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# A validated, stability indicating, LC and assay method for rosuvastatin

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## ABSTRACT

A simple, gradient, stability indicating, reversed phase high performance liquid chromatographic method (LC) has been developed for the quantification of rosuvastatin and its related substances and of degradation products generated by decomposition. When rosuvastatin was subjected to acid hydrolytic, oxidative, photolytic and thermal stress, degradation was observed. Chromatographic separation was achieved among rosuvastatin and related substances and degradation products, which were obtained from stress conditions like acid, base, water hydrolysis and oxidation. The optimized conditions are there by using a step wise gradient elution mode on a C18 column using a mixture of 0.02 M Potassium dihydrogen orthophosphate, pH adjusted to 3.0 and acetonitrile in the ratio of (80:20) (v/v) as solvent-A and further using a mixture of acetonitrile and water in the ratio (90:10) (v/v) as solvent-B. The method was completely validated in terms of linearity, accuracy, precision, specificity and robustness. The method can be used for quality control during manufacture and for assessment of the stability of samples of rosuvastatin. The LOD and LOQ values of rosuvastatin, rosuvastatin lactone and rosuvastatin ester are 0.05, 0.075, 0.077μgmL$^{-1}$ and 0.2, 0.30, 0.31 μgmL$^{-1}$ respectively. The present RPLC can be able to determine rosuvastatin and its related substances simultaneously in bulk drug and finished dosage forms of rosuvastatin.

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## KEYWORDS

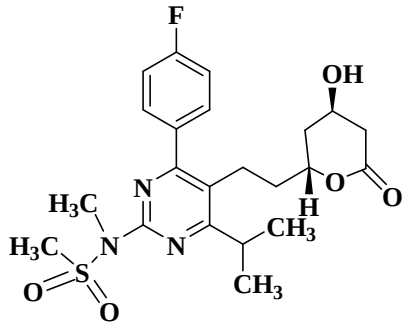
Rosuvastatin;
HPLC;
Method development;
Validation;
Solution stability;
Specificity.

## INTRODUCTION

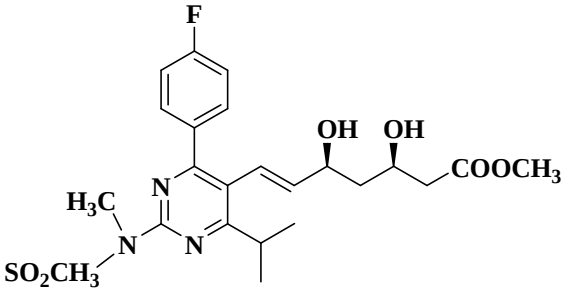
Rosuvastatin is chemically described as bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methyl sulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3, 5-dihydro xyhept-6-enoicacid] calcium salt. (Figure 1). Rosuvastatin is a new, synthetic, orally active and competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase with significant and specific low-density lipoprotein (LDL) cholesterol-lowering activity in vitro and *in vivo*[1,2]. Its empirical formula is $(C_{22}H_{27}FN_3O_6S)_2Ca$ and its molecular weight is,1001.14. Rosuvastatin is available as Crestor™ in tablet form (5, 10, 20, or 40 mg) for oral administration.

Limited LC methods have been reported in the literature. Furthermore, C.K.Hull et al. developed an assay method employing automated solid-phase extraction (SPE) followed by HPLC with positive ion Turbo Ion spray tandem mass spectrometry (LC–MS/MS)[3]. Kathalijne A et al. described a microbore LC method in combination with tandem mass spectrometry (MS/MS) for the sensitive detection of rosuvastatin

