks diluted to the volume with methanol to get the final concentration range of 10 of $60\,\mu g\,mL^{-1}$. The samples were then chromatographed using the following chromatographic conditions: stationary phase: a 250 mm × 4.6 mm, C_{18} analytical column, i.d. 5 μm analytical column, Waters (USA), mobile phase; water/acetonitrile/methanol (40:40:20, by volume).

The sample and the mobile phase were filtered through a 0.45 μm Millipore membrane filter.

The mobile phase was degassed for about 15 min in an ultrasonic bath prior to use, flow rate $1\,mL\,min^{-1}$ (~25°C), with UV detection at 245 nm. The prepared samples (20 μL) were injected by the aid of a 25 μL Hamilton® analytical syringe.

Records of the peak areas, the peak area ratios (ratios of the recorded peak areas to that of external standard ($30\,\mu g\,mL^{-1}$)), were plotted versus the corresponding concentrations of rosuvastatin calcium to get the calibration graph and to compute the corresponding regression equation. Concentrations of unknown samples of rosuvastatin calcium were determined using the obtained regression equation.

Assay of Laboratory Prepared Mixtures. Aliquots equivalent to 500–$100\,\mu g\,mL^{-1}$ were accurately transferred from rosuvastatin calcium working standard solution ($100\,\mu g\,mL^{-1}$) into a series of 10 mL volumetric flasks. To the previous solutions aliquots equivalent to $100–500\,\mu g\,mL^{-1}$ of acid degradation products ($100\,\mu g\,mL^{-1}$) were added. The volumes were completed with methanol and mixed thoroughly. Then we proceeded as mentioned under linearity and the concentrations were calculated from the corresponding regression equation.

System Suitability

Twenty microliters of the solvent mixture and the working standard solutions were injected. The system suitability parameters, retention time, tailing factor, theoretical plate count (N), height equivalent to theoretical plate (HETP), separation of rosuvastatin calcium peak, and its degradation product peak (resolution) and column capacity were studied.

Assay of Pharmaceutical Formulations

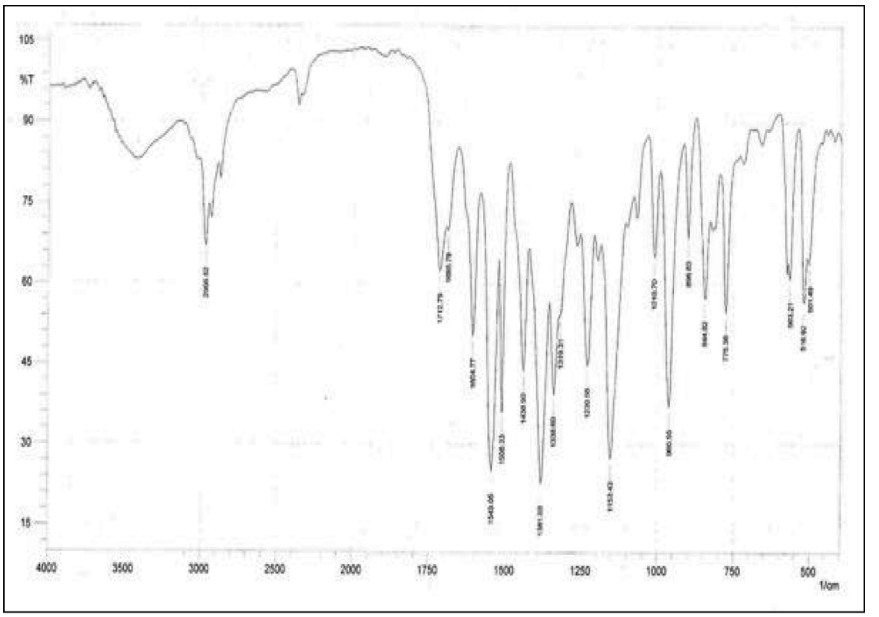
Twenty tablets were ground. A portion of the powder equivalent to 100 mg rosuvastatin calcium was accurately weighed into a 100 mL beaker, dissolved in methanol, for TLC spectrodensitometric method (4×20 mL) and filtered into a 100 mL measuring flask. The volume was completed with the same solvent (1 mg mL^{-1}). Ten milliliters of this tablet stock solution (1 mg mL^{-1}) were transferred into a 100 mL measuring flask and diluted to the mark with methanol to get a final concentration of 100 μg mL^{-1} for HPLC method, then the procedures under "Construction of calibration curves" for each method were followed.

