CHAPTER-4

Development and Validation of Stability Indicating HPLC Methods for Simultaneous Quantification of API’s in Pharmaceutical Dosage Form
Section 4.1
Simultaneous Quantification of Famotidine and Ibuprofen in Pharmaceutical Dosage by Using Validated Stability Indicating LC Method

4.1.1 Drug profile
Famotidine

Chemical name: 3-([2-(diaminomethyleneamino) thiazol-4-yl]methylthio)-N-sulfamoylpropanimidamide
Molecular formula: C₈H₁₅N₇O₂S₃
Molecular weight: 337.5
Description: Off white powder
Properties: In water: very slightly soluble.
In methanol: slightly soluble,
In glacial acetic acid: Freely soluble,
Therapeutic category: H₂-blockers

Fig. 4.1.F-1: Famotidine chemical structure
Ibuprofen

Chemical name: (RS) -2-9-(4-(2-methyl propyl) phenyl) propionic acid

Molecular formula: C_{13}H_{8}O_{2}

Molecular weight: 206.29

Description: Off white powder

Properties: Slightly soluble in water

Therapeutic category: Antiplatelet
4.1.2. Introduction

Famotidine is chemically 3-([2-(diaminomethyleneamino) thiazol-4-yl]methylthio)-N-sulfamoylpropanimidamide (Fig. 4.1.F-1) [1]. Famotidine is prescribed for peptic ulcer treatment and gastro esophageal disease. Famotidine belongs to therapeutic category of histamine H$_2$-receptor antagonist. Famotidine acts by blocking the histamine action on stomach cells and resulting in decreasing the production of acid. Famotidine is useful in treatment of duodenal ulcers, healing of stomach and reducing ulcer pain. Recurrence of ulcers is controlled by Famotidine in low doses in long duration of time. It is useful in treatment of healing ulceration, inflammation of the esophagus and heartburn. [2-6].

Ibuprofen is chemically (RS)-2-9(4-(2-methylpropyl)phenyl) propionic acid (Fig. 4.1.F-1). Ibuprofen is used for in the treatment of fever, primary and arthritis. It belongs to non-steroidal anti-inflammatory drug category [7-9].

4.1.3 Literature survey

Analysis of related impurities of Famotidine in pharmaceutical preparation was reported. Analysis time was within 10 minutes. Linear range was 1.5 to 100 μg/ml [11]. Quantitative determination by using HPLC for Famotidine was also reported [12]. Analysis of Famotidine and its degradation products by using HPLC were reported [13]. Famotidine was quantified in plasma by using RP-HPLC method was reported. [14]. Quantitative analysis of Famotidine by using IS in human plasma was reported [15].

Spectrophotometric method for estimation of Ibuprofen was reported [16].

Ibuprofen in combination with pseudoephedrine hydrochloride was analyzed using CE also reported [17]. Determination of Ibuprofen in ointments by RP-HPLC was reported [18].

A literature reveals that there is no HPLC method which is used to quantify both analyte simultaneously.
4.1.4 Present work

Material and reagents

Famotidine and Ibuprofen drug standards were provided by Hetero drugs, India. Their combine formulated tablet “Duexis” were purchased from market. Acetonitrile, sodium hydroxide and hydrochloric acid was obtained from Rankem (India). Ammonium acetate was purchased from Rankem laboratories, India. Hydrogen Peroxide procured from Acros Organics, USA. HPLC grade water was used to perform all experiments.

Instrumental conditions

Agilent-1100 HPLC was used for analysis. Analytical column used was YMC Cyano, (150 × 4.6 mm, 5 µm), at 30 °C oven temperature. Mobile phase consist of 50 mM ammonium acetate and 100% mobile phase B is acetonitrile. Gradient run starting from A/B = 95/05 upto 2 minute and changing to A/B = 80/20 to 10 minute. The condition was maintained upto 15 minute and the concentration was returned to A/B = 95/05 after 3 minute and hold for 2 minute. Instrument setting for mobile phase flow was at 1.0 ml/min. Famotidine and Ibuprofen was analyzed by using UV detector at 270 nm. 20µl injection volume was injected. Data processing was performed using Agilent chemstation software.

Standard stock solution

Accurately weighed and transferred about 27 mg Famotidine (99.13%) and 800 mg Ibuprofen (99.01%) into a volumetric flask (100 ml) and dissolved with diluent. Diluent used is acetonitrile and water 50:50; v/v. Standard solution concentration was achieved by stock diluting with diluent.