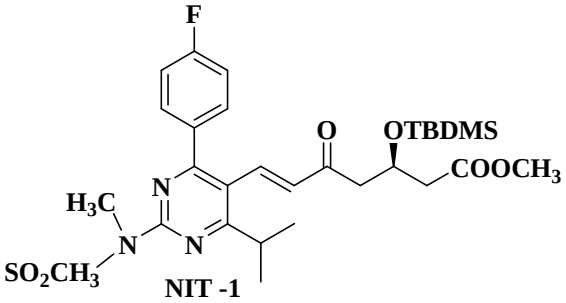
Note



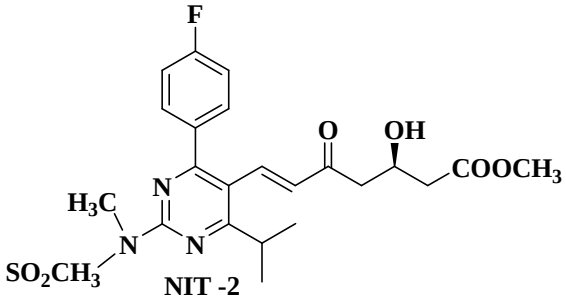
Imp-1: (Rosuvastatin lactone impurity)



Imp-2: (Rosuvastatin Ester impurity)



Imp-3: NIT-1 imp



Imp-4: NIT-2 imp

**Figure 1 : Chemical structures of Rosuvastatin and its related substances Rosuvastatin calcium**

(CrestorTM) in human plasma[4]. Ke Lan, Xuehua Jiang et al. reported a method for the determination of rosuvastatin in human plasma using liquid chromatography/tandem mass spectrometry in human plasma after being treated with acetic acid and tetra butyl ammonium hydroxide, the analyte was extracted by liquid-liquid extraction[5-13].

Attempts were made to develop a single LC method that could be used to determine rosuvastatin and its process related impurities bulk samples of rosuvastatin. This manuscript deals with the development of stability indicating analytical method using the samples, which are generated from the forced degradation studies and validation. Although five impurities including intermediates were detected and separated with reasonable resolution by this single method, but only two potential impurities were considered as known due to the absence of remaining impurities in the finished product. The developed method was validated to meet the stipulations of ICH guidelines[14-15].

## EXPERIMENTAL

### Chemicals

Samples of rosuvastatin and its impurities were received from Research and Development Department of IPDO of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade Acetonitrile was procured from Rankem , India. Analytical reagent grade potassium dihydrogen orthophosphate and Orthophosphoric acid were purchased from Merck, India. High pure water was prepared by using Millipore milli Q plus purification system.

### Equipment

The LC system, used for the method development, forced degradation studies was Agilent 1100 series (manufactured by Agilent Technologies, Waldbronn, Germany). LC system with PDA detector and VWD was used for method validation. The out put signal was monitored and processed by using Empower software (designed by waters) on $P_4$ (Digital Equipment Co).

### Chromatographic conditions

The chromatographic column used was Lichrospher RP-18e, 250mm×4.6 mm, 5µm particle size. The buffer is a solution of 20mM potassium di hydrogen ortho phosphate; pH was adjusted to 3.0 with diluted ortho phosphoric acid. Solvent A was buffer and acetonorile in the ratio 80:20(v/v). Solvent B was a mixture of acetonitrile and water in the ratio (90:10)(v/v). The flow rate of mobile phase was kept at 1.0mLmin$^{-1}$. The LC gradient was set as Time / % B: 0.01/35, 15/35, 25/55,

40/80, 55/80, 60/35, 65/35.The column temperature was maintained at $27^0$C and the wave length was monitored at 242 nm. The injection volume was 20µL. The diluent, Acetonitrile and water in the ratio 800:200v/v was used to prepare standard, blend and system suitability solutions.

**Sample/Standard preparation**

Standard and test solutions of 500 µg $mL^{-1}$ were prepared individually and a stock solution of impurity blend (mixture of Imp-1 and Imp-2)50 µg $mL^{-1}$ was prepared as well in the same diluent. A solution of rosuvastatin working standard was injected as a system suitability solution.

**Method validation**

**Specificity**

Specificity is the ability of method to measure the analyte response in the presence of its potential impurities and degradation products . The specificity of a developed LC method for rosuvastatin was carried out in the presence of its impurities namely Imp-1 and Imp-2.

