Stabilization of Rosuvastatin Calcium Formulation by Prevention of Intermolecular Esterification: An Experimental Design

Furquan Nazimuddin Khan⁎, Zahid Zaheer, Moizul Hasan, Imran Anees, Sarfaraz Khan, Iftequar Syed, Priti Sanjay Puranik
Department of Quality Assurance, Y.B. Chavan College of Pharmacy, Rauza Bagh, Aurangabad, Maharashtra, INDIA.

ABSTRACT
Background: Rosuvastatin calcium is the most effective molecule for the treatment and management of Hypercholesterolemia. This drug easily degrades by exposure to moisture and light and forms unstable formulation by formation of oxidation products, this degradation accelerates in acidic environment of the formulation. The acid form of Rosuvastatin calcium which is the active moiety has a tendency of conversion into lactone form. Inactive moiety through “intermolecular esterification” in acidic environment. Synthetic alkalis such agents as tribasic magnesium phosphate is usually added in the tablet formulation to overcome this degradation, but alkalis are harmful to gastric mucosa. Methods: Natural stabilizers such as Xanthan Gum, Chitosan and Guar Gum are used for stabilization. Selection of optimum stabilizer and drug/stabilizer ratio is obtained by quality by design approach by implementing 3² factorial design. Xanthan gum and Chitosan were selected as Critical quality attributes and material attributes with fixed Rosuvastatin Calcium concentration. Results: Tablets consisting of Xanthan Gum and Chitosan in 1:1.4 ratios exhibited maximum stabilization after stability study with 99% assay and dissolution was enhanced by more than 20% i.e. 98% within 30 min as compared to marketed tablets. Statistical test by Analysis of Variance revealed that the model is significant with p value <0.0001 and F-value of 50.58 that the model terms are significant for one factor i.e. the concentration of chitosan as natural stabilizer. Conclusion: The stabilisation of Rosuvastatin calcium was achieved by natural stabilizer i.e. chitosan with QBD approach and the stable tablet formulation was successfully prepared.
Key words: Rosuvastatin calcium, Quality by Design, Design of Experiment, Natural stabilizers, Chitosan, Xanthan Gum.
Correspondence
Dr. Furquan Nazimuddin Khan
Department of Quality Assurance, Y B Chavan College of Pharmacy, Rauza Bagh, Aurangabad, Maharashtra, INDIA.
Phone: +91 9730076135
Email: furkhankhan11@gmail.com;
ORCID: http://orcid.org/0000-0002-3729-5303
DOI: 10.5530/ijpi.2020.2.35

INTRODUCTION
Cardiac diseases such as atherosclerosis is the leading cause of death in the world and one of the most significant factor for these diseases is total/ high density lipoprotein (HDL) cholesterol ratio. Rosuvastatin calcium (RVS) inhibits the activation of the enzyme named HMG-CoA reductase, which is responsible for cholesterol synthesis in the body and prevents cholesterol formation.1 An important problem about RVS is conversion from acid form into lactone form through “intermolecular esterification” that take place between the carboxylic acid and hydroxyl groups that are present on the β and δ carbons. This reaction takes place in acidic environment and basic agents reverse the reaction. This phenomenon decreases the stability of the compound and as a result lowers the shelf life of the product. Various synthetic stabilisers such as tribasic calcium phosphate, tribasic magnesium phosphate salts were used to stabilise RVS compositions. However, high amounts of alkaline agents’ intake harms to gastric mucosa and causes stomach problems in patients.2 Thus there is a need for new and stable formulation for the most effective and the most frequently used medicament of statin group.3 The use of natural stabilizers such as various biopolymers and natural gums with higher alkaline nature can be used instead of these harmful alkalis to avoid such gastric irritation problems.4

MATERIALS AND METHODS
Materials
RVS is obtained as a gift sample by Lupin Ltd, Pune. The excipients such as Crospovidone, microcrystalline cellulose, Chitosan, Guar gum