Preparation of sample

Tablet powder containing 27 mg of Famotidine and 800 mg of Ibuprofen was transferred to flask (100 ml). to this added about 50 ml of diluent to dissolve by means of sonication and make up with diluent. Centrifuge this solution. Diluted this solution ten times to get assay concentration solution.

Forced degradation study
Stress degradation experiments for tablet were performed to define specificity.

**Acidic degradation**

Weighed and transfer tablet powder containing 27 mg of Famotidine and 800 mg of Ibuprofen to volumetric flask (100 ml). To this added 10 ml of 0.1N HCl, heated at 60 °C for 3 hrs on water bath. Neutralized with 0.1N NaOH solution diluted with diluent upto mark. Further dilution was made to get the assay level concentrations of both analytes.

**Alkali degradation**

Weighed and transfer tablet powder containing 27 mg of Famotidine and 800 mg of Ibuprofen to volumetric flask (100 ml). Added diluent to dissolve the drug and added 10 ml 0.1N NaOH solution, kept at 60 °C for 3 hrs on water bath. Neutralized with 0.1N hydrochloric acid make up with diluent. Further dilution was made to get the assay level concentrations of both analytes.

**Oxidative degradation**

Weighed and transfer tablet powder containing 27 mg of Famotidine and 800 mg of Ibuprofen to 100 ml flask. 30 ml diluent and 5 ml 30% hydrogen peroxide was added to it and heated the mixture at 60 °C on water bath for 1 hour duration. Allow attending room temperature. Further dilution was made to get the assay level concentrations of both analytes.

**Thermal degradation**

Powder was exposed at 90 °C for 24 hrs. Dilution was made to get the assay level concentrations of both analytes.

**UV-Short degradation**

Powder was kept in UV chamber to 254 nm for one day. Dilution was made to get the assay level concentrations of both analytes.

**UV-Long degradation**

Powder was kept in UV chamber to 366 nm for one day. Dilution was made to get the assay level concentrations of both analytes.
Peak purity was determined for Famotidine and Ibuprofen

4.1.5 Method validation

For each compound, method is validated according to ICH guidelines [19].

System suitability

To evaluate system suitability of the method six injections of standard solution was injected. Determine the system suitability parameters.

Precision

For system precision, injected standard solution six time at working level concentrations of Famotidine and Ibuprofen. The mean, SD and percentage RSD of peak area were calculated. For method precision purpose percentage RSD of for six tablet solution was calculated. Ruggedness was evaluated by analyzing six tablet solution and percentage RSD was determined by different analyst.

Linearity

Analyzed seven concentrations of Famotidine and Ibuprofen mix standard solutions making triplicate injections for each concentration. Calibration standards were prepared in the range 0.4 to 40 µg/ml for Famotidine and 0.7 to 1200 µg/ml for Ibuprofen.

Accuracy/recovery

To establish accuracy of method, spike preparation with 80, 100 and 120% of working level of Famotidine and Ibuprofen was prepared. Each spike level was prepared in triplicate.

Quantification limit and Detection limit

Calibration curve was constructed between analyte concentration and response of samples to determining the Detection limit and Quantification limit

Detection limit and Quantification limit were estimated by formula

Detection limit = Cd x Styx /b
Quantitation limit = Cq x Styx /b
Standard solution was prepared at detection and quantification level and precision of peak area was verified.

**Robustness**

Chromatographic parameters were purposely changed and assay, system suitability parameters and percentage RSD of peak area were evaluated. Flow rate was altered to ±0.1 units from set value. Column temperature varied ±2°C of set point. Other chromatographic condition kept remains constant. Organic strength impact was studied by changing initial composition of buffer and acetonitrile in ratio 97:0, v/v and 93:07, v/v. Tablet assayed in triplicate at each experiment. UV detector ruggedness was performed at detection wavelength 266 nm and 274 nm.

### 4.1.6 Results and discussion

**Chromatographic conditions optimization**

Famotidine and Ibuprofen are completely different in their structural polarity having pKa values about 7.1 and 4.5 respectively. Objective of chromatographic parameters optimization was to achieve optimal resolution between both analyte peaks, tailing of both peaks should be less than 2. From development trials it was clear that Cyano stationary phase have good selectivity than other stationary phases. The chromatographic separation of both analytes from its degradants was achieved using YMC Cyano, (150 × 4.6 mm, 5 µm) YMC INC. USA column, when employed isocratic mobile phase but retention time for Ibuprofen was too long. 0.05 M ammonium acetate and acetonitrile using gradient is an optimal mobile phase for separation of degradant from main peak. Column temperature was 30°C. Column saturation was performed at 1.0 ml/min. Retention time of Famotidine and Ibuprofen peaks were about 7.0 and 9.0 minute respectively.