Forced degradation studies were also performed to a bulk drug to provide an indication of stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of photolytic degradation (as per ICH recommended condition), thermal degradation (drug substance exposed at $105^0$C), acid hydrolysis (using 1.0 N HCl), base hydrolysis (using 0.1N NaOH), water hydrolysis (reflux at $100^0$C) and oxidative degradation (using 3% $H_2O_2$) to evaluate the ability of the proposed method to separate rosuvastatin from its degradation products. For heat and light studies, study period was seven days, whereas for acid, base, water hydrolysis and oxidative degradation it was 1 hr, 2hr, 48 hr and 3hr respectively. To check and ensure the homogeneity and purity of rosuvastatin peak in the stressed sample solutions, PDA detector was employed. Assessment of mass balance in the degraded samples was checked to see whether the amount of impurities detected in the stressed sample matches the amount present before the stress was applied. Assay studies were carried out on the stressed sample against rosuvastatin qualified reference standard and the mass balance (% assay + % sum of all impurities + sum of all degradants) was tabulated. Assay was also calculated for bulk sample by spiking the impurities (Imp-1, and Imp-2) at the level of 0.15%.

**Precision**

Assay method precision was evaluated by performing six independent assays of test sample of rosuvastatin against qualified reference substance and calculated %RSD for %assay content. The precision was checked by injecting six individual (n=6) preparations of (0.5mgm$L^{-1}$) rosuvastatin spiked with 0.15% of Imp-1 and Imp-2 w.r.to the analyte concentration. %RSD was calculated for %Imp-1 and %Imp-2. The intermediate precision of the method was also evaluated using different analyst, day and instrument in the same laboratory.

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

The LOD and LOQ for Imp-1 and Imp-2 were estimated at a S/N ratio of 3:1 and 10:1 respectively by injecting a series of diluted solutions with known concentrations[14]. Precision and accuracy studies were also performed at the LOQ level by injecting six individual preparations (n=6) of Imp-1and Imp-2 and the %RSD was calculated for peak area.

The accuracy was carried out by standard addition and recovery study at LOQ level.

**Linearity**

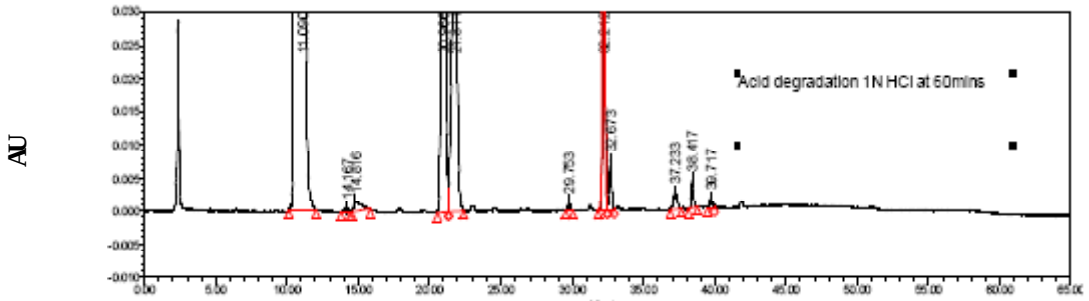
Linearity test solutions for assay method were prepared from stock solution at five concentration (n=5) levels from 50 % to 150% of assay analyte concentration (50,75,100,125 and 150%).The peak area versus concentration data was performed by least squares linear regression analysis.

Linearity test solutions for related substances method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at seven concentration (n=7) levels from LOQ to 150% w.r.to the impurities specification level of 0.15% (i.e, 0.03, 0.1875, 0375, 0.75, 0.5625, 1.125µgm$L^{-1}$). The calibration curve was drawn by plotting the peak areas of Imp-1and Imp-2 against its corresponding concentration. The %RSD and Y-intercept of the calibration curve was calculated.

