International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Research Article **Open Access**

Analytical Method Development and Validation for the Simultaneous Estimation of Metformine and Gemigliptin by RP-HPLC Method

Nair Ayana Ramesh[1], Dr. D. Naresh[1], Dr. Gampa Vijaya Kumar*[2]

[1]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[2]*Professor and Head, Department of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*

A B S T R A C T

The estimation of metformin and gemigliptin was done by RP-HPLC. The Phosphate buffer was p^{H}3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil ODS C_{18} column C18 (4.6 x 150mm, 5µm) .The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of metformin and gemigliptin were found to be from 100-500 µg/ml of metformin and 1-5µg/ml of gemigliptin. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of metformin and gemigliptin. LOD and LOQ were found to be within limit.

Keywords: Metformin, Gemigliptin, RP-HPLC

A R T I C L E I N F O

CONTENTS

Article History: Received 25 September 2016, **Accepted** 29 October 2016, **Available Online** 10 December 2016

***Corresponding Author**
Dr. Gampa Vijaya Kumar
Professor and Head, Dept. of Pharmacy,
KGR Institute of Technology and Management, Rangareddy, Telangana.
Manuscript ID: AJMPS3200

PAPER-QR CODE

Citation: Gampa Vijaya Kumar, *et al.* Analytical Method Development and Validation for the Simultaneous Estimation of Metformine and Gemigliptin by RP-HPLC Method. *Int. J. Med. Pharm. Res*., 2016, 4(6): 321-330.

1. Introduction

High-performance liquid chromatography also known as High-pressure liquid chromatography, HPLC is a form of column chromatography used frequently in analytical chemistry and biochemistry to identify, separate, and quantify compounds. It is a powerful tool in analysis. It is basically an improved form of column chromatography

which has been optimized to provide rapid high resolution separations. Early LC used gravity fed open tubular columns with particles 100s of microns in size; the human eye was used for a detector and separations often took hours or even days to develop. HPLC as compared with the classical technique is characterized by

- Small diameter (2-5 mm), reusable stainless steel columns without repacking & regeneration
- Column packings with very small (3, 5 and 10 µm) particles and the continual development of new substances to be used as stationary phases
- Relatively high inlet pressures and controlled flow of the mobile phase
- Precise sample introduction without the need for large samples
- Special continuous flow detectors capable of handling small flow rates and detecting very small amounts
- Automated standardized instruments
- Rapid analysis
- Greater reproducibility due to close control of the parameters affecting the efficiency of separation
- Capable of handling macro molecule & viscous solutions
- Efficient analysis of labile natural products
- Reliable handling of inorganic or other ionic species

HPLC is probably the most universal type of analytical procedure. In addition HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures. Its application areas include quality control, process control, forensic analysis, environmental monitoring and clinical testing. It has achieved this position as a result of the constant evolution of the equipment used in LC to provide higher and higher efficiencies at faster and faster analysis times with a constant incorporation of new highly selective column packings.

Metformin, *N*, *N* - dimethyl imido di carbonimidic diamide (Figure 1), is a biguanide hypoglycemic drug that is regarded as the main drug in mixed therapies of oral hypoglycemics. Literature survey reveals that some methods have been reported for determination of MET in mixtures including LC/MS/MS and HPLC [1-2].

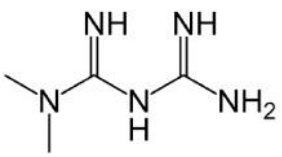

Figure 1: Structure of Metformin

Gemigliptin, (2S, 3R, 4R,5S ,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl]methyl]phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol, is used for Treating type 2 diabetes in certain patients. It is used along with diet and exercise. Gemigliptin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor [2]. It works by decreasing the amount of sugar the body absorbs and increasing the amount of sugar that leaves the body in the urine [3-5]. Literature survey reveals that only one spectrophotometric method [8] and one chromatographic method was reported for the determination of VDG in the presence of its synthetic intermediate [9].

