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Simultaneous Estimation of Sitagliptin Phosphate Monohydrate and Metformin Hydrochloride in Bulk and Pharmaceutical Formulation by RP-HPLC

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ABSTRACT

A new simple, accurate, precise and reproducible Reverse Phase-High Performance Liquid Chromatography method was developed for the simultaneous estimation of Sitagliptin and Metformin in bulk as well as in pharmaceutical dosage form using Symmetry C18 column (4.6 x 150mm, 3.5µm, Make: XTerra) in isocratic mode. The mobile phase was prepared by using Potassium Dihydrogen Phosphate and Acetonitrile in different ratio at different pH range. Several trials were performed and it was found that ratio of 65:35 of Potassium Dihydrogen Phosphate and Acetonitrile respectively was shown a good peak and the pH of the Buffer was adjusted to 5.8 by using Sodium Hydroxide. The detection was carried out at 254 nm. The method was linear over the concentration range for Sitagliptin 10-30ppm and for Metformin 100-300ppm. The % recoveries for Sitagliptin and Metformin were found to be 99.1 to 100.6% and 98.8 to 100.7% respectively. The validation of method was carried out utilizing International Conference on Harmonization (ICH) guidelines. The described High Performance Liquid Chromatography method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.

Keywords: Sitagliptin, Metformin, Simultaneous Estimation, Reverse Phase –High Performance Liquid Chromatography, Validation, ICH- Guideline.

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INTRODUCTION

As the number of individuals affected by diabetes is continuing to increase worldwide, the need for effective management assumes ever greater urgency. Newer classes of medications, particularly those which work via the incretin pathway, achieve glucose lowering and minimizing risks associated with more traditional therapies. Ideally, combination therapies should be well tolerated, convenient to take, have few contraindications, have a low risk of hypoglycemia and weight gain, and be reasonably effective over both the short and long term such as the combination of Metformin (MF) and the dipeptidyl peptidase-4 (DPP-4) inhibitor Sitagliptin (SG). The chemical structure of the drugs was represented in Figure 1 & 2 respectively.

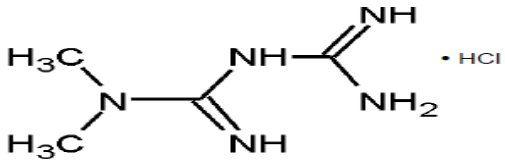

Figure 1 Structure of Metformin.

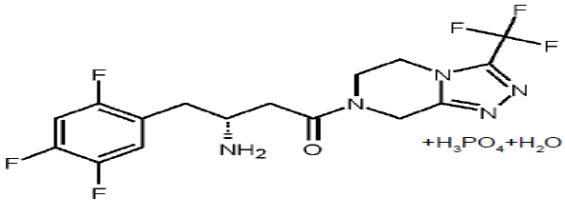

Figure 2 Structure of Sitagliptin

Sitagliptin phosphate monohydrate (SPM) chemically, (3*R*)-3-amino-1-[3-(trifluoromethyl)-5, 6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphate hydrate(Figure. 2) is oral hypoglycemic drug of the dipeptidyl peptidase-4(DPP-4) inhibitor class. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose dependent insulin release and reduce glucagons levels. This is done through inhibition of the inactivation of in cretins, particularly glucagon-like peptide- 1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control[1-3]. Several analytical methods based on UV[4-6], spectroflourimetry[6], RP-HPLC[7-8], LC-MS/MS[9-11] were reported for the determination of Sitagliptin phosphate monohydrate in plasma and urine of humans, rats and dogs. Metformin hydrochloride (MTF) ($C_4H_{11}N_5$.HCl) is 1:1 dimethylbiguanidine monohydrochloride is an anti-diabetic drug from the biguanide class of oral Hypoglycaemic agents, given orally in the treatment of non –insulin-dependent diabetes mellitus[12]. Major action of Metformin HCl in increasing glucose transport across the cell

membrane in skeletal muscle[13,14]. Several analytical methods based on UV[15-18], Spectroflourimetry [15], Reverse Phase-HPLC [19-27], HPTLC[28] and LC-MS/MS[29] was reported for the determination of Metformin. Although literature survey reveals that various methods were reported for Metformin (MTF) and Sitagliptin (SPM) both for single estimation and in combination with others drugs, but no method was reported for the analysis of these drugs in combination.