**Stress degradation study**

The stress conditions of the study were designed to get approximately 5 to 20% degradation of the drug product [20]. Avoid overstressing the samples which leads to generate irrelevant degradants. [21]. All degradation samples were analyzed.

Table 4.1.T-2 represents degradation condition, degradation percentage and assay in all stress conditions tested. Fig. 4.1.F-3 to 4.1.F-7 showed the
chromatograms of tablet solution, diluent, placebo solution, Famotidine specificity solution and Ibuprofen specificity solution respectively. Fig. 4.1.F-8 to 4.1.F-13 showed base hydrolysis blank, base hydrolysis degradation of tablet, oxidative blank, oxidative degradation of tablet, acid hydrolysis blank and acid hydrolysis degradation of tablet respectively. Spectral analysis report of Famotidine peak in all stress conditions is given in Fig. 4.1.F-14 to 4.1.F-16. Spectral analysis report of Ibuprofen peak is given in Fig. 4.1.F-17 to 4.1.F-19.

**Results of method validation**

Famotidine and Ibuprofen peaks system suitability parameters meets all acceptance criteria. Results are given in table 4.1.T-1.

In repeatability experiment Percentage RSD of assay determination for Famotidine and Ibuprofen was 0.8 and 1.1 respectively. Percentage RSD in the study of ruggedness (intermediate precision) for Famotidine and Ibuprofen was 0.7 and 1.3 respectively. Data is given in table 4.1.T-3.

Accuracy results shows the average recovery for both analytes was 100 ± 2% having percentage RSD less than 2.0. Results are tabulated in table 4.1.T-4.

Linear graph was obtained in range 0.7 to 1200 μg/ml for Ibuprofen and 0.4 to 40 μg/ml of Famotidine. Observed LOQ concentrations for Famotidine and Ibuprofen were 0.4 and 0.7 μg/ml respectively. Observed LOD concentrations for Famotidine and Ibuprofen were 0.13 and 0.22 μg/ml respectively. Data is tabulated in table 4.1.T-3.

In robustness study the all deliberated changes % assay of both analytes was 100 ± 2%. Results are tabulated in table 4.1.T-5.
4.1.7 Figures: Method validation chromatograms of Famotidine and Ibuprofen Tablet

Fig. 4.1.3: Tablet solution chromatogram

Fig. 4.1.4: Chromatogram of diluent
Fig. 4.1.5: Chromatogram of placebo solution

Fig. 4.1.6: Famotidine solution chromatogram
Fig 4.1.7: Chromatogram of Ibuprofen solution

Fig 4.1.8: Alkali blank chromatogram
Fig 4.1.9: Alkali hydrolysis degradation of the tablet chromatogram

Fig 4.1.10: Oxidative blank chromatogram
Fig 4.1.11: Oxidation degradation of tablet chromatogram

Fig 4.1.12: Acid blank chromatogram
Fig 4.1.13: Acid hydrolysis degradation of tablet chromatogram

Fig 4.1.14: Peak purity spectrum of Famotidine in alkali hydrolysis
Fig 4.1.15: Peak purity spectrum of Famotidine in oxidation

Fig 4.1.16: Peak purity spectrum of Famotidine in acid hydrolysis
Fig 4.1.17: Peak purity spectrum of Ibuprofen in alkali hydrolysis

Fig 4.1.18: Peak purity spectrum of Ibuprofen in oxidation
Fig 4.1.19: Peak purity spectrum of Ibuprofen in acid hydrolysis
4.1.8 Tables: Method validation data of Famotidine and Ibuprofen tablet

Table 4.1.T-1: System suitability data

<table>
<thead>
<tr>
<th>Analyte</th>
<th>RT (Min.)</th>
<th>Capacity factor</th>
<th>Resolution</th>
<th>Theoretical Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Famotidine</td>
<td>6.9</td>
<td>3.51</td>
<td>-</td>
<td>7863</td>
<td>1.2</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>8.8</td>
<td>4.47</td>
<td>5.3</td>
<td>9591</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 4.1.T-2: Forced degradation data