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.
The drug content is determined by weighing and powdering ten tablets equivalent to 10 mg of Rosuvastatin and dissolved in methanol and solution was filtered through a Whatman filter paper no.40. The absorbance was determined at 240 nm at UV visible spectrophotometer. The Tablet disintegration time was measured using disintegration test apparatus. One tablet was placed in each of the six tubes of the basket. The basket was immersed in water bath at 37 ± 2°C having phosphate buffer pH 6.8. The time in seconds required for complete disintegration was determined using a stop watch.9

Stabilization of Rosuvastatin Calcium

Drug stability study was carried out by preparing samples of drug and stabilizers in 1:1 ratio (100mg Rosuvastatin + 100mg stabilizer) such as Xanthan gum, Guar gum and Chitosan as natural excipients. The drug is stable in higher alkaline environment which agree with the literature.10 Thus the three stabilizers were selected based upon their alkaline nature for providing alkaline environment to the formulation so that the drug degradation is prevented. The mixtures were filled in closed vials and placed in stability chamber at three different storage conditions such as Set A (Long Term stability), Set B (Intermediate stability), Set C (Accelerated stability as per ICH guidelines).11

Identification of Critical Quality Attributes and Material Attributes

In pharmaceutical development, factors or input variables are the raw material attributes and critical process parameters while outputs are the CQAs such as percent purity or percent assay and dissolution.12 From the stabilisation study, the material attributes were identified as factors for applying statistical response surface design.13

Design of Experiment

A 3^3 factorial experimental design was used to optimize and evaluate the effect of material attributes on stability of RVS. A two-factor, three-level design was suitable for exploring linear response surfaces with Design expert software (Version 10.0.0, Stat-ease Inc. Minneapolis, MN, USA), as per the two-factor, three-level design 9 batches were prepared and abbreviated as F1-F9. All ingredients were accurately weighed according to batches F1-F9 and mixed in a glass mortar-paste and again sifted for uniform mixing. RVS and each stabilizer are separately sifted through sieve number 40. The samples are subjected to stability study as per ICH guidelines. Results obtained are statistically analysed by Analysis of Variance (ANOVA) using design expert software. The batch showing maximum response i.e. percent assay is selected as optimised combination of drug-stabilizer ratio to be used in the formulation of stable RVS tablets.

Formulation of Tablets

Based on the optimised drug-stabilizer ratio obtained from factorial design and other excipients, immediate release tablet of RVS was formulated. All ingredients i.e. RVS 10mg, Chitosan 14mg, Xanthan Gum 10mg, Crospovidone 60mg, Manesium Stearate 2.5mg, Talcum 2.2mg and Microcrystalline Cellulose (MCC PH102) quantity sufficient are weighed accurately and sifted separately through sieve no: 40 to remove the lumps present if any.

Step-I: RVS, chitosan and Xanthan Gum were mixed in glass mortar-pestle and again sifted for uniform mixing.

Step-II: This mixture from step I and other ingredients except lubricant are placed in a poly-bag and blended for 5 min.

Step-III: The resulting mixture is then lubricated by magnesium stearate and t alc just before compression.

Step-IV: The mixture obtained in Step III is compressed to produce tablets by using 8 station rotary tablet compression machine with 8.5mm concave punch.14

Evaluation of pre-compression parameters for prepared tablet

The evaluation of pre-compression parameters includes Angle of Repose i.e. the maximum angle possible between the surfaces of pile of powder or granules and horizontal surface. It is given as: \( \tan \theta = h/r \), \( \theta = \tan^{-1} h/r \). Where \( \theta \) is angle of repose, \( h \) is height of pile of powder or granules and \( r \) is the radius of the pile. Then Bulk density was measured by pouring the blend into a measuring cylinder and initial volume was noted, called as bulk volume. Bulk density was calculated as \( \rho_B = M / V_b \). Where \( M \) is the mass of blend and \( V_b \) is the bulk volume. Tapped Density is determined by tapping the measuring cylinder containing blend for 100times and the tapped volume was noted. It is expressed in g/ml and is given by formula: \( \rho_T = M / V_t \). Where, \( M \) is the mass of powder blend and \( V_t \) is the tapped volume.