MATERIALS AND METHOD

Chemical and Reagent Used:

The following chemicals were procured for the process Water [HPLC Grade], Metformin & Sitagliptin **[**Working Standards], Methanol [HPLC Grade] & Sodium Hydroxide all the chemicals were procured from STANDARD SOLUTIONS and Metformin & Sitagliptin Tablets 500mg&50mg respectively were collected from the Local market and the manufacture was MSD, Brand name Janumet.

Apparatus and Chromatographic Conditions:

Equipment Used	**High Performance Liquid Chromatography Equipped with Auto Sampler & DAD or UV Detector**
Column Used	Symmetry C18 (4.6 X 150nm, 3.5 µm, Make: XTerra) or Equivalent
Flow Rate Maintained	0.9 mL per min.
Wavelength Selected	254 nm
Injection Volume	20µL
Column Oven Maintained	Ambient
Run Time	7 min.

Preparation of Phosphate buffer[30, 31]:

The Buffer Solution was prepared by weighing 7.0 grams of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC grade water. Then the pH was adjusted to 5.8with Sodium hydroxide.

Preparation of mobile phase:

The Mobile Phase was prepared by mixing a mixture of above buffer 650 ml (65%) and 350 ml of Acetonitrile HPLC (35%) and degassed in ultrasonic water bath for 5 minutes. Then it was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The same Mobile Phase was used as Diluent.

Preparation of the Metformin & Sitagliptin Standard & Sample Solution:

Standard Solution Preparation:

The Standard Stock Solution for the drugs were prepared by weighing accurately and transferred 10 mg of Metformin and 10mg of Sitagliptin working standard into a 10ml&100ml clean dry volumetric flask respectively. About 7ml&70ml of Diluent was added and sonicated to dissolved it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Sample Solution Preparation:

The Sample Stock Solution was prepared by weighing accurately and transferred 936.9 mg of Metformin and Sitagliptin Tablet powder into a 100ml clean dry volumetric flask. About 70ml of the diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution pipette out 0.4ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Standard & Sample Solution Injected inside the Column[32]:

About 20µL of Standard and Sample Solutions was injected into the chromatographic system and the area was measured for the Metformin and Sitagliptin peaks respectively. Then the %Assay was calculated by using the suitable formulae.

System Suitability[33]:

The Tailing factor for the peaks due to Metformin & Sitagliptin in Standard solution should not be more than 1.5. The Theoretical plates for the Metformin& Sitagliptin peaks in Standard solution should not be less than 2000.

Formulae for Calculating the Metformin Assay:

$$\textbf{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$$

Where:

AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT= Weight of Sample taken in mg.

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

LC = Label Claim of Metformin mg/ml.

Results Obtained for the Assay of Metformin:

System Suitability Results:

1) Tailing factor Obtained from the standard injection is 1.5

2) Theoretical Plates Obtained from the standard injection is 4817.5

Assay Results of Metformin:

$$\frac{2015521}{2020755} \times \frac{10}{10} \times \frac{2}{10} \times \frac{100}{936.9} \times \frac{10}{0.4} \times \frac{99.9}{100} \times \frac{936.9}{500} \times 100 = 99.6\%$$

Assay Calculation for Sitagliptin:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$$

Where: AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

WS = Weight of working standard taken in mg.

WT= Weight of Sample taken in mg.