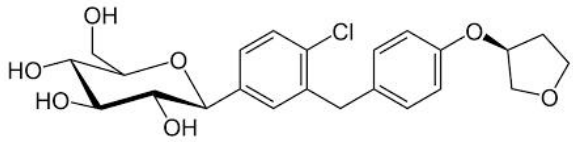

Figure 2: Structure of Gemigliptin

Due to the lack of reported HPLC methods describing determination of the mixtures under investigation, it was deemed useful to develop simple, sensitive and selective HPLC method that could be useful for the simultaneous determination of Metformin and Gemigliptin. The proposed method was designed to be suitable for the quality assessment of these mixtures in a tablet dosage form.

2. Materials and Methods

Table 1: Chemicals Instruments used

S.No	Instrument	Model
1	HPLC	WATERS, Empower, 2695 separation module, PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV 3000$^+$
3	pH meter	Adwa – AD 1020
4	Weighing machine	Afcoset ER-200A
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil

Table 2: Chemicals used

S.No	Chemical	Brand
1	Metformin	Mylon
2	Gemigliptin	Cipla
3	KH_2PO_4	FINER chemical Ltd
4	Water and Methanol	Lichrosolv (MERCK)
5	Acetonitrile for HPLC	Molychem
6	Ortho phosphoric Acid	MERCK

HPLC Method Development:

Optimized Chromatogram is obtained by following Conditions:

Trial 1:

Mobile phase : Water: Methanol (50:50%v/v)
Column : Thermosil C8 (4.6*100mm) 5µm
Flow rate : 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl

Observation:

From the above chromatogram it was observed that the Metformin peak was eluted

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively.

Wave length selection:

UV spectrum of 10 µg / ml Metformin and Gemigliptinin diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance.

Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

Optimized Chromatographic Conditions

Instrument used : Waters HPLC with auto sampler and PAD ordetector.

Temperature : Ambient

Column : Inertsil ODS (4.6 x 150mm, 5µm)

Buffer : 6.8 grams of potassium dihydrogenortho phosphate in 1000 ml water pH adjusted with orthophaosparic acid.

pH : 3.0

Mobile phase : 30% buffer 70% Methanol

Flow rate : 1 ml per min

Wavelength : 260 nm

Injection volume : 10µl

Run time : 10min.

Preparation of Buffer and Mobile Phase: [4, 5, 6]

Preparation of Phosphate buffer:

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml of Methanol HPLC (70%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Preparation of the Metformin & Gemigliptin Standard & Sample Solution:[5, 6]

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Metformin and 10mg of Gemigliptin working standard into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Metformin & Gemigliptin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3 ml of Metformine and Gemigliptinof the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for Metformin and Gemigliptin peaks & calculate the %Assay by using the formulae.

System Suitability: Tailing factor for the peaks due to Metformin and Gemigliptinin Standard solution. Should not be more than 2.0. Theoretical plates for the Metformin and Gemigliptinpeaks in Standard solution should not be less than 2000.

Calculation: (For metformin)

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

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of Metformin mg/ml.

Calculation: (For Empagliflozin)

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

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = label claim of gemigliptin mg/ml.

Method Validation Summary

Precision: [7, 8]

Preparation of stock solution:

Accurately weigh and transfer 25 mg of Metformin and Gemigliptin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3 ml of Metformin & Gemigliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: 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.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%**.**

Intermediate Precision/Ruggedness: [9, 10]

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:

Accurately weigh and transfer 25 mg of Metformin and 10mg of Gemigliptin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml of Metformin & Gemigliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

Accuracy: [11, 12]

Preparation of Standard stock solution:

Accurately weigh and transfer 10 mg of Metformin andGemigliptin10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):[13]

Accurately weigh and transfer 5mg of Metformin and 5.3mg of Gemigliptin, working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock Solution:

Further pipette 3 ml of Metformine& 0.3 ml of Gemigliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration): Accurately weigh and transfer 10 mg of Metformin and 10 mg of Gemigliptin working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock Solution

Further pipette 3 ml of Metformin& 0.3 ml of Gemigliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 14.4mg of Metformin and 14.5mg of Gemigliptin working standards into a 10mL and 100ml of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 3 ml of Metformin& 0.3 ml of Gemigliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Metformin and gemigliprin and calculate the individual recovery and mean recovery values.