RESULTS AND DISCUSSION

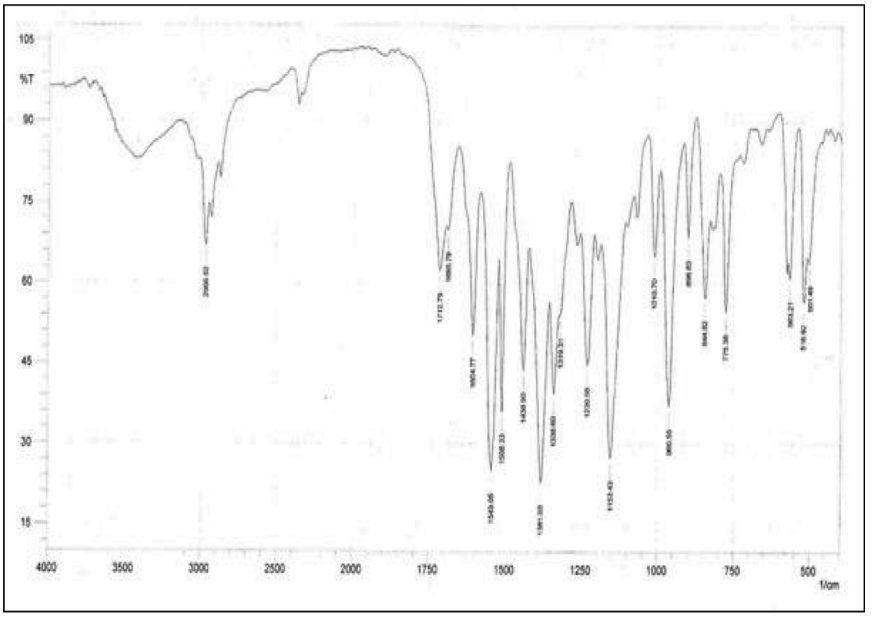
Degradation of Rosuvastatin Calcium

Many pharmaceutical compounds undergo degradation during storage or even during the different processes of their manufacture. Several chemical or physical factors can lead to the degradation of drugs.[14] Hydrolysis is the most famous chemical degradation route of drugs.[15,16] Rosuvastatin calcium was liable to oxidation[11], but it was found to be stable in alkaline medium. The main classes of drugs that are subject to hydrolysis are esters, amides, and lactams. It was found that rosuvastatin calcium was liable to degradation upon refluxing in an acid medium to give two degradation products, demonstrated in Figure 1. Different concentrations of HCl were tried and it was found that 0.1 N HCl was capable to produce degradation, but 1 N HCl solution was used for the preparation of the degradation products to ensure complete degradation in a short time. In this work, acid degradation products of rosuvastatin calcium ware prepared and separated and their structures are identified by IR and mass spectroscopy. The IR spectrum of intact rosuvastatin calcium showed stretching band at 3394.72 cm^{-1} for carboxylic group and stretching band of carbonyl group at 1604.77 cm^{-1} (Figure 2a), and rosuvastatin acid degradation product I showed stretching band of six-membered lactone ring at 1735.93 cm^{-1} (Figure 2b). The IR spectrum of rosuvastatin acid degradation product II showed change in fingerprint region, indicating a change of its nature (Figure 2c). Rosuvastatin calcium molecular weight is 1001.14; its acid degradation product I showed molecular ion peak at 445.9 m/z (Figure 3a) whereas its acid degradation product II showed molecular ion at 403.8 m/z (Figure 3b), which are equivalent to their molecular weights.

The present work was conducted for the selective determination of rosuvastatin calcium in the presence of its acid degradation products with the application to pharmaceutical dosage forms.