Carr's Index is calculated by the given formula: \( \text{Carr's Index} = (\rho_T - \rho_B)/\rho_T \times 100 \). Where, \( \rho_T \) is tapped density and \( \rho_B \) is bulk density. Lastly Hausner's ratio is calculated by following formula: \( \text{Hausner's ratio}=\rho_T/\rho_B \). Where, \( \rho_T \) is tapped density and \( \rho_B \) is bulk density.15

Evaluation of Prepared Tablet

Prepared Rosuvastatin Calcium tablets were evaluated for organoleptic properties such as colour, odour and shape. Uniformity of weight was determined by taking 20 tablets randomly from each formulation and weighed collectively and average weight was calculated using digital balance. The individual weights were compared with the average weight for obtaining weight variation. Tablet hardness was measured using Monsanto hardness tester on 3 tablets randomly. Tablet thickness was evaluated on 10 tablets by the Vernier-caliper. Tablet Friability was determined by placing 20 pre-weighed samples of tablets in the Tablet Friabilator and was subjected to 100 revolutions. Tablets were dusted using a soft muslin cloth and reweighed. Compressed tablets should not lose more than 1% of their original weight. The drug content estimation was done by taking a quantity of crushed tablet powder equivalent to 10 mg of Rosuvastatin and dissolved in methanol; the solution was filtered through a Whatman filter paper no. 40 and absorbance was taken at 244 nm at UV visible spectrophotometer after appropriate dilution with methanol. The drug content was determined using calibration curve. The mean percent drug content was calculated as an average of three dimensions. The disintegration time of tablets was measured using D isintegration test apparatus in which one tablet was placed in each of the six tubes of the basket. The basket was immersed in water bath at 37 ± 2°C having phosphate buffer pH 6.8. The assembly is raised and lowered between 30 cycles per minute in the solution. The time in seconds required for complete disintegration was determined using a stop watch.

Comparative in-vitro Drug Dissolution Study

Prepared and marketed RVS immediate release tablets were subjected to dissolution study and then the dissolution profile was compared with each other. In vitro release studies were carried out using tablet dissolution test apparatus (USP type-II).16 Dissolution medium containing 900 ml of phosphate buffer pH 6.8 rotating at a speed of 75 rpm and temperature conditions at 37 ± 0.5°C was used in in-vitro dissolution studies. Aliquots, each of 5 ml, were withdrawn at 5, 10, 15, 20, 25 and 30 min and replenished by an equal volume of fresh dissolution medium to maintain sink conditions.17 The samples were filtered and analysed by using phosphate buffer pH 6.8 as blank and the absorbance taken at 240 nm. Drug concentration was calculated from the standard calibration curve.18
Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) analysis is done by using a Shimadzu DSC-60 (Shimadzu Instruments, Japan) differential scanning calorimeter. The sample were sealed in aluminium pans and heated at a constant rate 10°C/min over a temperature range of 80°C-300°C. An inert atmosphere was maintained with standard cooling chamber by purging nitrogen gas at a flow rate of 50 ml/min.19

RESULTS

Characterisation of Procured Drug

Procured RVS sample’s physical appearance is white amorphous powder without any specific odour, melting point observed was between 142-144°C. The FTIR spectrum shows major peaks at 3316.96 cm⁻¹, 1739.28 cm⁻¹, 330.78 cm⁻¹ and 2996.84 cm⁻¹. The λmax of the drug in methanol is obtained at 240 nm.

Characterisation of Marketed Tablet

Marketed Rosuvastatin Calcium tablet had a weight variation of 75±1.12 mg, hardness of 2.5±0.3 kg/cm², thickness of 2.56±0.05 mm, friability of 0.46±0.15%, drug content of 98.76±1.13% and disintegration time of 4.59±0.59 in min.