<table>
<thead>
<tr>
<th>condition</th>
<th>Peak purity</th>
<th>Assay (%)</th>
</tr>
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<tr>
<td>Acidic (60 °C/3 hrs)</td>
<td>999.368</td>
<td>999.868</td>
</tr>
<tr>
<td>Alkali (60 °C/3 hrs)</td>
<td>999.523</td>
<td>999.731</td>
</tr>
<tr>
<td>Oxidative (60 °C/1hrs)</td>
<td>999.725</td>
<td>999.699</td>
</tr>
<tr>
<td>Thermal (90 °C/24 hrs)</td>
<td>999.829</td>
<td>999.629</td>
</tr>
<tr>
<td>UV-short (24 hrs)</td>
<td>999.925</td>
<td>999.801</td>
</tr>
<tr>
<td>UV-long (24 hrs)</td>
<td>999.919</td>
<td>999.933</td>
</tr>
</tbody>
</table>
Table 4.1.T-3: Validation summary

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Famotidine</th>
<th>Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Precision (% RSD)</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Intermediate Precision (% RSD)</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.13</td>
<td>0.22</td>
</tr>
<tr>
<td>Linearity range (µg/ml)</td>
<td>0.4 to 40</td>
<td>0.7 to 1200</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 7.0212x + 3.1592</td>
<td>y = 5.2896x + 2.9358</td>
</tr>
<tr>
<td>r²</td>
<td>0.9992</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

Table 4.1.T-4: Accuracy study results

<table>
<thead>
<tr>
<th>% Level</th>
<th>Spiked Amount (mg)</th>
<th>Found Amount (mg)</th>
<th>Mean Recovery (%)</th>
<th>RSD (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Famotidine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>21.45</td>
<td>21.32</td>
<td>99.19</td>
<td>1.1</td>
</tr>
<tr>
<td>100</td>
<td>27.56</td>
<td>26.99</td>
<td>99.65</td>
<td>0.7</td>
</tr>
<tr>
<td>120</td>
<td>32.96</td>
<td>32.56</td>
<td>99.16</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>643.12</td>
<td>632.11</td>
<td>98.39</td>
<td>1.2</td>
</tr>
<tr>
<td>100</td>
<td>804.23</td>
<td>801.59</td>
<td>99.13</td>
<td>0.9</td>
</tr>
<tr>
<td>120</td>
<td>965.23</td>
<td>960.12</td>
<td>98.99</td>
<td>0.8</td>
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### Table 4.1.T-5: Robustness study data

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<tr>
<th>Parameter</th>
<th>Variation</th>
<th>Rs</th>
<th>Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fam.</td>
</tr>
<tr>
<td>Flow rate (± 10%)</td>
<td>0.9 ml/min.</td>
<td>5.3</td>
<td>99.12</td>
</tr>
<tr>
<td></td>
<td>1.1 ml/min.</td>
<td>5.1</td>
<td>99.10</td>
</tr>
<tr>
<td>Organic modifier (± 2%)</td>
<td>3 ml</td>
<td>4.4</td>
<td>98.97</td>
</tr>
<tr>
<td></td>
<td>7 ml</td>
<td>5.4</td>
<td>99.30</td>
</tr>
<tr>
<td>Column Oven temperature (± 2 °C)</td>
<td>28 °C</td>
<td>5.3</td>
<td>99.89</td>
</tr>
<tr>
<td></td>
<td>32 °C</td>
<td>5.1</td>
<td>99.20</td>
</tr>
<tr>
<td>Wavelength ((± 4 nm)</td>
<td>266 nm</td>
<td>5.0</td>
<td>99.78</td>
</tr>
<tr>
<td></td>
<td>274 nm</td>
<td>5.0</td>
<td>99.32</td>
</tr>
</tbody>
</table>

Rs - resolution
Section 4.2
Simultaneous Quantification of Sitagliptin and Simvastatin in Dosage form by Using Stability Indicating LC Method

**Fig. 4.2.F-1:** Chemical structure of Sitagliptin

4.2.1 Drug profile

Sitagliptin:

**Chemical name:** (R2)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine

**Molecular formula:** C_{16}H_{15}F_{6}N_{5}O

**Molecular weight:** 407.31

**Description:** Off white powder

**Properties:** Freely soluble in water, N,N dimethyl formamide

In methanol slightly soluble.