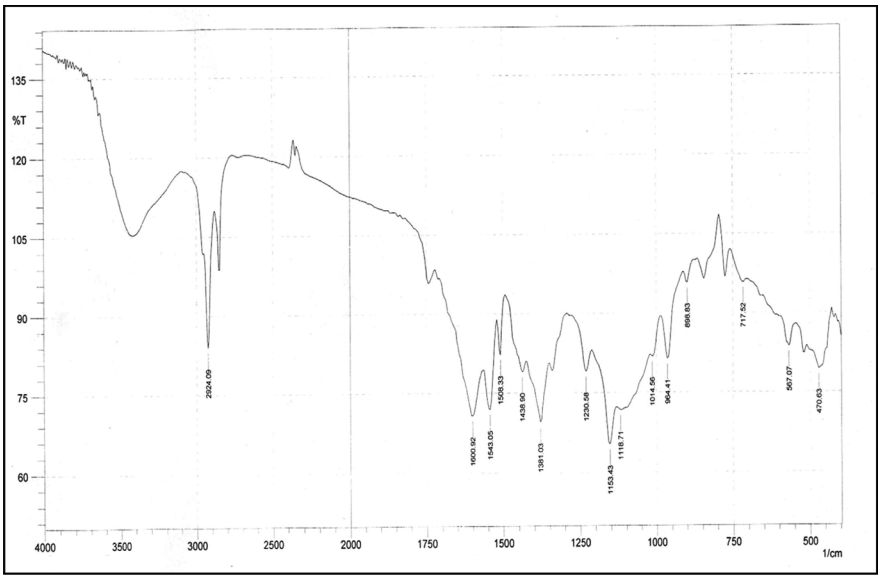


(a)



(b)

FIGURE 2 (a) IR spectrum of intact rosuvastatin calcium; (b) IR spectrum of rosuvastatin calcium acid degradation product I; (c) IR spectrum of rosuvastatin calcium acid degradation product II.



(c)

FIGURE 2 Continued.

TLC Fractionation

TLC monitoring of the drug degradation was done on thin layer plates of silica gel F_{254} using ethyl acetate/methanol/ammonia (7:3:0.01, by volume) as the developing solvent. The developed plates were visualized under short UV lamp.

The degradation products' R_f values were found to be 0.45 and 0.8 for acid degradation products II and I, respectively, and these could be separated from the intact drug (R_f value = 0.32).

TLC Spectrodensitometric Method

TLC spectrodensitometry overcomes the problem of overlapping absorption spectra of a mixture of drugs by separating these components on TLC plates and determining each ingredient by scanning the corresponding chromatogram. The TLC–UV densitometric method has the advantage of simultaneously determining the active ingredients in multicomponent dosage forms.[17]

The proposed procedure is based on the difference in R_f values of rosuvastatin calcium ($R_f = 0.32$) and its acid degradates ($R_f = 0.45$, 0.8 for acid degradates II and I, respectively). Various developing systems were