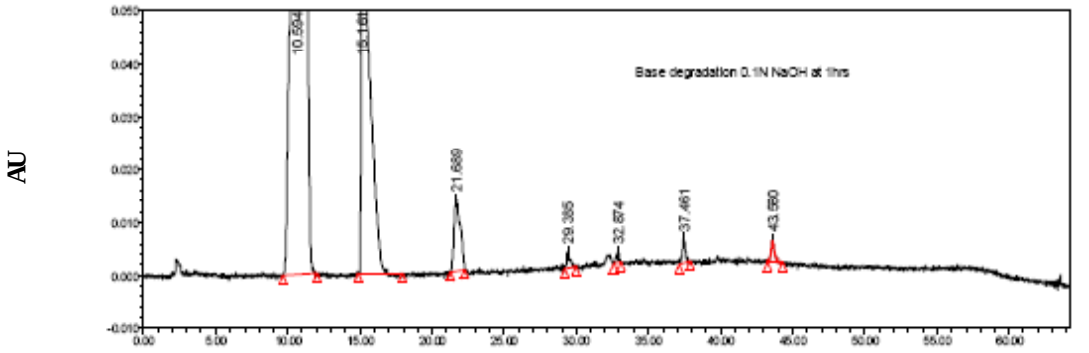
**Accuracy**

The accuracy of the assay method was evaluated

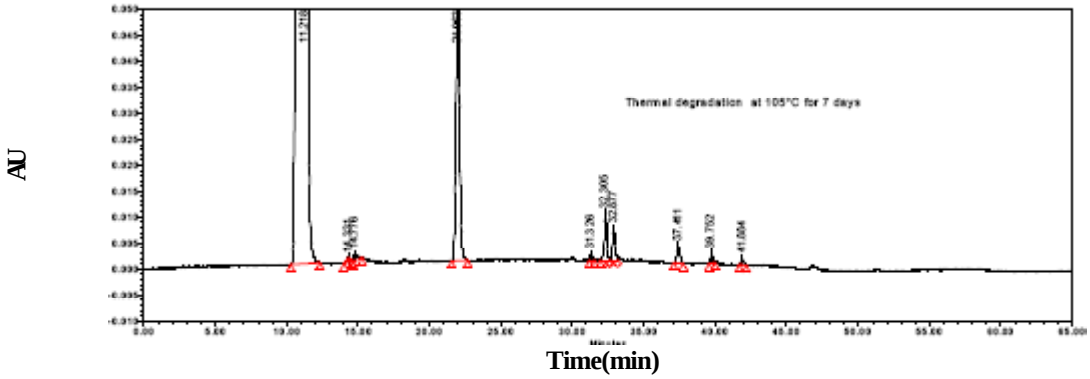
Note




(a) Acid degradation of Rosuvastatin: 1N HCl 1 hr at 60$^0$C




(b) Base degradation of Rosuvastatin: 0.1N NaOH




(c) Thermal degradation of Rosuvastatin: Thermal degradation at 105$^0$C for 7 days

**Figure 2 : Typical HPLC chromatograms of Rosuvastatin and its related substances**

in triplicate (n=3) at three concentration levels i.e, 25, 50, 75 μg mL$^{-1}$ in bulk drug sample.

Standard addition and recovery experiments were conducted to determine accuracy of related substances method for quantification of the impurities in bulk drug samples.

The study was carried out in triplicate at 0.0375, 0.75 and 1.125μg mL$^{-1}$ concentrations, where as the analyte concentration 500 μg mL$^{-1}$.The percentage recoveries of Imp-1and Imp-2 were calculated.

## Solution stability and mobile phase stability

The solution stability of rosuvastatin and its impurities in this method was carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48hours. Content of imp-1 and imp-2 were determined for every 6h interval up to the study performed. Mobile phase was also carried out for 48 hours by injecting the freshly prepared sample solutions for every 6h interval. Content of imp-1 and imp-2 were checked in test solutions. Mobile phase prepared was kept constant during the study period.

## Robustness

To determine the robustness of the developed method, the chromatographic conditions were deliber-

ately altered and verified the system suitability criteria. Established the selectivity factor in all the robustness studies for each impurity and compared with regular experiment.

The flow rate of mobile phase was 1.0 $mLmin^{-1}$. To study the effect of flow rate on the peak USP tailing and USP theoretical plates, flow rate was altered by 0.2 units, i.e, from 0.8 to 1.2 mL $min^{-1}$. The effect of pH on peak tailing and theoretical plates of rosuvastatin was studied by varying ± 0.2 pH units (at 2.8 and 3.2 buffer pH). The effect of column temperature was studied at $27^0C \pm 5^0C$.

