Determination of Rosuvastatin in the Presence of Its Degradation Products by a Stability-Indicating LC Method

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A forced degradation study was successfully applied for the development of a stability-indicating assay method for determination of rosuvastatin Ca in the presence of its degradation products. The method was developed and optimized by analyzing the forcefully degraded samples. Degradation of the drug was done at various pH values. Moreover, the drug was degraded under oxidative, photolytic, and thermal stress conditions. Mass balance between assay values of degraded samples and generated impurities was found to be satisfactory. The proposed method was able to resolve all of the possible degradation products formed during the stress study. The developed method was successfully applied for an accelerated stability study of the tablet formulation. The major impurities generated during the accelerated stability study of the tablet formulation were matches with those of the forced degradation study. The developed method was validated for determination of rosuvastatin Ca, and the method was found to be equally applicable to study the impurities formed during routine and forced degradation of rosuvastatin Ca.

Rosuvastatin Ca (Figure 1) is a new synthetic HMG-CoA reductase inhibitor (1). Compared with several other HMG-CoA reductase inhibitors, rosuvastatin Ca does not appear to be metabolized significantly by cytochrome P450 3A4 (2) and, therefore, may not possess the same potential for drug interactions as seen for some other statins, e.g., lovastatin (3) and simvastatin (4). It exhibits a high degree of specificity for uptake into the liver and is a potent in vitro and in vivo competitive inhibitor of HMG-CoA reductase. Analytical methods are reported for the determination of rosuvastatin Ca in human plasma along with its metabolites (5, 6).

To date, no method is published for the determination rosuvastatin Ca as an active pharmaceutical ingredient (API) and in its dosage form along with its degradation products. This paper describes the development and application of a stability-indicating assay method for determination of rosuvastatin Ca in the presence of its various degradation products. The developed method was successfully applied for determination of rosuvastatin Ca in its tablet dosage forms and for routine (long-term and accelerated) stability study of the tablet formulation. The generated impurities during the routine stability study were compared with these of the forced degradation study. The method was validated for its intended purpose.

Received October 2, 2004. Accepted by JM February 8, 2005.
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Experimental

Materials

(**a**) *Rosuvastatin Ca.*—Working standard grade, supplied by Torrent Research Center (Gandhinagar, India); its claimed purity was 98.2 ± 0.5%.

(**b**) *Tablets, placebo, and granules*.—Batch No. 002 (10 mg), manufactured by Torrent Research Center.

Reagents

(**a**) *NaH_2PO_4 dihydrate*.—Merck (New Bombay, India).
(**b**) *H_3PO_4*.—Merck.
(**c**) *KH_2PO_4*.—Merck.
(**d**) *Boric acid*.—Merck.
(**e**) *Methanol and acetonitrile*.—HPLC grade Rankem (New Delhi, India).
(**f**) *Milli-Q water*.—Purified with a Millipore system (Millipore Corp., Banglore, India).
(**g**) *Hydrochloric acid*.—0.2M aqueous solution (Merck).
(**h**) *Sodium hydroxide*.—1M aqueous solution (Merck).

Instruments

(**a**) *Walk-in stability chamber*.—Newtronic (Bombay, India).
(**b**) *Photostability chamber*.—SVI (Binder GmbH, Tuttlingen, Germany).
(**c**) *BOD incubator*.—Nova Instruments (Ahmedabad, India).
(**d**) *Autoclave and hot-air oven.*—Labline (Ahmedabad, India).

Chromatographic System

A Shimadzu Model LC-2010C chromatograph with autoinjector, sample cooler, and UV-Vis and photodiode array

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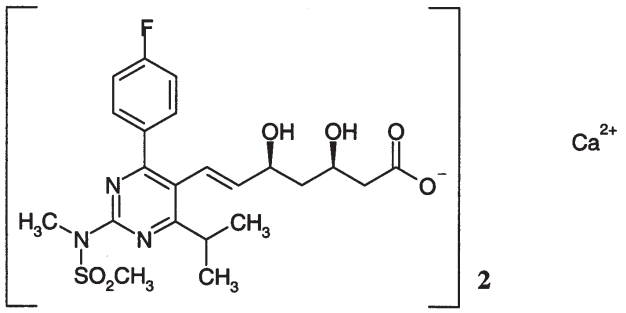

Figure 1. Structure of rosuvastatin as its Ca salt.