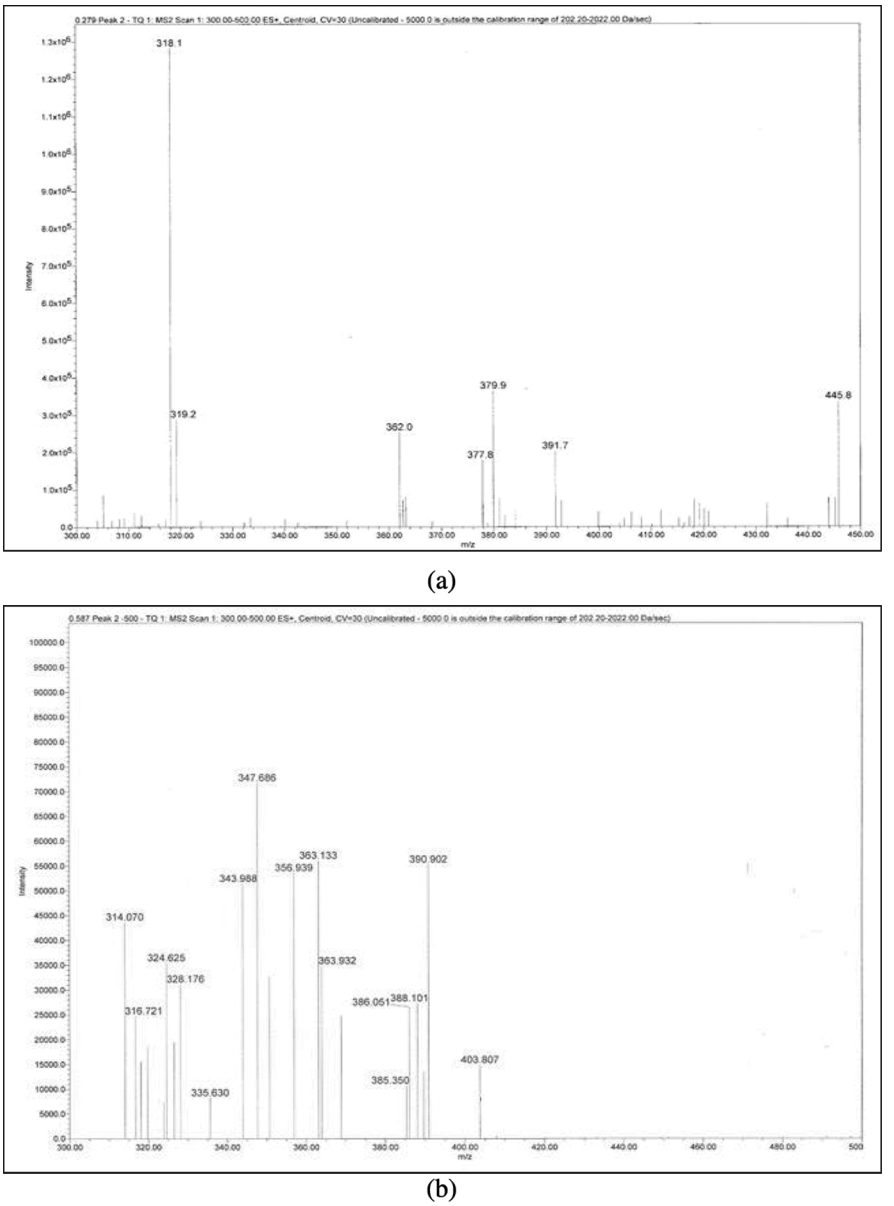


FIGURE 3 (a) Mass spectrum of rosuvastatin calcium acid degradation product I; (b) Mass spectrum of rosuvastatin calcium acid degradation product II.

tried, but complete separation was achieved using ethyl acetate/methanol/ammonia (7:3:0.01, by volume).

The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 245 nm over a range of

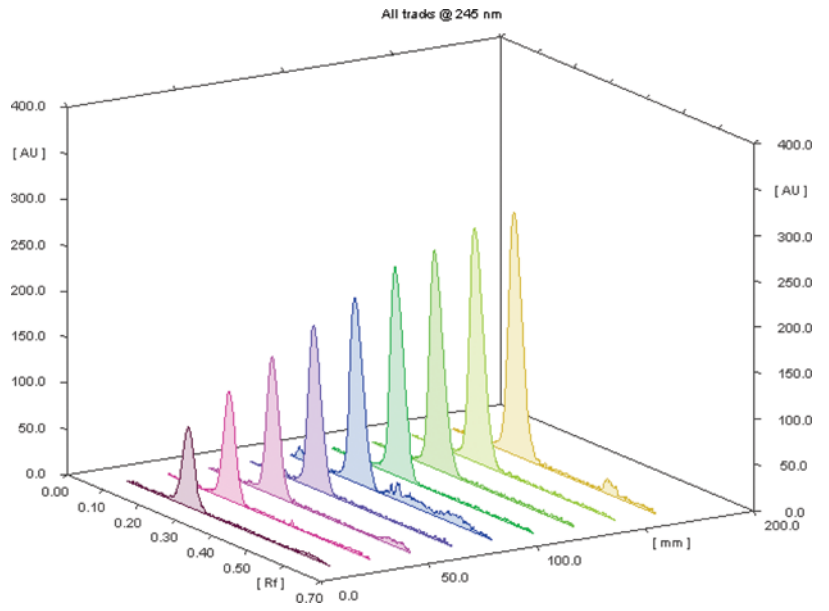


FIGURE 4 Scanning profile of the TLC chromatogram of rosuvastatin calcium (0.6–3.4 µg/band) at 245 nm.

0.6–3.4 µg/ band, where a linear response was obtained. Scanning profile of different concentrations of rosuvastatin calcium at 245 nm was shown in Figure 4. The linear regression equation was found to be the following:

$$A = 0.2653C + 0.166 \qquad r = 0.9996$$

where A is the integrated area under the peak $\times 10^{-4}$, C is the concentration in µg/band, and r is the correlation coefficient.

HPLC Method

A simple HPLC method was adopted for the simultaneous determination of rosuvastatin calcium in the presence of its acid degradation products without pervious separation.

Different mobile systems were tried; the best resolution was achieved when using a mobile phase consisting of water/acetonitrile/methanol (40:40:20, by volume) using UV detection at 245 nm to obtain a retention time 4.03 min for rosuvastatin calcium, 7.6 min for its acid degradation product II, and 9.56 min. for acid degradation product I (Figure 5).