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

LC = Label Claim of Sitagliptin mg/ml**.**

Results Obtained for the Assay of Sitagliptin:

System Suitability Results:

1). Tailing factor Obtained from the standard injection is 1.2

2). Theoretical Plates Obtained from the standard injection is 4267.5

Assay Calculation for Sitagliptin:

$$\frac{130152}{130835} \times \frac{10}{100} \times \frac{2}{10} \times \frac{100}{936.9} \times \frac{10}{0.4} \times \frac{99.8}{100} \times \frac{936.9}{50} \times 100 = 99.2\%$$

VALIDATION METHOD

Precision:

The precision of an analytical procedure expresses the closeness of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Preparation of stock solution:

The Standard Stock Solution was prepared by weighing accurately and transferred 10 mg of

Metformin and 10mg of Sitagliptin working standard into a 10ml&100ml clean dry volumetric Flask. About 7ml&70ml of Diluent was added and sonicated to dissolved it completely and the volume was made up to the mark with the same solvent. Further from above Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure for Injecting the Standard Stock Solution:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits and the results are summarized in Table 1 & 2.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

The Stock Solution was prepared by weighing accurately and transferred 10 mg of Metformin and 10mg of Sitagliptin working standard into a 10ml&100ml clean dry volumetric flask. Added about 7ml&70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure for Injecting the Standard Solutions:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits and the results are summarized in Table 3 & 4.

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found.

Preparation of Standard stock solution:

The Standard Solution was prepared by weighing accurately and transferred 10 mg of Metformin and 10mg of Sitagliptin working standard into a 10ml&100ml clean dry volumetric flask. Added about 7ml&70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation Sample solutions:

The Sample Solution was prepared as follows:

For preparation of 50% solution (With respect to target Assay concentration):

The Stock Solution was prepared by weighing accurately and transferred 5 mg of Metformin and 5mg of Sitagliptin working standard into a 10ml&100ml clean dry volumetric flask. Added about 7ml&70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

The Stock Solution was prepared by weighing accurately and transferred 10mg of Metformin and 10mg of Sitagliptin working standards into a 10ml&100ml clean dry volumetric flask. Added about 7ml&70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

The Stock Solution was prepared by weighing accurately and transferred 15mg of Metformin and 15 mg of Sitagliptin working standards into a 10ml&100ml clean dry volumetric flask. Added about 7ml&70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the to the mark with the same solvent. Further from the above Stock Solution pipette out 2ml of Metformin & Sitagliptin into a 10ml volumetric flask and diluted up to the mark with diluent.

Procedure for Injecting the Stock Solution:

Injected the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculated the Amount found and Amount added for Metformin & Sitagliptin and calculated the individual recovery and mean recovery values and the results were summarized in Table. 5 & 6.

Linearity:

The linearity of the analytical procedure is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample.

Preparation of stock solution:

The Stock Solution was prepared by weighing accurately and transferred 10 mg of Metformin and Sitagliptin working standard into a 10ml &100ml clean dry volumetric flask. Added about 7ml&70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. From the above Stock Solution following preparation was made:

Preparation of Level – I (100ppm of Metformin & 10ppm of Sitagliptin):

1mlof stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (150ppm of Metformin & 15ppm of Sitagliptin):

2mlof stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (200ppm of Metformin &20ppm of Sitagliptin):

3mlof stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (250ppm of Metformin &25ppm of Sitagliptin):

4mlof stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (300ppm of Metformin & 30ppm of Sitagliptin)

5mlof stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Procedure for Injecting the Solutions: Injected each level into the chromatographic system and measured the peak area. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculated the correlation coefficient. The Results are summarized in Table 7 & 8.

Limit of Detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non instrumental or instrumental.

Limit of Detection for Metformin:

Preparation of 200µg/ml solution:

The Stock Solution was prepared by weighing accurately and transferred 10mg of Metformin working standard into a 10ml clean dry volumetric flask. Added about 7ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.3% solution At Specification level (0.06µg/ml solution):

Further from the above Stock Solution pipette out 1ml into a 10ml volumetric flask and diluted up to the mark with diluent. Further from the above Stock Solution pipette out 1ml into a 10ml volumetric flask and diluted up to the mark with diluent. Pipette out 0.3ml of solution into a 10 ml of volumetric flask and diluted up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOD solution (0.3% of target assay concentration) : 131 µV

S/N = 131/44 = 2.97

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.