Stabilization of Rosuvastatin Calcium

Drug and excipients physical mixtures were studied for percent assay, colour and appearance at different storage conditions according to ICH guideline. The stability study data of samples kept at long term (25°C ± 2°C/60% RH ± 5% RH), intermediate (30°C ± 2°C/65% RH ± 5% RH) and accelerated (40°C ± 2°C/75% RH ± 5% RH) stability conditions are summarised in Table 1.

Identification of Critical Quality Attributes and Material Attributes

The material attributes selected from stability data are different ratios of xanthan gum and chitosan with constant drug concentration for stabilisation of the drug in formulation.

Table 1: Percent assay of samples studied at 25°C ±2°C/60% ±5%RH, 30°C ±2°C/65% ±5%RH and 40°C ±2°C/75% ±5%RH.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Samples</th>
<th>Percent Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 Days</td>
</tr>
<tr>
<td>1.</td>
<td>Pure RVS</td>
<td>101±1.23</td>
</tr>
<tr>
<td></td>
<td>25°C ±2°C/60% ±5%RH</td>
<td>100.04±1.23</td>
</tr>
<tr>
<td></td>
<td>30°C ±2°C/65% ±5%RH</td>
<td>100.2±1.23</td>
</tr>
<tr>
<td></td>
<td>40°C ±2°C/75% ±5%RH</td>
<td>99.98±1.05</td>
</tr>
<tr>
<td>2.</td>
<td>RVS + Xanthan gum</td>
<td>100.62±1.05</td>
</tr>
<tr>
<td></td>
<td>25°C ±2°C/60% ±5%RH</td>
<td>100.33±1.05</td>
</tr>
<tr>
<td></td>
<td>30°C ±2°C/65% ±5%RH</td>
<td>100.1±1.52</td>
</tr>
<tr>
<td></td>
<td>40°C ±2°C/75% ±5%RH</td>
<td>100.08±1.52</td>
</tr>
<tr>
<td>3.</td>
<td>RVS + Chitosan</td>
<td>100.06±1.52</td>
</tr>
<tr>
<td></td>
<td>25°C ±2°C/60% ±5%RH</td>
<td>100.1±1.47</td>
</tr>
<tr>
<td></td>
<td>40°C ±2°C/75% ±5%RH</td>
<td>100.16±1.47</td>
</tr>
<tr>
<td>4.</td>
<td>RVS + Guar gum</td>
<td>100.11±1.47</td>
</tr>
</tbody>
</table>

Design of Experiment

The Variables / factors and levels for applied design of experiment the coded values for the each independent and dependent variable are given in Table 2. The results of the assay after performing stability studies of the factorial batches prepared by applying design of experiments are summarised in Table 3. The significance of the design of experiment was analyzed statistically by (ANOVA) analysis of variance and the results are shown in Table 4. The response surface 3D plot of effect of concentration of natural stabilizers such as Xanthan gum and Chitosan on percent assay of drug is depicted in Figure 1.

Formulation of Tablet

The immediate release tablets were prepared by direct compression method using 12.5×8.5mm round shaped punches. The angle of repose of pre-compressed blend was 28.09 ± 1.2°. Bulk density and tapped density was found to be 0.323 ± 0.010 gm/cm³ and 0.354 ± 0.013 gm/cm³ respectively. Carr’s index of tablet blend of the drug was 10.41± 2.24% and Hausner’s ratio of tablet blend was found to be 1.11 ± 0.03. The prepared tablets were evaluated for various parameters such as weight variation i.e. 250± 1.12 mg, hardness 2.5± 0.25 kg/cm², thickness 3.83± 0.28 mm, drug content 95.76±1.2 %, friability 0.80± 0.04 % and in vitro disintegration time is 1.34± 0.5.

Comparative in vitro Drug Dissolution Study

The graphical representation of dissolution profile of comparative plot of marketed and prepared tablet is shown in Figure 2. It represents the graph of percent drug release with respect to time. The prepared Rosuvastatin tablet exhibited 93.02 ± 0.11% of drug release within 30 min as compared to 69.58 ± 1.62% of marketed tablet.