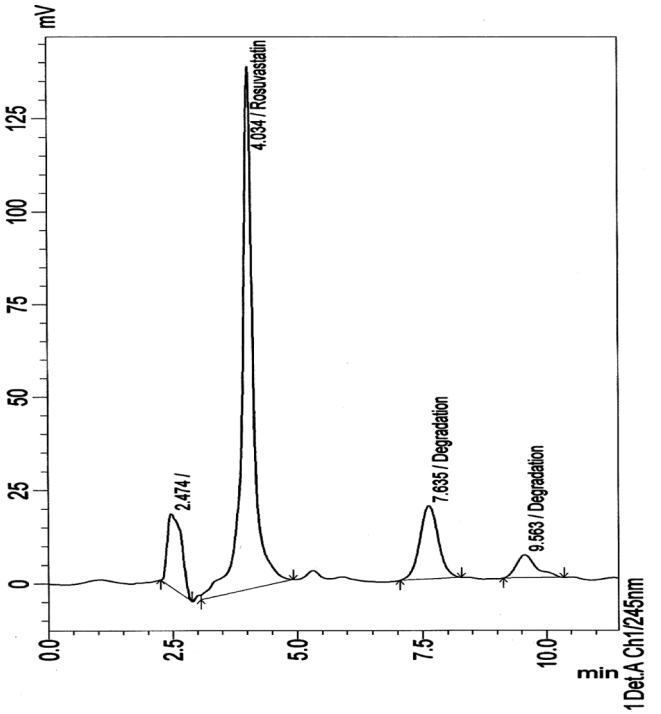


FIGURE 5 HPLC chromatogram of rosuvastatin calcium (20 μg mL^{-1}; R_t, 4.03 min), its acid degradation product II (10 μg mL^{-1}; R_t, 7.635 min), and its acid degradation product I (10 μg mL^{-1}; R_t, 9.56 min).

A linear relation was obtained between peak area and the concentration of rosuvastatin calcium in the range of 10 to 60 μg mL^{-1}. The linear regression equation was found to be the following:

$$A = 0.033C + 0.0054 \qquad r = 0.9998$$

where A is the relative peak area, C is the concentration in μg mL^{-1}, and r is the correlation coefficient.

Stability Indication

To assess the stability-indicating efficiency of the proposed methods, the acid degradation products of rosuvastatin calcium were mixed with its intact sample in different ratios and analyzed by the proposed methods. Table 1a illustrates good selectivity in the determination of rosuvastatin calcium in the presence of up to 82% acid degradation products by the TLC spectrodensitometric method and up to 83% acid degradation products by HPLC method (Table 1b).

TABLE IA: Determination of rosuvastatin calcium in laboratory prepared mixtures by the proposed TLC-spectrodensitometric method

Mixture number	% Degradation product	Concentration (μg/band)		Recovery %
		Rosuvastatin calcium	Acid degradates	
1	17.7	2.80	0.60	99.97
2	23.5	2.60	0.80	100.05
3	35.3	2.20	1.20	99.92
4	47.1	1.80	1.60	98.54
5	58.8	1.40	2.00	98.29
6	70.6	1.00	2.40	99.91
7	82.4	0.60	2.80	101.15
Mean				99.69
S.D.				0.977
R.S.D.%				0.980

TABLE IB: Determination of rosuvastatin calcium in laboratory prepared mixtures by the proposed HPLC method

Mixture number	% Degradation products	Concentration (μg/ml)		Recovery %
		Rosuvastatin calcium	Acid degradates	
1	16.7	50.00	10.00	99.74
2	33.3	40.00	20.00	100.09
3	50	30.00	30.00	99.68
4	66.7	20.00	40.00	99.24
5	83.3	10.00	50.00	100.11
Mean				99.77
S.D.				0.356
R.S.D.%				0.357

TABLE 2 Determination of Rosuvastatin Calcium in Pharmaceutical Formulations by the Proposed Methods and Results of Application of Standard Addition Technique

Product	Method	Found* (%)	Recovery (%)
Rosuvast 10 tablets Batch No.100333A	TLC densitometric method	101.54 ± 0.62	99.25 ± 1.067
Sovikan 10 tablets Batch No.003		99.69 ± 1.01	99.06 ± 1.005
Sovikan 20 tablets Batch No.001		99.39 ± 0.85	99.67 ± 1.767
Rosuvast 10 tablets Batch No.100333A	HPLC method	100.25 ± 0.74	99.66 ± 0.221
Sovikan 10 tablets Batch No.003		100.65 ± 0.86	99.51 ± 0.611
Sovikan 20 tablets Batch No.001		101.12 ± 0.51	100.21 ± 0.385

*Average of three different determinations.