Limit of Detection for Sitagliptin:

Preparation of 20µg/ml solution:

The Stock Solution was prepared by weighing accurately and transferred 10mg of Sitagliptin working standard into a 100ml clean dry volumetric flask. Added about 70ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 0.5% solution At Specification level (0.1µg/ml solution):

Further from the above prepared Stock Solution pipette out 1ml solution into a 10ml volumetric flask and diluted up to the mark with diluent. Further from the above Stock Solution pipette out 0.5ml solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOD solution (0.5% of target assay concentration) : 129 µV

S/N = 129/44 = 2.93

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.

Limit of Quantification:

The Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/ or degradation products. Several approaches for determining the Quantification limit are possible, depending on whether the procedure is a non- instrumental or instrumental.

Limit of Quantification of Metformin:

Preparation of 200µg/ml solution:

The Stock Solution was prepared by weighing accurately and transferred 10mg of Metformin working standard into a 10ml clean dry volumetric flask. Add about 7ml of Diluent and sonicated to dissolved it completely and the volume was made upto the mark with the same solvent. Further from the above Stock Solution pipette out 2ml into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 1.0% solution At Specification level (0.2µg/ml solution):

Further from the above Stock Solution pipette out 1ml into a 10ml volumetric flask and diluted up to the mark with diluent. Further from the above Stock Solution pipette out 1ml into a 10ml volumetric flask and diluted up to the mark with diluent. Further Pipette out 1ml of solution into a 10 ml of volumetric flask and diluted up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOQ solution (1.0% of target assay concentration) : 437µV

S/N = 437/44 = 9.93

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

Limit of Quantification of Sitagliptin:

Preparation of 20µg/ml solution:

The Stock Solution was prepared by weighing accurately and transferred 10mg of Sitagliptin working standard into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicated to dissolved it completely and make volume up to the mark with the same solvent. Further from the above Stock Solution pipette out 2ml solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of 2.0% solution At Specification level (0.4µg/ml solution):

Further from the above Stock Solution pipette out 1ml solution into a 10ml volumetric flask and diluted up to the mark with diluent. Further from the above Stock Solution pipette out 2ml of solution into a 10 ml of volumetric flask and diluted up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOQ solution (2.0% of target assay concentration) : 435µV

S/N = 435/44=9.88

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.8 ml/min to 1.0 ml/min.

The Standard solution 200ppm of Metformin &20ppm of Sitagliptin was prepared and analysed using the varied flow rates along with the developed flow rate.. On evaluation of the above results, it can be concluded that the variation in flow rate does not affected the method

significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. The results are summarized in Table 9 & 10.

The Organic composition in the Mobile phase was varied from 25% to 15%.

The Standard solution 200 µg/ml of Metformin & 20 µg/ml of Sitagliptin was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase does not affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±1. The results are summarized in Table 11 & 12.

RESULTS & DISCUSSION

Present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of Metformin & Sitagliptin in Bulk as well as in pharmaceutical dosage forms. In order to method development under isocratic conditions, mixtures of Phosphate Buffer with the pH 5.8 and Acetonitrile [HPLC grade] in different combinations were tested as mobile phase on a Symmetry C18 (4.6 x 150mm, 3.5µm, Make: XTerra) column. A binary mixture of Phosphate Buffer pH 5.8 and Acetonitrile in 65:35 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for Metformin & Sitagliptin were around 2.592 & 4.307 min respectively. A model chromatogram was shown in Figure 3.

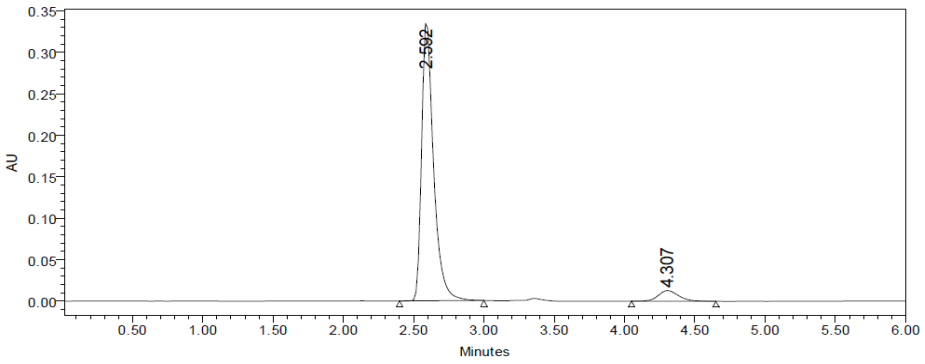

Figure 3A model Chromatograph showing the separation of the Drug

The Precision data was represented by Table. 1 & 2.