**Therapeutic category:** Antidiabetic
Simvastatin

Chemical name: \((1S,3R,7S,8S,8aR)-8\{-2\{(2R,4R)-4\text{-hydroxy\text{-}6-oxoxan-2\text{-}yl}\text{-}ethyl\}\text{-}3,7\text{-dimethyl\text{-}1,2,3,7,8,8a\text{-hexahydranaphthalen\text{-}1\text{-}yl}\text{-}2,2\text{-dimethylbutanoate}\}

Molecular formula: \(C_{25}H_{38}O_5\)

Molecular weight: 418.57

Description: Off white powder

Properties: Water insoluble

Therapeutic category: Cardiovascular

Fig. 4.2.F-2: Chemical structure of Simvastatin
2.2. Introduction

Sitagliptin is chemically known as (R2)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine. It has very high selectivity towards DPP-4. Sitagliptin is useful in the management of diabetes (type 2). Secretion of hormones glucagon and gastric inhibitory polypeptide levels were increased by Sitagliptin. For lowering HbA1c and postprandial glucose sitagliptin is useful with combination of other oral drugs. For treatment of hyperglycemic in patient, sitagliptin works by increasing the level of insulin, which is useful for inhibition of glucagon secretion [22-25].

Simvastatin is chemically (1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxoxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl2,2dimethylbutanoate. Simvastatin mode of action is inhibiting the 3-hydroxy-3-methylglutarylcoenzyme reductase. The biosynthesis of cholesterol is controlled by this enzyme. Lipoproteins (high and low density) were oxidized [26]. LDL cholesterol and triglyceride levels in blood were controlled by drug class “statin” [27]. Now day’s these drugs are more favorable to prescribe [28]. β-hydroxy acid is active form of these drugs, Formation of β-hydroxy acid is due to lactone ring cleavage. In body, these metabolite hydrolyzed to its hydroxyl acid pharmacophore by enzymatic action [29].

4.2.3. Review of Literature

Metformin and Sitagliptin was quantified using C18 column (Phenomenex, 250 × 4.6 mm, 5 μm. The UV detector was kept at 252 nm. linear response was observed over the concentration range for Sitagliptin and Metformin. The recovered amount of Sitagliptin and Metformin was 100.27 and 100.73% respectively [30].

A selective RP-HPLC method for Sitagliptin using C18 column (25 cm × 0.46 cm, 5μm) was reported. Drug was well resolved from all its stress degradations [31].

Sitagliptin quantification in bulk drug and in formulation was also reported by extractive spectrophotometric method. [32].

LC-MS method involving sample extraction was reported for the analysis of Simvastatin. Method is applied for analysis of drugs in human plasma.[33].
Determination of Simvastatin (I) by using LC-MS was reported. Analysis was performed on C18 column [34].

Determination of Simvastatin in presence of other statins was reported for analysis of Simvastatin levels in patients [35].

Two UV methods also reported for Simvastatin estimation in bulk drug and in tablets. The methods involve complexometric reaction by using cerium (IV) [36].

4.2.4. Present work

Material and reagents

Sitagliptin and Simvastatin drug standards were provided by Biocon Ltd. India. Their combine formulated tablet “Juvisync” were purchased from market. Acetonitrile was procured Rankem, sodium hydroxide and concentrated hydrochloride acid (37% purity) was procured from Rankem. Phosphate buffer, Hydrogen Peroxide was obtained from Rankem, Mumbai. HPLC grade quality water used to perform the study.

Instrumental setup

Agilent-1100 HPLC system was used for analysis. Analyte separation was achieved by using YMC Cyano, (150 x 4.6 mm x 5µ) YMC Ltd. Column temperature was 40 °C. Acetonitrile and buffer in the ratio of 35: 65, v/v used as eluent (mobile phase). Buffer used was 0.01 M KH2PO4 solution in water adjusted pH 7.0 with 0.01M K2HPO4 solution in water. UV detector was used at 254 nm and Mobile phase flow rate set at 1.2 ml/min. To quantify Sitagliptin and Simvastatin. Injection volume was kept 20 µl. Agilent chemstation software was used to process the chromatograms.

Standard stock solution

Weighed and transferred about 400 mg Sitagliptin and 160 mg Simvastatin to a volumetric flask (100 ml) and added diluent to dissolve the content. Diluent used was acetonitrile and water as 50:50, v/v. A working level solution (160 µg/ml and 400 µg /ml Simvastatin and Sitagliptin respectively) was prepared from stock solution.
Chapter 4

Preparation of sample

Weighed and transferred powder of tablet containing 400 mg of Sitagliptin and 160 mg of Simvastatin to volumetric flask (100 ml). Added 50 ml of diluent to dissolve and make up the volume with diluent. After centrifuging this solution, take 5 ml to 50 ml with diluent.