## RESULTS AND DISCUSSION

### Optimization of chromatographic conditions

Imp-1 and imp-2 are the potential impurities that related to the corresponding lactone and ester compounds of rosuvastatin. The main target of the chromatographic method is the separation of Imp-1, imp-2, intermediates and degradation products from rosuvastatin peak. Due to the dissimilar polarities among rosuvastatin, Imp-1 and imp-2, the separation of these impurities is not much critical, but the elimination of interferences due to degradation products from rosuvastatin and its impurities is only the tough task in the initial stages of development. Impurities were co-eluted by using different stationary phases like cyano, phenyl and C8 and different mobile phases containing buffers like phosphate, sulphate and acetate with different pH (2.5-7) and using organic modifiers like acetonitrile and methanol in the mobile phase. Satisfactory chromatographic separation was achieved using a solvent A, which is a solution of 20 mM potassium dihydrogen ortho phosphate and acetonitrile in the ratio of (80:20) *(v/v)*, wherein the pH of the potassium dihydrogen ortho phosphate was adjusted to 3.0 and solvent-B is a mixture of acetonitrile and water in the ratio (90:10) (v/v). The LC gradient of solvent B was kept as Time / % B: 0.01/35, 15/35, 25/55, 40/80, 55/80, 60/35, 65/35. In the optimized conditions, the rosuvastatin, imp-1,imp-2 and degradation products were well separated with resolution ($R_s$) grater than 2 and the typical relative retention times of imp-1 and imp-2 were about 2.03 and 4.34 respectively. Various C18 columns i.e Zodiac C18, Symmetry C18, Kromasil C18 and venusil C18 were checked with optimized conditions and found the best resolutions in Licchrospher RP18e. The developed LC method was found to be specific for Rosuvastatin and its impurities namely imp-1 and imp-2.

Selection of wavelength at 242 nm is appropriate for rosuvastatin, process related impurities and for degradation products as well. The relative response factors of known impurities are in between 0.8 to 1.2. While selecting the wave length, the UV spectra and absorbance of degradation products at 242 nm was also taken into consideration.

### Results of forced degradation studies

Considerable degradation observed in rosuvastatin bulk samples, under stress conditions such as acid, base and water hydrolysis and moderate degradation was observed in photolytic and thermal stress. To achieve this level of degradation, different test solutions were prepared in 1.0N HCl, 0.1N NaOH, 3% hydrogen peroxide and water respectively. These solutions were further subjected to stress conditions stressed to maximum 48h at $60^0C$. Under these conditions the degradation of drug substance was observed during acid and base hydrolysis. rosuvastatin was degraded into Imp-1(Figure 2) under acidic conditions (treated with 1.0N HCl at $60^0C$ for 1 hr) and it was confirmed by co-injection with a qualified imp-1 standard. rosuvastatin is highly sensitive to base, because it was rapidly degraded, even at room temperature with one tenth normal solution of sodium hydroxide. Moderate degradation of drug substance was observed under water hydrolysis conditions (treated water at $100^0C$ for 48 hrs) leads to the formation of a few unknown degradation peaks. In oxidation, no degradation peaks were observed. Peak purity test results obtained from PDA confirm that the rosuvastatin peak is homogeneous and pure in all the analyzed samples. The mass balance of stress samples was close to 99.6%. The assay of rosuvastatin is unaffected in the presence of imp-1 and imp-2 which confirm the stability indicating power of the developed method.

### Results of method validation of experiments

### Precision

The %RSD of rosuvastatin during assay method

Note

precision study was well within 0.5% and the %RSD of imp-1 and imp-2 in related substances method precision study was within 2%. The % RSD of assay results obtained in intermediate precision study was within 1.0% and the % RSD of %area of imp-1 and imp-2 confirming excellent precision of the method.

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

The LOD of rosuvastatin, imp-1 and imp-2 are 0.05, 0.075 and 0.077μg $mL^{-1}$. The LOQ of rosuvastatin, imp-1 and imp-2 are 0.2, 0.30, 0.31?g $mL^{-1}$ (of analyte concentration i.e., 500μg $mL^{-1}$) for 10 μL injection volume. The method precision for imp-1, imp-2 at LOQ level was below 5% RSD.