Table 1The Precision results are summarized Metformin.

Injection	Area
Injection-1	1988914
Injection-2	2025739
Injection-3	2019189

Injection-4	2018510
Injection-5	2033936
Average	2017258
Standard Deviation	17020.5
%RSD	0.84

Table 2The Precision results are summarized Sitagliptin.

Injection	**Area**
Injection-1	128478
Injection-2	130962
Injection-3	130097
Injection-4	130484
Injection-5	130460
Average	130096
Standard Deviation	955.3
%RSD	0.73

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%. When Metformin & Sitagliptin were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation results, a low coefficient of variation was observed Table 3 & 4.

Table 3The Ruggedness results are summarized Metformin.

Injection	**Area**
Injection-1	1960848
Injection-2	1940400
Injection-3	1942932
Injection-4	1947900
Injection-5	1952215
Average	1948859
Standard Deviation	8102.3
%RSD	0.42

Table .4The Ruggedness results are summarized Sitagliptin.

Injection	**Area**
Injection-1	122532
Injection-2	126721
Injection-3	125998
Injection-4	126435
Injection-5	126663
Average	125670
Standard Deviation	1777.0
%RSD	1.41

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%. Above data showed the preciseness of present HPLC method and it was represented by

Figure 4.

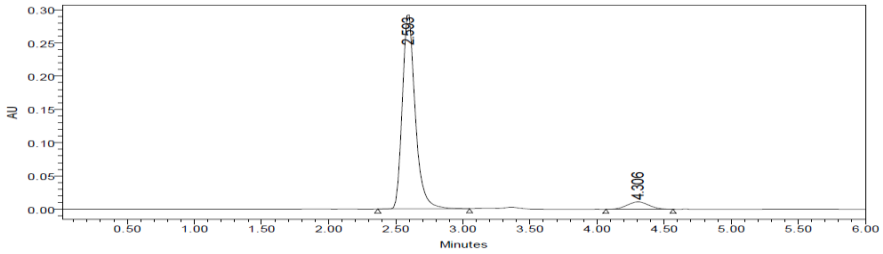

Figure.4The Ruggedness Chromatograph for the Drug Metformin & Sitagliptin.

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found were represented in Table. 5 & 6.

Table 5The Accuracy results are summarized for Metformin.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1009442	5.0	4.94	98.8%	99.9%
100%	2047373	10.0	10.0	100.2%	
150%	3085210	15.0	15.1	100.7%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table 6 The accuracy results are summarized for Sitagliptin.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65699.3	5.0	4.95	99.1%	100.1%
100%	133312	10.0	10.0	100.5%	
150%	200131	15.0	15.0	100.6%	

Table 7The Linearity results are summarized for Metformin.

S.No	Linearity Level	Concentration	Area
1	I	100ppm	1322402
2	II	150ppm	1669399
3	III	200ppm	2032985
4	IV	250ppm	2365299
5	V	300ppm	2688465
Correlation Coefficient			0.999

Acceptance Criteria: The Correlation coefficient should be not less than 0.999

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0% In order to test the linearity of the method, five dilutions of the working standard solutions of the drug in the range of 100ppm

to 300ppm for the drug Metformin and 10ppm to 30ppm for the drug Sitagliptin were prepared respectively and represented in Table. 7 & 8.

Table 8 The Linearity results are summarized for Sitagliptin.

S.No	Linearity Level	Concentration	Area
1	I	10ppm	85152
2	II	15ppm	108768
3	III	20ppm	130477
4	IV	25ppm	152589
5	V	30ppm	177212
Correlation Coefficient			0.999

Acceptance Criteria: The Correlation coefficient should be not less than 0.999.

Each of the dilutions was injected into the column and the graph for the Linearity Curve was represented in Figure 5 & 6.