Acceptance Criteria:

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

Linearity:

Preparation of stock solution:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Metformin and Gemigliptin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Preparation of Level – I (100ppm ofMetformin&1ppm of Gemigliptin): 1ml and 0.1 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (200ppm of Metformin&2ppm of Gemigliptin): 2ml and 0.2 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level– III (300ppm of Metformin&3ppm of Gemigliptin): 3ml and 0.3 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level– IV (400ppm of Metformin&4ppm of Gemigliptin): 4ml and 0.4 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (500ppm of Metformin&5ppm of Gemigliptin): 5ml and 0.5 ml of stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Procedure: Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

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

Limit of Detection:

Limit of Detection: (For Metformin):

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Metformin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.12µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Pipette 0.4mL of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 152/52 = 2.9

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

Limit of Detection: (For Gemigliptin)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Gemigliptin working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.015µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOD solution

S/N = 156/52 = 3.0

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

Limit of Quantification:

Limit of Quantification (for Metformin HCL)

Preparation of 300µg/ml solution:

Accurately weigh and transfer 10 mg of Metformin working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.42µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Pipette 1.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

S/N = 522/52 = 10.03

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution

Limit of Quantification: (for Gemigliptin)

Preparation of 3µg/ml solution:

Accurately weigh and transfer 10mg of Gemigliptin working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.05µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.7mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank

Signal Obtained from LOQ solution

S/N = 524/52 = 10.

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.

a). The flow rate was varied at 0.8 ml/min to 1.2ml/min.

Standard solution 300ppm of Metformin & 3ppm ofGemigliptin was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly.

Hence it indicates that the method is robust even by change in the flow rate ±10%**.**

***** Results for actual flow (1.0ml/min) have been considered from Assay standard. : 156 µV

b). The Organic composition in the Mobile phase was varied from 50% to 50%.

Standard solution 300 µg/ml of metformin& 3µg/ml of Gemigliptin 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 affected the method significantly. Hence it

Indicates that the method is robust even by change in the Mobile phase ±10

*****Results for actual Mobile phase composition (55:45Methanol: Buffer (ph-2.8) has been considered from Accuracy stand

3. Results and Discussion

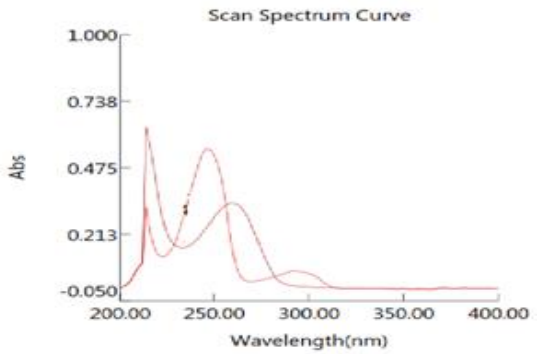

Figure 3: Spectrum showing wavelength of Metformin

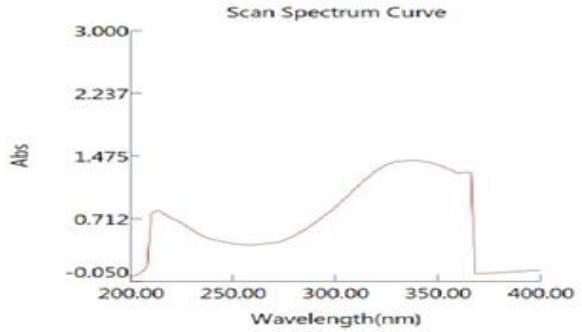

Figure 4: Spectrum showing wavelength of gemigliptin

Optimized Chromatogram is obtained by following Conditions [Trial -1]:

Mobile phase : Water: Methanol (50:50%v/v)
Column : Thermosil C8 (4.6*100mm) 5µm
Flow rate : 1.0 ml/min
Wavelength : 260 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl
Rt : 2.258

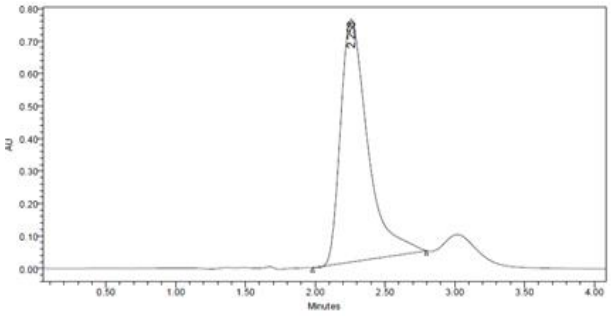

Figure 5: Optimized Chromatogram

Observation: From the above chromatogram it was observed that the Metformin peak was eluted and gemigliptin was not eluted.

Chromatogram for Metformin and Gemigliptin

Column : Inertsil ODS C18 (4.6 x 250mm, 5µm)
Buffer pH : 3.0.
Mobile phase : 30% buffer 70% Methanol
Flow rate : 1.0ml per min
Wavelength : 260 nm
Temperature : ambient.
Run time : 10 min.

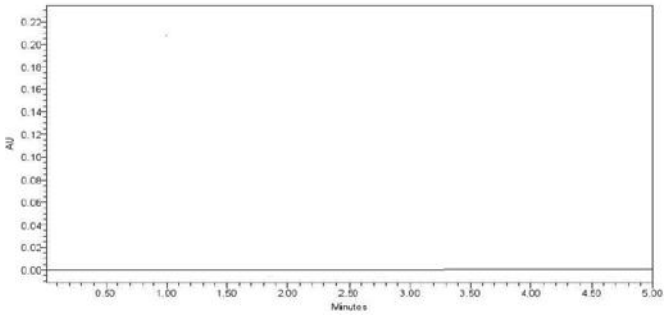

Figure 6: Chromatogram for blank

Observation: From the above chromatogram it was observed that there are no interferences.

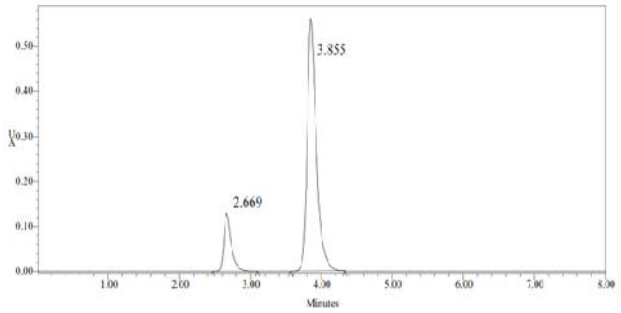

Figure 7: Chromatogram for Metformin and Gemigliptin sample Preparation

Observation: From the above chromatogram it was observed that the Metformin and
Gemigliptin peaks are well separated
Retention time of Metformin : 2.669min
Retention time of Gemigliptin : 3.855 min.

Validation Parameters:

Precision: Precision of the method was carried out for standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

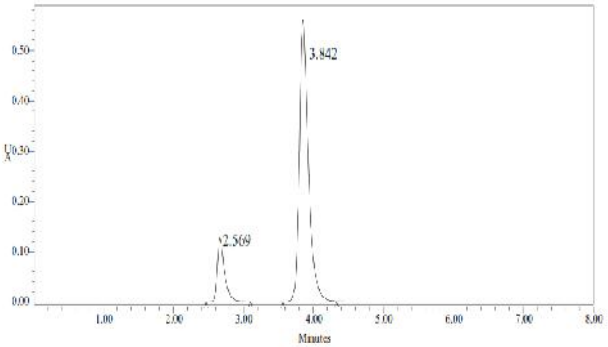

Figure 8: Chromatogram for standard injection -1

Table 3: Results of method precession for Metformin

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 4: Results of method precession for Gemigliptin