Differential Scanning Calorimetry

The DSC thermogram of pure drug, physical mixture of drug and Chitosan and final tablet blend was carried out and compared. Figure 3 shows thermal behaviour of pure drug component. The sharp endothermic peak appears at 134°C. The DSC thermogram of physical mixture of RVS + chitosan is shown in Figure 4.20
Characterisation of procured Rosuvastatin Calcium and excipients sample’s physical appearance is white amorphous powder without any specific odour, melting point observed was between 142-144°C. The FTIR spectrum of drug shows characteristic peaks of OH stretching at 3316.96 cm\(^{-1}\) and C=O stretching at 1739.28 cm\(^{-1}\) for carboxylic acid, aromatic amines C-N stretching at 1330.78 cm\(^{-1}\) and alkanes C-H stretching at 2996.84 cm\(^{-1}\) which corroborate with the literature, hence confirming the purity of the drug.

Table 2: Variables / factors and levels for applied design of experiment.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Variables / factors</th>
<th>Lower (-1)</th>
<th>Medium (0)</th>
<th>Upper (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Xanthan Gum (in mg) ([X_1])</td>
<td>60</td>
<td>100</td>
<td>140</td>
</tr>
<tr>
<td>2.</td>
<td>Chitosan (in mg) ([X_2])</td>
<td>60</td>
<td>100</td>
<td>140</td>
</tr>
</tbody>
</table>

Table 3: Composition of factorial batches and responses of prepared RVS physical mixtures at room temperature and accelerated stability conditions.

<table>
<thead>
<tr>
<th>No of Runs</th>
<th>Factor 1 ((X_{gum}) X_1)</th>
<th>Quantity (mg)</th>
<th>Factor 2 ((Chitosan) X_2)</th>
<th>Quantity (mg)</th>
<th>Response (% assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>+1</td>
<td>140</td>
<td>1</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>F2</td>
<td>+1</td>
<td>100</td>
<td>1</td>
<td>94</td>
<td>93</td>
</tr>
<tr>
<td>F3</td>
<td>-1</td>
<td>60</td>
<td>1</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>100</td>
<td>-1</td>
<td>88</td>
<td>87</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>100</td>
<td>-1</td>
<td>94</td>
<td>90</td>
</tr>
<tr>
<td>F6</td>
<td>-1</td>
<td>60</td>
<td>1</td>
<td>95</td>
<td>92</td>
</tr>
<tr>
<td>F7</td>
<td>-1</td>
<td>140</td>
<td>+1</td>
<td>96</td>
<td>95</td>
</tr>
<tr>
<td>F8</td>
<td>0</td>
<td>140</td>
<td>+1</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>60</td>
<td>-1</td>
<td>90</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 4: ANOVA for response surface linear model [Partial sum of squares - Type III].

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
<th>significant/ non-significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Model</td>
<td>241.67</td>
<td>2</td>
<td>120.83</td>
<td>50.58</td>
<td>0.0002</td>
<td>Significant</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>A-A Xanthan gum</td>
<td>13.50</td>
<td>1</td>
<td>13.50</td>
<td>5.65</td>
<td>0.0550</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>B-B Chitosan</td>
<td>228.17</td>
<td>1</td>
<td>228.17</td>
<td>95.51</td>
<td>&lt;0.0001</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Residual</td>
<td>14.33</td>
<td>6</td>
<td>2.39</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Correlation</td>
<td>256.00</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: The response surface (3D) plot of effect of concentration of natural stabilizers such as xanthan gum and chitosan on percent assay of drug.

Figure 2: Comparative dissolution profile of marketed and prepared RVS tablets.

Figure 3: DSC thermogram of pure RVS.

Figure 4: DSC thermogram of physical mixture of RVS and Chitosan.