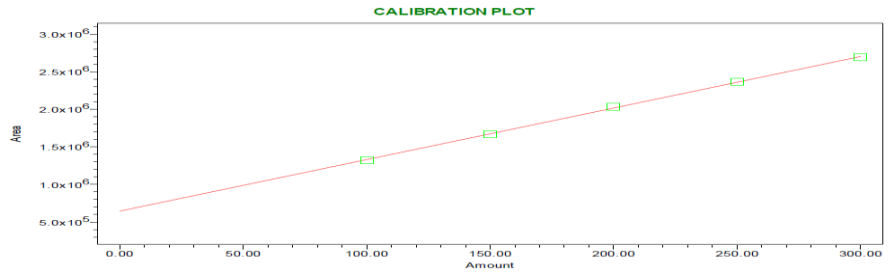

Figure.5The Linearity curve for Metformin.

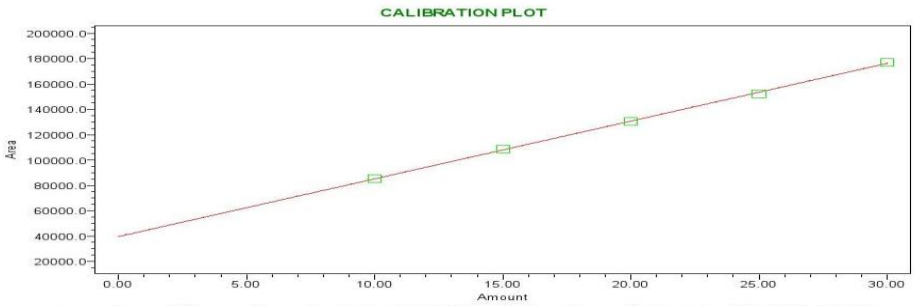

Figure 6 The Linearity curve for Sitagliptin.

The method was duly validated by evaluation of the required parameters. Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate buffer and Acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The Figure 7, 8, 9 & 10 was represented the Robust nature of the chromatograph.

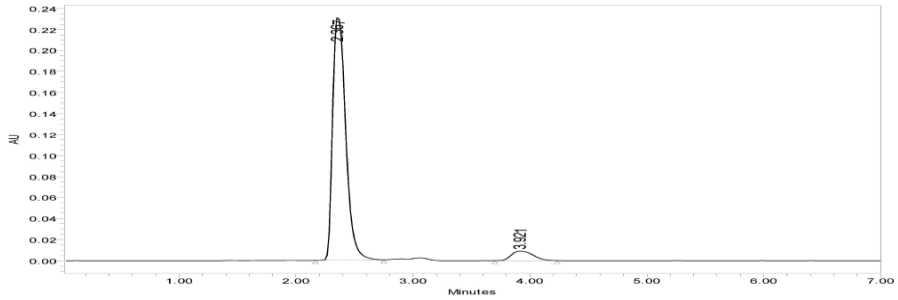

Figure 7 The Robustness Chromatograph with increase in composition of the Mobile Phase.

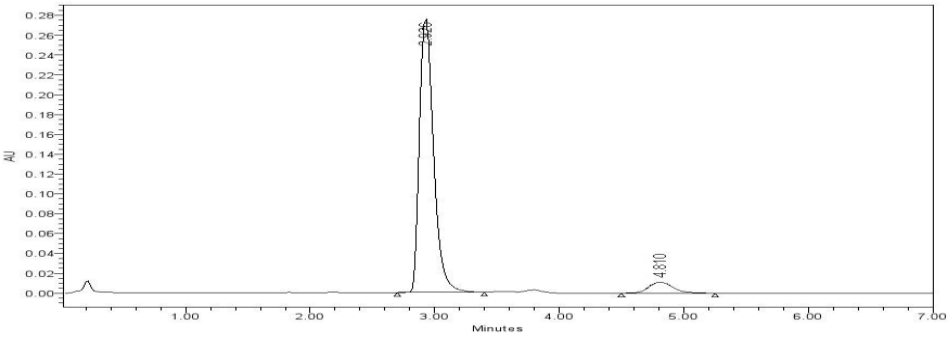

Figure 8The Robustness Chromatograph with decrease in composition of the Mobile Phase.