(PDA) detector (SPD 10Ma vp) was used. In the developed method, a C18 column (Inertsil-3V, 250 × 4.6 mm id, 5 μm particle size) with a mobile phase consisting of NaH_2PO_4 dihydrate buffer (50 mM, pH 2.0)–acetonitrile–methanol (40 + 20 + 40, v/v/v) was used, and the UV-Vis detector was set at 242 nm. During the course of method development, the PDA detector was set in the 190 to 400 nm range. The flow was adjusted to 1.0 mL/min. The diluent used for standard and sample preparation was water–acetonitrile–methanol (40 + 50 + 10, v/v/v).

Design of the Forced Degradation Study

The forced degradation study of rosuvastatin Ca was done under various extreme conditions. Degradation of the drug was done in solutions having various pH values. Acid-induced degradation was done in 0.2M HCl. Degradation of the drug at neutral pH was done in 6.8 pH buffer (0.2M KH_2PO_4, pH adjusted with NaOH). Borate buffer (0.2M boric acid, adjusted to pH 9.0 with NaOH) and 1N NaOH were used to induce degradation at basic pH. Oxidative degradation of rosuvastatin Ca was performed in 0.5% H_2O_2 aqueous solution. All of the above-mentioned solutions were kept in a hot air oven at 80°C for 20 h. Thermal degradation of drug and tablet dosage form was performed by exposing them at 100°C for 24 h and additionally by autoclaving at 121°C with 15 lb pressure for 20 min. Photostability of drug, drug granules, and coated and uncoated tablets was also studied by exposing them for 1.2×10^9 lux/h in the photostability chamber. All the samples mentioned above were prepared at 0.1 mg/mL concentration of rosuvastatin Ca in the diluent mentioned earlier, after adjusting the pH to 7 with diluted aqueous solutions of HCl/NaOH, if required.

Standard and Test Solutions

(**a**) *Standard preparation*.—0.05 mg/mL in the diluent.

(**b**) *Test sample preparation*.—Tablet powder equivalent to 50 mg rosuvastatin Ca was transferred to a 200 mL volumetric flask, 150 mL diluent was added, and the solution was sonicated for 60 min. After cooling, the solution volume was adjusted to the mark with the diluent, and 10 mL of the solution was centrifuged at 3500 rpm for 10 min. The supernatant was suitably diluted to give a 0.05 mg/mL concentration of rosuvastatin Ca.

The standard and test solutions of rosuvastatin Ca were found to be stable after storage for 24 h at room temperature and for 3 days at 15°C.

Table 1. Data indicating various validation parameters of the developed method

Parameter	Rosuvastatin at 242 nm
Limit of detection	0.01 μg/mL
Limit of quantitation	0.03 μg/mL
Linearity range	0.05—70 μg/mL
Linearity equation	$y = 104288x - 1591.8$
Correlation coefficient	0.9999
System precision (RSD)[a]	
Intraday (n = 5)	0.6–0.9%
Interday (n = 5)	0.35–1.0%
Repeatability of measurement (n = 5)	Batch No. 002
Mean ± SD, RSD	100.38 ± 0.39, 0.39%
Reproducibility of measurement (n = 3 x 3)[b]	
Mean ± SD[c]	RSD
Day 1	99.93 ± 0.15, 0.15%
Day 2	100.50 ± 0.5, 0.50%
Day 3	100.57 ± 0.40, 0.40%
Overall RSD	0.45%

[a] RSD = Relative standard deviation.
[b] Three analyses on each of 3 different days.
[c] SD = Standard deviation.

Method Validation

The developed method was validated for linearity at various concentration ranges (0.05–70 μg/mL) of rosuvastatin Ca. System precision was evaluated by analyzing the standard solution 5 times (n = 5). Method precision (repeatability) was evaluated by performing 5 replicate assays of the same sample (n = 5) on same day. Reproducibility of the method was evaluated by analyzing the same sample on 3 different days in

Table 2. Data from the recovery study of rosuvastatin Ca in the presence of placebo

Amount of drug added, mg	Amount of drug found, mg	Recovery, %	Mean, %
8	8.05	100.63	
10	10.02	100.20	100.53
12	12.09	100.75	

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Table 3. Data indicating specificity of the developed method