**Linearity**

Linear calibration plot for assay method was obtained over the calibration ranges tested i.e, 125 to 750μgm$L^{-1}$ and the correlation coefficient obtained was greater than 0.999. Linearity was checked for the assay method over the same concentration range for three consecutive days. The % RSD values of the slope and Y-intercept of the calibration curves were 3.1 and 4.3 respectively. The results show that an excellent co-relation existed between the peak area and concentration of the analyte.

Linear calibration plot for related substance method was obtained over the calibration ranges tested i.e, 0.03 to 1.125 μg $mL^{-1}$ for imp-1 and 2. The correlation coefficient obtained greater than 0.998 linearity was checked for the related substances method over the same concentration range for three consecutive days. The % RSD values of the slope and Y-intercept of the calibration curves were 4.6 and 5.5 respectively. The results (TABLE 1) show that an excellent co-relation existed between the peak area and concentration of imp-1and imp-2.

**Accuracy**

The % recovery of rosuvastatin in bulk drug samples was ranged from 99.1 to 100.5 The % recovery of imp-1 and 2 in bulk drug samples was ranged from 94.6 to 103.0. The results showing excellent accuracy of the method.

**Solution stability and mobile phase stability**

No significant change was observed in the content of imp-1 and imp-2 and rosuvastatin during solution and mobile phase stability experiments when performed using this method. The solution and mobile phase stability experiments data confirms that sample solutions and mobile phase used during imp-1 and imp-2 and rosuvastatin content determination were stable up to 48 hours.

**TABLE 1: Linearity data for rosuvastatin and related substances**

| Component | Calibration range (μgm$L^{-1}$) | Regression equation | SES[1] | SEI[2] | CC[3] (r) |
|---|---|---|---|---|---|
| Rosuvastatin | 0.03-1.125 | y = 213.7x+ 7.3 | 29±20 | 21±15 | 0.999 |
| Imp-1 | 0.03-1.125 | y =214.3x+10.3 | 31±21 | 19±12 | 0.999 |
| Imp-2 | 0.03-1.125 | y = 192.9x+8.4 | 29±23 | 19±15 | 0.998 |

SES[1]:Standard error of slope± 95% confidence interval, SEI[2]: Standard error of intercept± 95% confidence interval

**TABLE 2: Robustness data for rosuvastatin related substances**

| Robustness data for Rosuastatin related substances | | | | | |
|---|---|---|---|---|---|
| Parameter | Variation | $T_f$ | N | %Imp-1 | %Imp-2 |
| -0.2 units | pH | 1.20 | 5119 | 0.05 | 0.01 |
| Regular 3.0 | | 1.47 | 5808 | 0.03 | 0.01 |
| +0.2 units | | 1.19 | 5200 | 0.06 | 0.01 |
| Temp -5°C | Column temperature | 1.47 | 5824 | 0.03 | 0.01 |
| Regular 27°C | | 1.47 | 5808 | 0.03 | 0.01 |
| Temp +5°C | | 1.21 | 5526 | 0.04 | 0.01 |
| Flow -0.2 | Flow rate (mLmin$^{-1}$) | 1.21 | 6070 | 0.04 | 0.004 |
| Regular 1.0 | | 1.47 | 5808 | 0.03 | 0.01 |
| Flow +0.2 | | 1.14 | 5042 | 0.05 | 0.01 |
| -10% | %Organic ratio | 1.17 | 5904 | 0.05 | 0.01 |
| Regular 100% | | 1.47 | 5808 | 0.03 | 0.01 |
| +10% | | 1.23 | 5016 | 0.06 | 0.01 |

**Robustness**

In all the deliberate varied chromatographic conditions (pH, column temperature, flow rate and percentage of organic ratio). The USP tailing and USP theoretical plates were less than 1.5 and more than 5000 respectively (TABLE 2).

## CONCLUSIONS

In this manuscript the simple, accurate and well defined stability indicating gradient LC method for the determination of rosuvastatin in the presence of its related substances and degradation products was described for the first time. The behavior of rosuvastatin under various stress conditions were studied and presented. The information presented herein could be very

useful for quality monitoring of bulk samples, finished dosage forms and as well as employed to check the quality during the stability studies.

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