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Acceptance criteria:

%RSD for sample should be NMT 2**.** The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate Precession Ruggedness:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

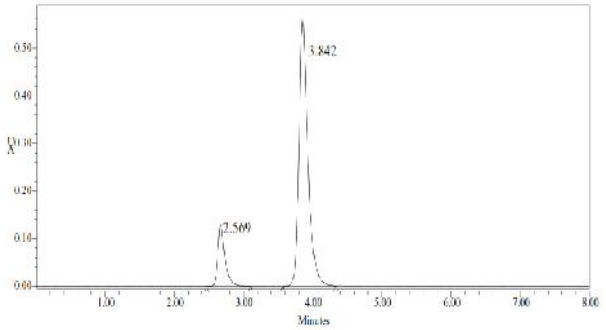

Figure 9: Chromatogram for sample injectiocn-1

Table 5: Results of Intermediate precision for Metformin

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 6: Results of Intermediate precision for Gemigliptin

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Linearity:

The linearity range was found to lie from 100µg/ml to 500µg/ml of Metformin, 5µg/ml to 25µg/ml 0f Gemigliptin and chromatograms are shown below.

Table 7: Area of different concentration of Metformin

S.N	Linearity Level	Conc.	Area
1	I	100ppm	468934
2	II	200ppm	856781
3	III	300ppm	1313873
4	IV	400ppm	1763458
5	V	500ppm	2167084
Correlation Coefficient			0.999

Table 8: Area of different concentration of Gemigliptin

S.N	Linearity Level	Conc.	Area
1	I	1ppm	48510
2	II	2ppm	91701
3	III	3ppm	134802
4	IV	4ppm	172731
5	V	5ppm	209732
Correlation Coefficient			0.999

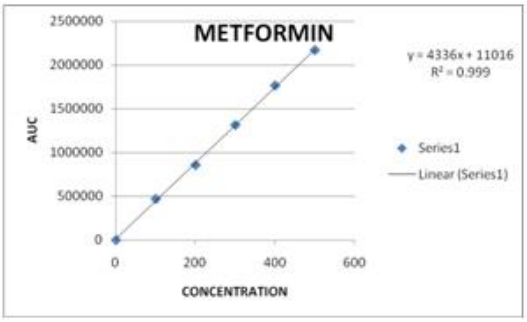

Figure 10: Calibration graph for Metformin at 225 nm

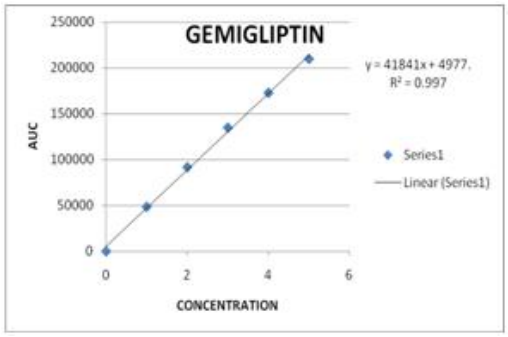

Figure 11: Calibration graph for Gemigliptin at 260 nm

Table 9: Analytical performance parameters of Metformin and Gemigliptin

Parameters	Metformin	Gemigliptin
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.997

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit. The linearity was established in the range of 100% to 500% of Metformin and 1% to 5% of Gemigliptin.