Forced degradation study

Specificity

Stress study of tablet was carried in specificity. Tablet powder was subjected to acidic, basic hydrolysis, oxidation, thermolysis and photolysis.

Acidic degradation

Weighed and transfer tablet powder containing 400 mg of Sitagliptin and 160 mg of Simvastatin to volumetric flask (100 ml). To this added 10 ml of 0.1N HCl, heated at 70°C for 3 hrs on water bath. Neutralized with 0.1N NaOH solution diluted with diluent upto mark. Further dilution was made to get the assay level concentrations of both analytes.

Alkali degradation

Weighed and transfer tablet powder containing 400 mg of Sitagliptin and 160 mg of Simvastatin to volumetric flask (100 ml). Added diluent to dissolve the drug and added 10 ml 0.1N NaOH solution, kept at 70°C for 30 Minutes on water bath. Neutralized with 0.1N HCl and diluted up to mark. Further dilution was made to get the assay level concentrations of both analytes.

Oxidative degradation

Weighed and transfer tablet powder containing 400 mg of Sitagliptin and 160 mg of Simvastatin to 100 ml flask. 30 ml diluent and 5 ml 1.0% hydrogen peroxide was added to it and heated the mixture at 70°C on water bath about 30 Minute duration. Allow attending room temperature. Further dilution was made to get the assay level concentrations of both analytes.

Thermal degradation

Powder was exposed at 70°C for 30 Minute. Dilution was made to get the assay level concentrations of both analytes.
UV-Short degradation

Powder was kept in UV chamber to 254 nm for one day. Dilution was made to get the assay level concentrations of both analytes.

UV-Long degradation

Powder was kept in UV chamber to 366 nm for one day. Dilution was made to get the assay level concentrations of both analytes.

Peak purity of Sitagliptin and Simvastatin peaks were determined.

4.2.5. Method validation

System suitability

To evaluate suitability of system standard solution was injected six times for evaluation of system suitability

System suitability parameters were calculated from the standard solution chromatogram.

Precision

For system precision, injected standard solution six time at working level concentrations of Sitagliptin and Simvastatin. The mean, SD and percentage RSD of peak area were calculated. For method precision purpose percentage RSD of for six tablet solution was calculated. Ruggedness was evaluated by analyzing six tablet solution and percentage RSD was determined by different analyst.

Linearity

Seven concentrations of Sitagliptin and Simvastatin mix standard solutions in triplicate were analyzed for linearity. 1.0 mg/ml Sitagliptin stock and 0.4 mg/ml of Simvastatin stock were prepared and linearity levels were prepared form detection concentration to 200%.

Accuracy/Recovery

For recovery, known amount of drugs were spiked in placebo at 100 ± 20 % of working level of Sitagliptin and Simvastatin. Each level was prepared in triplicate.
Quantification limit and Detection limit

Calibration curve was constructed between analyte concentration and response of samples to determining the Detection limit and Quantification limit.

Detection limit and Quantification limit were estimated using formula:

Detection limit = Cd x Syx /b

Quantitation limit = Cq x Syx /b

Standard solution was prepared at detection and quantification level and precision of peak area was verified.

Robustness

Chromatographic parameters were purposely changed and assay, system suitability parameters and percentage RSD of peak area were evaluated. Flow rate robustness was studied at ± 0.1 ml/min. Oven temperature effect was studied at ±2 °C of set point. The effect of organic composition in mobile phase was investigated by changing initial composition of acetonitrile and buffer (30:70, v/v) and (40:60, v/v). Tablet was assayed in triplicate at each deliberated change. UV detector ruggedness was performed at detection wavelength 254 ± 4 nm.
4.2.6. Result and Discussion

Chromatographic conditions optimization

Sitagliptin and Simvastatin are completely different in their structural polarity. To develop stability indicating assay method, resolution between both analyte peaks from other degradants is minimum requirement.