No.[a]	Degradation condition (degradation of rosuvastatin Ca, %)	Total peak purity (rosuvastatin)
1	At pH 1.5 (50–55)	0.9997
2	At pH 6.8 (4–5)	0.9997
3	At pH 9.0 (4–5)	0.9987
4	In 1M NaOH (10–12)	0.9988
5	Thermal degradation, dry heat (100°C) (4–5)	0.9991
6	Oxidative (0.5% H_2O_2 in water) (20–25)	0.9999
7	Photolytic degradation (25–28)	0.9987
8	Tablet autoclaved at 121°C with 15 lb pressure for 20 min degradation (3–5)	0.9992
9	Tablet kept at 60°C for 1 month degradation (3–5)	0.9999

[a] For 1–7, degradation of rosuvastatin Ca (drug substance) was done.

triplicate ($n = 3$). Recovery of the method was evaluated at 3 different concentration levels by addition of known amounts of standard to a placebo preparation. The limit of detection (LOD) and limit of quantitation (LOQ) of the method were derived by calculating the signal-to-noise (S/N) ratio of diluted standard. Data related to method validation are given in Tables 1 and 2.

Specificity of the method was established by verifying the purity of the peaks of the drugs in a stress sample using the PDA detector. Placebo preparation was also analyzed for specificity. Data related to specificity of the method are given in Table 3.

Application of Developed Method

The developed method was used for the optimization of the dosage form, and in this context the method has been used for the evaluation of the compatibility of the drug with various probable excipients. Data related to the excipient compatibility study are summarized in Table 4. The developed method has been successfully used for the routine (long-term and accelerated) stability study of drug substance and tablet formulation. After characterization of degradation products, the method is equally applicable for analysis of related substances.

Table 4. Data indicating compatibility of rosuvastatin Ca with various excipients

No.	Sample[a]	Total impurity, %
1	Drug	0.54
2	Drug + microcrystaline cellulose	0.60
3	Drug + lactose	0.75
4	Drug + calcium phosphate	0.55
5	Drug + crospovidone	0.58
6	Drug + BHT[b]	0.58
7	Drug + magnesium stearate	0.70

[a] Drug was admixed with each excipient individually and kept in a walk-in chamber at 60°C for a month.

[b] Butylated hydroxytoluene.

Results and Discussion

Application of the forced degradation study was considered a very important aspect for the development of a stability-indicating assay method for rosuvastatin Ca. Degraded samples prepared by systematic forced degradation were used for method development trials to optimize the method as a stability-indicating method for determination of rosuvastatin Ca and possible impurities forms under stressed and routine (accelerated and long-term) stability studies.

During the course of the forced degradation study of rosuvastatin Ca, stability in various pH solutions was determined. Rosuvastatin Ca was found to be highly unstable at acidic pH and to degrade very quckly with formation of more than 10 different impurities. An impurity that elutes near to the rosuvastatin peak observed in the standard preparation (Figure 2) was increased in acidic conditions (Figure 3). Development trials were performed by analyzing the acid-stressed sample to resolve the major impurity found. In the developed method, all of the generated impurities were

Table 5. Data indicating photolytic degradation of rosuvastatin Ca

Rosuvastatin Ca exposed as	Impurity, %
Drug	26.5
Drug granules	13.2
Uncoated tablet	2.5
Coated tablet	0.75

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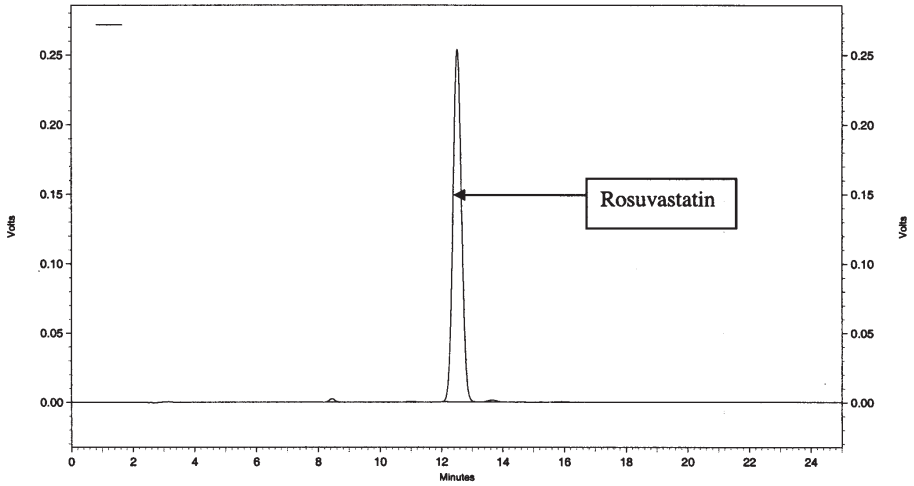

Figure 2. Chromatogram indicating standard preparation of rosuvastatin Ca.