Limit of Detection (LOD): The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio

Limit of Quantification (LOQ): The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Robustness:

The standard and samples of Metformin and Gemigliptin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 10: Accuracy (recovery) data for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	
50%	656659.5	5.0	5.036	100.7%	
50%	656659.5	5.0	5.036	100.7%	
100%	1304258	10.0	10.003	100.0%	
100%	1304258	10.0	10.003	100.0%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	
150%	1854608	14.4	14.224	98.780%	
150%	1854608	14.4	14.224	98.780%	

Table 11: Accuracy (recovery) data for Gemigliptin

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	
50%	65800	5.3	5.34	100.8%	
50%	65800	5.3	5.34	100.8%	
100%	124353	10	10.10	100.01%	
100%	124353	10	10.10	100.01%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	
150%	177940	14.2	14.45	99.68%	
150%	177940	14.2	14.45	99.68%	

Table 12: Flow Rate (ml/min) data for Metformin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 13: Flow rate (ml/min) data for Gemigliptin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 14: Change in Organic Composition in the Mobile Phase for Metformin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 15: Change in Organic Composition in the Mobile Phase for Gemigliptin

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2

2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

Table 16: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Metformin	52	152	2.9
Gemigliptin	52	156	3

Table 17: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Metformin	52	522	10.03
Gemigliptin	52	524	10.1

.

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of metformin and gemigliptin was done by RP-HPLC. The Phosphate buffer was p^H3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil ODS C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 1ml/min. the linearity range of metformin and gemigliptin were found to be from 100-500 µg/ml of metformin and 1-5µg/ml of gemigliptin. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of metformin and gemigliptin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

[1] Zeng W, Xu Y, Constanzer M, Woolf EJ. Determination of sitagliptinin human plasma using protein precipitation and tandem mass spectrometry. J.Chromatogr. B. 2010; 878(21):1817-1823.

[2] Wei Zeng, Donald Musson G, Alison Fisher L, Li Chen, Michael Schwartz S, Eric Woolf J, Amy Qiu Wang. Determination of sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry. J. Pharm. Biomed.Anal.2008; 46(3): 534-542.

[3] Ramakrishna Nirogi, VishwottamKandikere, KoteshwaraMudigonda, PrashanthKomarneni, Raghupathi Al eti, RajeshkumarBoggavarapu. Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid–liquid extraction. Biomed. Chromatogr. 2008; 22(2): 214–222.

[4] Campbell DB, Lavielle R, Nathan C. The mode of action and clinical pharmacology of gliclazide: a review. Diab Res Clin Prac.1991; 14:S21-S36.

[5] Robert Moses, Fixed combination of repaglinide and metformin in the management of type 2 diabetes, Diabetes, Metabolic syndrome and obesity: Targets and Therapy Dove press, open access to scientific and medical research.2009; 2:101-9.

[6] Tripathi K.D. Essential of Medical Pharmacology, 5th Edn, Jaypee Brothers Medical publisher New Delhi. pp: 515-516.

[7] Hassa Saad S.M., Mahmoud Wagiha H., Elmosallamy Mohamed A.F, Othman Abdel Hammeed M. Determination of metformin in pharmaceutical preparations using potentiometry, spectrofluorimetry and UV–visible spectrophotometry. Anal. ChimicaActa .1999; 378(1-3): 299-311.

[8] Patil S.S., Bonde C. G .Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk &Tablets using UV visible spectroscopy, Int. J. Chem. Tec. Res.2009; 1(4): 905-909.

[9] Lakshmi KS, Rajesh T, Sharma S, Lakshmi S. Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Metformin, Pioglitazone and Glimepiride in Pharmaceutical Formulations. Der Pharma Chemica.2009; 1 (1): 238-246.

[10] Freddy H. Havaldar, DharmendraL.Vairal. Simultaneous estimation of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride in the tablets dosage form. Int. J. Appl .Bio. Pharm.Tec.2010;1(3):1000-1005.

[11] AbuRuz, S., Millership J, McElnay J. The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. J Chromatogr. B, 2005; 817(2): 277-286.

[12] Jain D, Jain S,Jain D ,Maulik A. Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by

RP-HPLC in Tablet Formulation. Journal of Chromatogr. Sci. 2008; 46:501-504.

[13] Lakshmi KS, Rajesh T,Sharma S. Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. Int.J Pharm. Pharm. Sci. 2009; 1(2): 162-166.