Main target for chromatographic parameter optimization was to get optimal separation between both analyte peaks, tailing factor should be not more than 2, RT up to 10 minutes. From development trials, Cyano stationary phase found having good selectivity than other column stationary phases. Separation of both analytes peaks and degradant peaks was achieved using YMC Cyano, (150 × 4.6 mm) YMC INC. USA column, with 5 µm particles. Buffer used was 0.01 M KH₂PO₄ solution in water adjusted pH 7.0 with 0.01M K₂HPO₄ solution in water. Mobile phase was composed of acetonitrile and buffer as 65:35, v/v. Mobile phase flow rate setting is kept at 1.2 ml/min. and column temperature 40 °C. Typical retention time of Sitagliptin and Simvastatin peaks is about 5.0 and 9.0 minutes respectively.
**Forced degradation results**

The stress conditions of the forced degradation study were designed to get approximately 5 to 20% degradation of the drug product [20]. Avoid overstressing the samples which leads to generate irrelevant degradants. [21]. All degradation samples were analyzed.

In oxidative, alkali hydrolysis and acid hydrolysis, thermolysis and photolysis degradation was observed. Table 4.1.T-2 indicates the stress conditions percentage degradation, peak purity and assay of both analytes. Fig. 4.2.F-3 to 4.1.F-6 shows the chromatograms of tablet solution, diluent, Sitagliptin standard and Simvastatin standard solution.

Fig. 4.2.F-7 to 4.1.F-15 shows the chromatograms base hydrolysis blank, base hydrolysis degradation of tablet, oxidation degradation blank, oxidation degradation of tablet, acid hydrolysis blank, acid hydrolysis degradation of tablet, thermal degradation of tablet and photolytic degradation of tablet respectively. Fig 4.2.F-16, Fig 4.2.F-18 shows spectral analysis report for Simvastatin in attempted stress conditions. Fig 4.2.F-19 to Fig 4.2.F-21 shows spectral analysis report for Simvastatin in attempted stress conditions.
Results of method validation

Sitagliptin and Simvastatin peaks system suitability data are within acceptance criteria. Results are tabulated in table 4.2.T-1.

Percentage RSD in method precision for Sitagliptin and Simvastatin was 1.1 and 0.9 respectively. RSD in the study of ruggedness (intermediate precision) for Sitagliptin and Simvastatin was 0.5 and 0.8 respectively. The results of the recover experiment showed that the average recovery for both analytes was 100 ± 2% having percentage RSD less than 2.0. Data is given in table 4.2.T-4.

Linear response was observed in concentration range 1.5 to 600 μg/ml for Sitagliptin and 0.9 to 240 μg/ml for Simvastatin. Correlation coefficient for Sitagliptin and Simvastatin was 0.9991 and 0.9990 respectively. Observed LOQ for Sitagliptin and Simvastatin were 1.5 μg/ml and 0.9 μg/ml respectively. For Sitagliptin and Simvastatin observed LOD concentration were 0.5 μg/ml and 0.3 μg/ml respectively. Data is tabulated in table 4.2.T-3.

In robustness study, at all deliberated changes the percentage assay of both analytes was 100 ± 2% which prove robustness of analytical method. Data is given in table 4.2.T-5.
4.2.7 Figures: Method validation chromatograms of Sitagliptin and Simvastatin tablet

Fig. 4.2.3: Tablet solution chromatogram

Fig. 4.2.4: Chromatogram of diluent
**Fig. 4.2.5:** Sitagliptin solution chromatogram

**Fig 4.2.6:** Simvastatin solution chromatogram
Fig 4.2.7: Alkali blank chromatogram

Fig 4.2.8: Alkali hydrolysis degradation of tablet chromatogram
Fig 4.2.9: Chromatogram oxidative blank

Fig 4.2.10: Chromatogram of oxidative degradation of tablet
Fig 4.2.11: Acid blank chromatogram

Fig 4.2.12: Acid hydrolysis degradation of tablet chromatogram
Fig 4.2.13: Photolysis (short) degradation of tablet chromatogram

Fig 4.2.14: Photolysis (long) degradation of tablet chromatogram
Fig 4.2.15: Thermal degradation of tablet chromatogram

Fig 4.2.16: Peak purity spectrum of Sitagliptin in alkali hydrolysis.
Fig 4.2.17: Peak purity spectrum of Sitagliptin in oxidation

Fig 4.2.18: Peak purity spectrum of Sitagliptin in acid hydrolysis
Fig 4.2.19: Peak purity spectrum of Simvastatin in alkali hydrolysis

Fig 4.2.20: Peak purity spectrum of Simvastatin in oxidation
**Fig 4.2.21:** Peak purity spectrum of Simvastatin in acid hydrolysis

**Fig 4.2.21:** Peak purity spectrum of Simvastatin in UV short degradation
Fig 4.2.22: Peak purity spectrum of Simvastatin in UV long degradation
4.2.8 Tables: Method validation data of Sitagliptin and Simvastatin tablet