TABLE 3 System Suitability Parameters of the Elaborated HPLC Method for the Analysis of Rosuvastatin Calcium in the Presence of Its Acid Degradation Products

Parameter	Obtained Value			Reference Value
	Rosuvastatin Calcium	Degradation Product I	Degradation Product II	
Resolution (R)	2.24	1.33		$R>1$
T (tailing factor)	1.56	1.05	1.22	$T=1$ for a typical symmetric peak
α (selectivity factor)	1.26*	2.24**		>1
K (column capacity)	2.89	8.19	6.48	1–10 acceptable
N (column efficiency)	115.60	2097.57	287.76	Increases with efficiency of the separation
HETP (height equivalent to theoretical plates)	0.22	0.01	0.09	The smaller the value, the higher the column efficiency

*Relative to degradation product I.
**Relative to degradation product II.

The suggested methods were successfully applied for the determination of rosuvastatin calcium in its pharmaceutical formulations, showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique (Table 2).

System suitability tests, which are used to ensure system performance before or during the analysis of drugs, were performed. The obtained values of rosuvastatin calcium and its acid degradation products were agreed with the stated reference values (Table 3).

Assay validation was done by repeating the procedures three times on three different days (inter-day) and three times on different time intervals

TABLE 4 Validation of the Results Obtained by Applying the Suggested Methods for the Determination of Rosuvastatin Calcium

Parameters	TLC Spectrodensitometric Method	HPLC Method
Range	0.6–3.4 µg/band	10–60 µg mL^{-1}
Slope	0.2653	0.033
Standard error of the slope	0.003021	0.000323
Intercept	0.166	0.0054
Standard error of the intercept	0.006647	0.01258
Accuracy	99.78 ± 1.419	100.22 ± 0.859
Specificity*	99.69 ± 0.977	99.77 ± 0.356
Correlation coefficient (r)	0.9996	0.9998
Repeatability**	0.759	0.448
Intermediate precision***	0.922	0.630

*Specificity was calculated from the analysis of laboratory prepared mixtures.
Precision was determined by calculating R.S.D.% of the intra-day** and the inter-day*** analysis of samples of rosuvastatin calcium (10, 30, and 40 µg mL^{-1}) for HPLC method and (1.00, 1.80, and 2.60 µg/band) for TLC densitometric method.

TABLE 5 Statistical Analysis of the Results Obtained by Applying the Proposed Methods and a Reported Spectrophotometric Method for the Analysis of Pure Rosuvastatin Calcium

Values	TLC Densitometric Method	HPLC Method	Reported* Method[13]
Mean	99.78	100.22	99.75
±SD	1.419	0.859	1.057
n	8	6	7
Variance	2.014	0.738	1.117
t	0.052	0.858	–
t theoretical	(2.160)**	(2.201)**	
F	1.803	1.514	–
F theoretical	(4.21)**	(4.90)**	

*Direct UV spectrophotometric method at 244 nm in methanol.
**The values in parentheses are the corresponding tabulated *t*- and *F*-values at $P = 0.05$.

within the same day (intra-day) for the analysis of different concentrations of rosuvastatin calcium (Table 4). The results show that the methods were accurate, precise, and specific.

Results of the suggested methods for determination of rosuvastatin calcium were statistically compared with those obtained by applying reported spectrophotometric method.[13] The calculated *t*- and *F*-values were found to be less than the corresponding theoretical ones, confirming good accuracy and precision (Table 5).

CONCLUSION

Two methods TLC densitometry and HPLC were developed for the determination of rosuvastatin calcium in the presence of its acid degradation products. The proposed HPLC method gives a good resolution between rosuvastatin calcium and its acid degradation products within 10 min. The methods provide simple, accurate, rapid, and reproducible quantitative analysis of rosuvastatin calcium in bulk powder, laboratory-prepared mixtures, and dosage forms. These methods can be used as stability-indicating procedures in quality control laboratories.

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