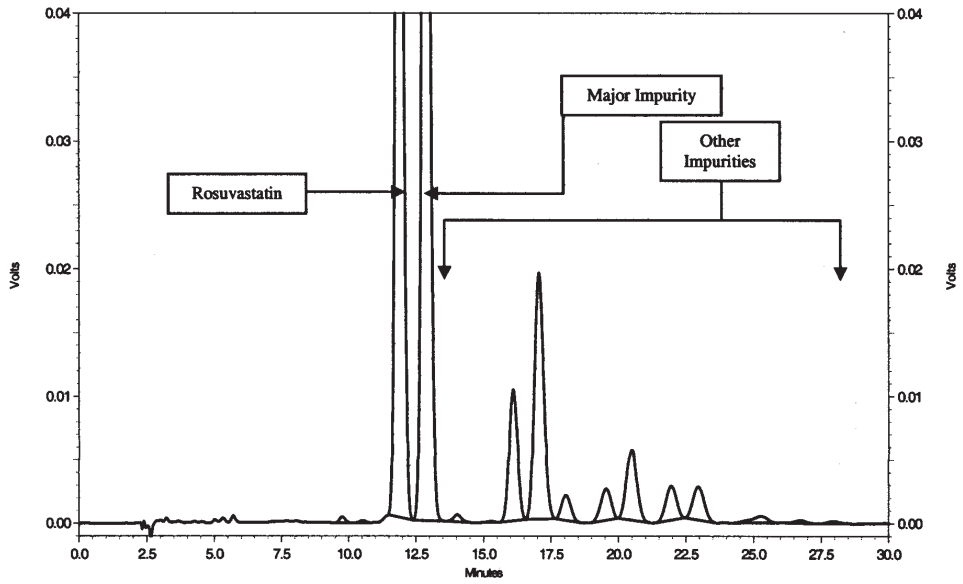

Figure 3. Chromatogram indicating acid-induced degradation of rosuvastatin Ca.

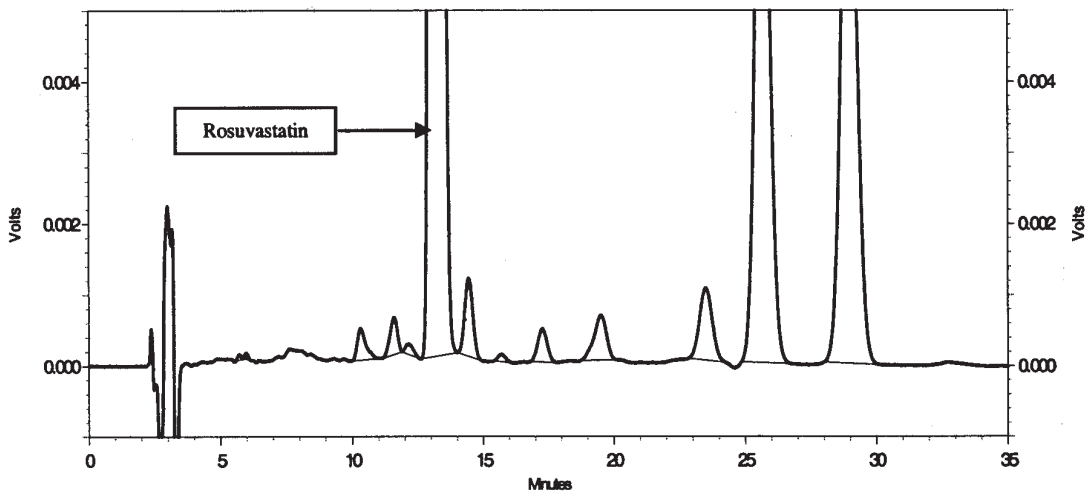

Figure 4. Chromatogram indicating photolytic degradation of rosuvastatin Ca.