**Table 4.2.T-1: System suitability data**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>RT (Min.)</th>
<th>Capacity factor</th>
<th>Resolution</th>
<th>Theoretical Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitagliptin</td>
<td>5.3</td>
<td>2.2</td>
<td>-</td>
<td>9651</td>
<td>1.3</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>8.8</td>
<td>4.1</td>
<td>8.0</td>
<td>16521</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**Table 4.2.T-2: Forced degradation data**

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Peak purity</th>
<th>Assay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STG</td>
<td>SMT</td>
</tr>
<tr>
<td>Acidic (0.1NHCl/70°C/3 hrs)</td>
<td>999.156</td>
<td>999.167</td>
</tr>
<tr>
<td>Alkali (0.1N NaOH/70°C/30 Min.)</td>
<td>999.511</td>
<td>999.481</td>
</tr>
<tr>
<td>Oxidative(1% H₂O₂/70°C/30 Min.)</td>
<td>999.801</td>
<td>999.792</td>
</tr>
<tr>
<td>Thermal (70 °C/30 Min.)</td>
<td>999.623</td>
<td>999.655</td>
</tr>
<tr>
<td>UV-short (24 hrs)</td>
<td>999.702</td>
<td>999.601</td>
</tr>
<tr>
<td>UV-long (24 hrs)</td>
<td>999.312</td>
<td>999.945</td>
</tr>
</tbody>
</table>
### Table 4.2.T-3: Validation summary

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sitagliptin</th>
<th>Simvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Precision (% RSD)</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Intermediate Precision (% RSD)</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Linearity range (µg/ml)</td>
<td>1.5 to 600</td>
<td>0.9 to 240</td>
</tr>
<tr>
<td>r²</td>
<td>0.9991</td>
<td>0.9990</td>
</tr>
</tbody>
</table>

### Table 4.2.T-4: Accuracy study results

<table>
<thead>
<tr>
<th>% Level</th>
<th>Spiked Amount (mg)</th>
<th>Found Amount (mg)</th>
<th>Mean Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sitagliptin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>320.12</td>
<td>318.25</td>
<td>99.42</td>
<td>1.1</td>
</tr>
<tr>
<td>100</td>
<td>402.23</td>
<td>396.88</td>
<td>98.67</td>
<td>1.5</td>
</tr>
<tr>
<td>120</td>
<td>485.12</td>
<td>477.12</td>
<td>98.35</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Simvastatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>129.05</td>
<td>127.26</td>
<td>98.61</td>
<td>1.5</td>
</tr>
<tr>
<td>100</td>
<td>160.56</td>
<td>157.91</td>
<td>98.34</td>
<td>0.9</td>
</tr>
<tr>
<td>120</td>
<td>190.22</td>
<td>188.20</td>
<td>98.94</td>
<td>1.0</td>
</tr>
<tr>
<td>Parameter</td>
<td>Variation</td>
<td>Rs</td>
<td>Assay (%)</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------</td>
<td>----</td>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>STG</td>
</tr>
<tr>
<td>Flow rate (± 10%)</td>
<td>1.1 ml/min.</td>
<td>8.6</td>
<td>99.50</td>
<td>99.19</td>
</tr>
<tr>
<td></td>
<td>1.3 ml/min.</td>
<td>8.2</td>
<td>99.11</td>
<td>99.25</td>
</tr>
<tr>
<td>Organic modifier (± 5%)</td>
<td>30 ml</td>
<td>9.4</td>
<td>99.22</td>
<td>98.99</td>
</tr>
<tr>
<td></td>
<td>40 ml</td>
<td>8.1</td>
<td>99.37</td>
<td>99.09</td>
</tr>
<tr>
<td>Column Oven temperature (± 2°C)</td>
<td>38 °C</td>
<td>9.0</td>
<td>99.81</td>
<td>98.88</td>
</tr>
<tr>
<td></td>
<td>42 °C</td>
<td>8.0</td>
<td>99.25</td>
<td>98.92</td>
</tr>
<tr>
<td>Wavelength ((± 4 nm)</td>
<td>250 nm</td>
<td>8.5</td>
<td>99.03</td>
<td>99.11</td>
</tr>
<tr>
<td></td>
<td>258 nm</td>
<td>8.5</td>
<td>99.10</td>
<td>99.51</td>
</tr>
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</table>

Rs – resolution
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lipophilicities, solubility’s, structure pharmacological considerations of
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