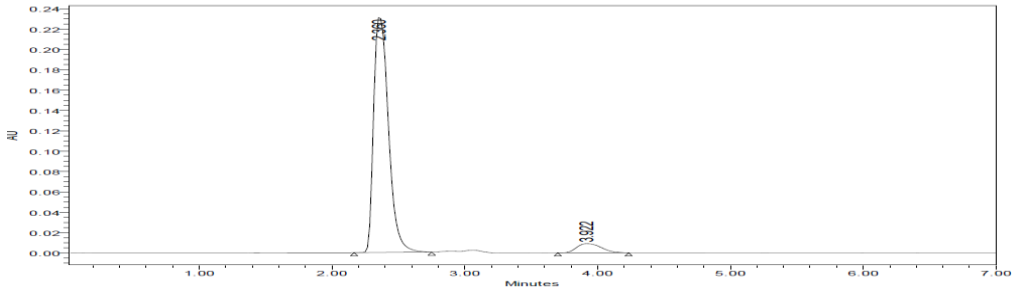

Figure 9 The Robustness Chromatograph with increase in the Flow Rate.

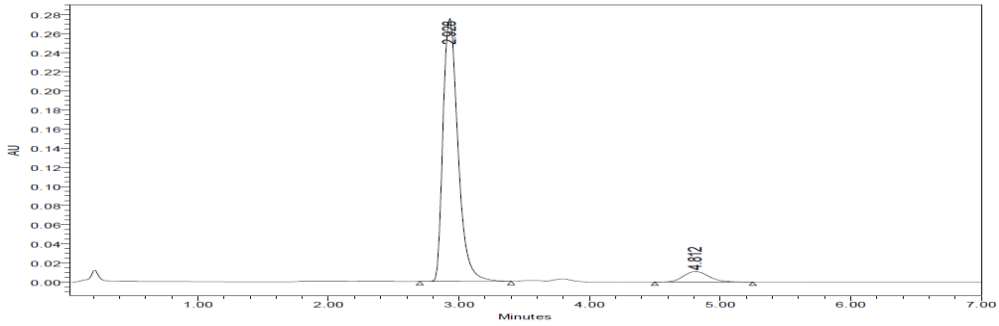

Figure 10 The Robustness Chromatograph with decrease in the Flow Rate.

The system suitability parameters were within the limits as shown in Table 9, 10, 11 and 12 for the drug.

Table 9 The results are summarized for System suitability results for Metformin.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
1	0.8	3421.6	1.4
2	0.9	4817.5	1.5
3	1.0	2398.9	1.4

Table 10. The results are summarized for System suitability results for Sitagliptin.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
1	0.8	3023.0	1.2
2	0.9	4267.5	1.2
3	1.0	2264.6	1.3

Table 11The results are summarized for System suitability results for Metformin.

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	SP Tailing
1	10% less	3815.9	1.4
2	*Actual	4817.5	1.5
3	10% more	2891.5	1.4

Table 12The results are summarized for System suitability results for Sitagliptin.

.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	SP Tailing
	10% less	3128.9	1.2
	*Actual	4267.5	1.2
	10% more	2759.6	1.3

Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for the drug Metformin was found to be 0.06µg/ml and LOQ for the Drug Metformin was found to be 0.2µg/mL & the LOD for the drug Sitagliptin was found to be 0.1 µg/mL, LOQ for the drug Sitagliptin was found to be 0.4 µg/mL. The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data indicates the reproducibility of the method in dosage forms. It was concluded that the proposed HPLC method was sufficiently sensitive and reproducible for the analysis of Metformin & Sitagliptin in the Tablet formulation dosage forms within a short analysis time.

CONCLUSION

It was concluded that the proposed RP-HPLC method developed for the quantitative determination of Metformin & Sitagliptin in bulk and in its formulations was simple, selective,

sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence this method can easily be adopted as an alternative method to reported ones for the routine determination of Metformin & Sitagliptin depending upon the availability of chemicals and nature of other ingredients present in the sample. The method will also find use in clinical, biological and pharmacokinetic studies of Metformin & Sitagliptin at future.

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