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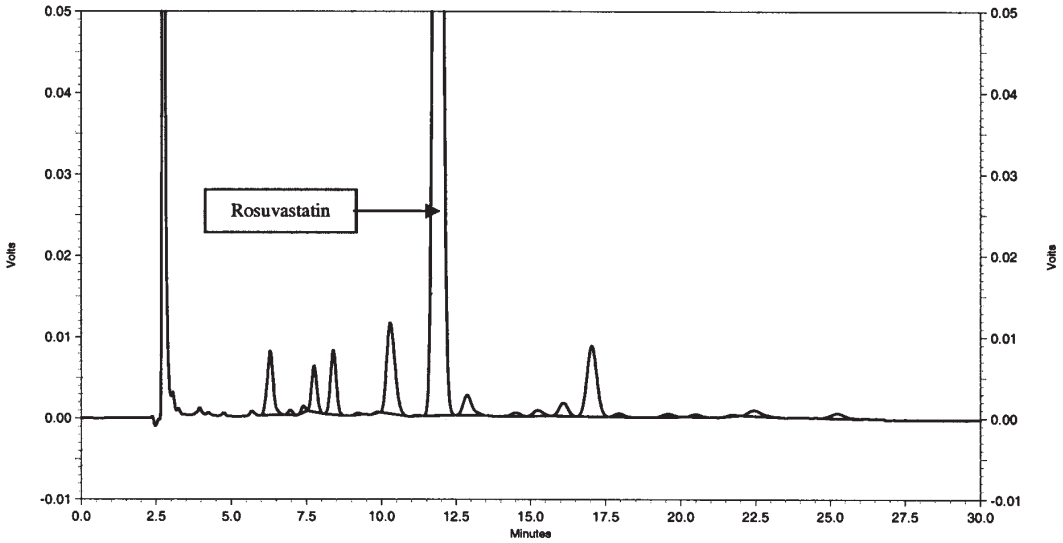

Figure 5. Chromatogram indicating oxidative degradation of rosuvastatin Ca.

separated with high resolution along with the closely eluting major impurity.

A systematic approach was applied for development of the method for rosuvastatin Ca. A C18 Inertsil ODS-3V column (250 × 4.6 mm) having 5 μm particles was used because of its advantages of better reproducibility, high resolving power due to symmetrical particle size, lower back pressure, and high degree of retention.

Various trials were performed using potassium buffer, and it was not found to be suitable for resolution of closely eluting peaks of degradation products from the main peak. The use of $NaH_2PO_4 \cdot 2H_2O$ buffer for statins was found suitable because of high eluting power compared to acid base and phosphate buffer, and it was suitable for closely eluting degradation products.

Resolution between the major impurity [found in acid degradation (Figure 3)] and rosuvastatin was optimum with the mobile phase at pH 2.0. Methanol could be used to get better resolution compared to acetonitrile, but the use of acetonitrile was necessary for the best peak shape and theoretical plate number for the main peak. Acetonitrile was used to optimize the retention time of late-eluting impurities generated in the photostability sample (Figure 4). The ratio of buffer, methanol, and acetonitrile was selected on the basis of resolution between the major degradation peaks and the main peak, and it was finalized as (400 + 200 + 400, v/v/v) after analyzing all the degraded samples with the PDA detector and evaluating the peak purity, resolution, specificity, and stability-indicating ability of the method.

The degradation study of rosuvastatin Ca indicated that it is considerably stable under neutral and basic condition, but it degrades in acidic and oxidative conditions. In oxidative conditions, some polar impurities are generated as some of the oxidative degradation products having lesser retention time than rosuvastatin Ca (Figure 5).

Photolytic degradation of rosuvastatin Ca as drug substance, drug granules, and uncoated tablet was found to be very prominent (Figure 4). The photolytic degradation of rosuvastatin Ca in coated tablets was very much less than that found as drug substance, uncoated tablet, and drug granules (Table 5). These findings suggested the requirement of an opaque film coating on the tablet to retard the photolytic degradation.

The developed method was applied for routine and accelerated study of tablet formulation of rosuvastatin Ca. It is stability-indicating and very specific for determination of rosuvastatin Ca in the presence of its degradation products and various excipients used for its tablet dosage from (Table 3).