DISCUSSION

Characterisation of procured Rosuvastatin Calcium and excipients sample’s physical appearance is white amorphous powder without any specific odour, melting point observed was between 142-144°C. The FTIR spectrum of drug shows characteristic peaks of OH stretching at 3316.96 cm\(^{-1}\) and C=O stretching at 1739.28 cm\(^{-1}\) for carboxylic acid, aromatic amines C-N stretching at 1330.78 cm\(^{-1}\) and alkanes C-H stretching at 2996.84 cm\(^{-1}\) which corroborate with the literature, hence confirming the purity of the drug. Calibration curve of drug was made in methanol. The absorbance of five replicate samples were recorded.
and the observed absorbance was processed for linear regression plot and the linear regression equation i.e. $y = 0.005x + 0.0116$ with correlation coefficient of $R^2 = 0.998$. Characterisation of marketed tablet of Rosuvastatin 100 mg tablet was done by purchasing the tablet from the local Market and its physical characterization was performed and all the parameters such as weight variation, hardness, thickness, friability, drug content and disintegration time were found to be within specified limits. For stabilization of drug among all the other natural stabilizers, drug + Chitosan combination showed maximum stabilization followed by drug + Xanthan Gum, drug + Guar gum and lastly pure drug, kept at long term, intermediate and accelerated stability conditions. According to QBD, pharmaceutical development includes identifying critical quality attributes (CQA) of the drug product and determining material attributes and defining the control strategy. As the study targets on stable formulation of Rosuvastatin, the CQA selected is the drug content in various combinations of the drug and stabilizers. The effect of natural stabilizers on stability of the drug was studied by applying factorial design. From the pre-formulation stability study it is found that the Chitosan and Xanthan gum gave maximum protection against drug degradation. Thus 3² factorial experimental design was applied using Chitosan and Xanthan gum with the drug. Based on the factorial design, 9 batches were prepared are analysed for response i.e. percent assay at both room temperature and accelerated stability conditions. From responses of nine batches B8 batch consisting of 140 mg of chitosan and 100 mg of Xanthan gum exhibited enhanced stabilisation. The F-value of 50.58 and probability value less than 0.0500 indicate that the model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. The “Predicted R-Squared” of 0.8754 is in reasonable agreement with the “Adjusted R-Squared” of 0.9253; i.e. the difference is less than 0.2. The response surface 3D plots shows that as the concentration of Chitosan and Xanthan Gum increases drug degradation decreases, thus enhanced stabilisation is achieved. From the factorial analysis by Design of experiment, batch F1 having maximum concentration of Chitosan was selected as the ratio of drug-stabilizer for the formulation of tablet. The angle of repose, Bulk density, Carr’s index, Hausner’s ratio and tapped density of pre-pressed blend was determined and the results show the blend possess good flow and compressibility properties. The weight variation, hardness, thickness, drug content, friability and in vitro disintegration time were within the acceptable specifications. The dissolution profile of marketed formulation was compared with prepared tablet after one month accelerated stability study. The prepared tablet exhibited enhanced drug dissolution as compared to marketed Rosuvastatin tablet. Thus prepared tablets showed better drug dissolution as compared to the marketed formulation. The Differential Scanning thermogram of pure drug, physical mixture of drug and Chitosan and final tablet blend was carried out and compared. The thermal behaviour of pure drug component was studied and the sharp endothermic peak at 134°C is characteristic peak of pure drug. The thermogram of physical mixture of drug and Chitosan demonstrates a slight shift in endothermic peak towards higher side due to the colligative property of mixture of excipients.

ACKNOWLEDGEMENT

We express our sincere thanks to Honourable Chairman Maulana Azad Education Trust’s Padmashree Mrs. Fatma Rafiq Zakaria, for providing all the necessary facilities for carrying out research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UV: Ultraviolet; USP: United States Pharmacopoeia; mm: Millimeter; cm: Centimeter; mg: Milligram; Kg: Kilogram; λ: Wavelength.

REFERENCES


Article History: Submission Date : 28-01-2020; Revised Date : 14-03-2020; Acceptance Date : 07-04-2020.