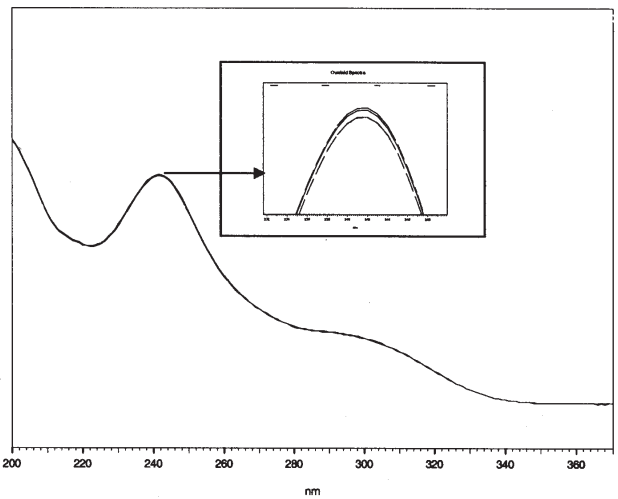

Figure 6. Overlaid UV spectra of rosuvastatin Ca standard preparation (—), and degraded samples of it in acidic (----), basic (•••) and oxidative (— • —) stressed conditions.

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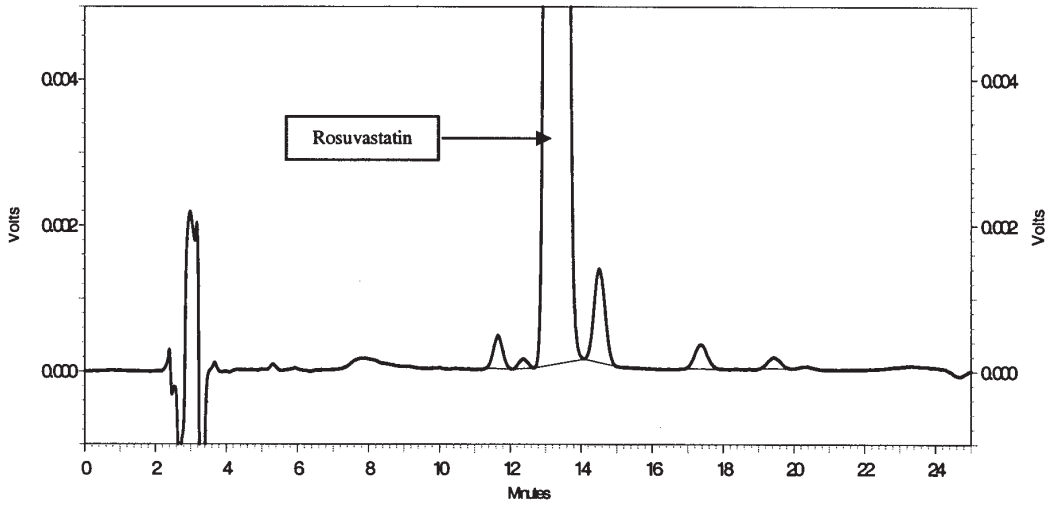

Figure 7. Chromatogram indicating degradation of rosuvastatin Ca in tablet dosage form after exposed for 1 month at 60°C.

Data in Table 3 indicate the percent degradation of rosuvastatin Ca as drug and in its tablet dosage form when exposed to various extreme conditions. The data of peak purity of rosuvastatin for each of the degraded samples indicate specificity of the developed method. Additionally, ultraviolet (UV) spectra of rosuvastatin in standard preparation and degraded samples were compared (Figure 6). The total resemblance of the UV spectra indicates specificity of the method for determination of rosuvastatin in the presence of various degradation products. The developed method was successfully applied for a compatibility study of the drug with some common excepients, and the drug was found compatible at 60°C for 1 month because no significant increase of any degradation products was observed (Table 4). Major impurities found in the tablet formulation after exposure for 1 month at 60°C matched those found during the forced degradation study (Figure 7).

Conclusions

In this study, rosuvastatin Ca was subjected to a stress study under various extreme conditions. The drug was found to degrade under acidic, oxidative, and photolytic conditions but was comparatively stable under neutral and basic pH conditions. The drug was analyzed specifically in the presence of different degradation products having chromophoric groups by way of simple isocratic conditions. The method was validated for parameters such as linearity, precision, accuracy, specificity, and ruggedness. The method was also applied to a preformulation study by evaluating compatibility of the drug with various excepients and tablet formulation. Thus, the method can be used for analysis of the drug during stability studies, and good mass balance between drug percentage and degradation products was established. The proposed method is suitable for quality control laboratories, where economy and time are essential.

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