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Research

Estimation of Sitagliptin and Metformin by Using RP-HPLC

T. Naveen Kumar*, Dr. V. Jagadeesh Babu, Dr. D. Varun

Department of Pharmaceutical Analysis, Sri Indu Institute of Pharmacy, Sheriguda (V), Ibrahimpatnam, Telangana, 501510

*Author for Correspondence: T. Naveen Kumar
Email: naveenkumart848@gmail.com

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Abstract

The present study focuses on the development and validation of a simple, precise, and accurate Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Sitagliptin and Metformin in bulk and pharmaceutical dosage forms. Chromatographic separation was achieved using a Waters HPLC system equipped with an autosampler and PDA detector (model 996), and an Altima C18 column (4.6 × 150 mm, 5 µm) maintained at 35°C. The mobile phase consisted of Methanol and Acetonitrile in a 50:50 (v/v/v) ratio, delivered at a flow rate of 1.0 mL/min. Detection was performed at 260 nm with an injection volume of 10 µL and a total run time of 14 minutes. The method was validated as per ICH guidelines, demonstrating excellent linearity, precision, accuracy, specificity, and sensitivity for both Sitagliptin and Metformin. The method was successfully applied for the analysis of commercial tablet formulations without interference from excipients. This validated RP-HPLC method is suitable for routine quality control and analysis of Sitagliptin and Metformin in pharmaceutical preparations.

Keywords: RP-HPLC, Sitagliptin, Metformin, Altima C18 column, simultaneous estimation, validation.

1. INTRODUCTION

HPLC is an analytical technique in which solutes are resolved by differential rates of elution as they pass through a chromatographic column. The method of separation by this instrument is governed by distribution between the mobile phase and stationary phase. The instrumentation is made-up of eight basic components, mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing and computer, integrator or recorder. The successful use of HPLC for the possible problem requires the

right combination of variety of operating conditions such as the type of column packing and mobile phase, column length and diameter, mobile phase flow rate, column temperature and sample size [1].

Now a day reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% (possibly up to 90%) of all HPLC separations are carried out in the reversed phase mode. The reasons for this include the simplicity, versatility and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass [2-4].

PRINCIPLE

In isocratic HPLC the analyte is forced through a column of the stationary phase (usually a tube packed with small round particles with a certain surface chemistry) by pumping a liquid (mobile phase) at high pressure through the column. The sample to be analyzed is introduced in a small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time and is considered a reasonably unique identifying characteristic of a given analyte. The use of pressure increases the linear velocity (speed) giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combinations of water or various organic liquids (the most common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components. A further refinement to HPLC has been to vary the mobile phase composition during the analysis, this is known as gradient elution. A normal gradient for reverse phase chromatography might start at 5% methanol and progress linearly to 50% methanol over 25 minutes, depending on how hydrophobic the analyte is. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase composition relative to the stationary phase. This partitioning process is similar to that which occurs during a liquid-liquid extraction but is continuous, not step-wise. In this example, using a water/methanol gradient, the more hydrophobic components will elute (come off the column) under conditions of relatively high methanol; whereas the more hydrophilic compounds will elute under conditions of relatively low methanol. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte. Often a series of tests are performed on the analyte and a number of generic runs may be processed in order to find the optimum HPLC method for the analyte - the method which gives the best separation of peaks.

APPLICATIONS

Preparative HPLC refers to the process of isolation and purification of compounds. Important is the degree of solute purity and the throughput, which is the amount of compound produced per unit time. This differs from analytical HPLC, where the focus is to obtain information about the sample compound. The information that can be obtained includes identification, quantification, and resolution of a compound. Chemical Separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. Thus, the chromatographer can separate compounds (more on chiral separations) from each other using HPLC; the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. To alter the retention time of a compound, several parameters can be manipulated. The first is the choice of column, another is the choice of mobile phase, and last is the choice in flow rate. All of these topics are reviewed in detail in this document. Identifying a compound by HPLC is accomplished by researching the literature and by trial and error. A sample of a known compound must be utilized in order to assure identification of the unknown compound. Identification of compounds can be assured by combining two or more detection methods.

1.1. Types of HPLC methods

1. Reverse Phase HPLC Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution [5]. Uses water-organic as mobile phase, columns may be C18 (ODS), C8, phenyl, Trimethyl Silane (TMS), cyano as a stationary phase. It is first choice for most samples especially neutral or non-ionized compounds, that dissolve in water organic mixtures.

2. Normal Phase HPLC In normal-phase chromatography, the stationary phase is polar and the mobile phase is nonpolar. In reversed phase we have just the opposite; the stationary phase is nonpolar and the mobile phase is

polar. Typical stationary phases for normal-phase chromatography are silica or organic moieties with cyano and amino functional groups. In this the mixtures of organic solvents for mobile phase and columns i.e. cyano, diol and amino silica can be used as stationary phase. It is first choice for mixtures of isomers and for preparative scale HPLC and second choice for lipophilic samples that cannot dissolve well in water organic mixtures [6].

1.2. STEPS FOR HPLC METHOD DEVELOPMENT

The wide variety of equipment, columns, eluant and operational parameters involved makes high performance liquid chromatography (HPLC) method development seem complex. The process is influenced by the nature of the analytes and generally follows the following steps:

Step 1 - Selection of the HPLC method and initial system
Step 2 - Selection of initial conditions
Step 3 - Method optimization
Step 4 - Method validation

EXPERIMENTAL METHODS

INSTRUMENTS USED

HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
pH meter	Lab India
Weighing machine	Sartorius
Volumetric flasks	Borosil
Pipettes and Burettes	Borosil

CHEMICALS USED

Sitagliptin	Procured from Sun Pharma, Provided by Sura Pharma labs
Metformin	Procured from Sun Pharma, Provided by Sura Pharma labs
Water and Methanol for HPLC	LICHROSOLV (MERCK)
Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Sitagliptin and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1 ml of the above Sitagliptin and 0.1 ml of the Metformin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase	: Methanol: ACN (50:50v/v)
Column	: Altima C18 (4.6×150mm, 5.0 µm)
Flow rate	: 1 ml/min
Wavelength	: 260 nm
Column temp	: 35ºC
Injection Volume	: 10 µl
Run time	: 14 minutes

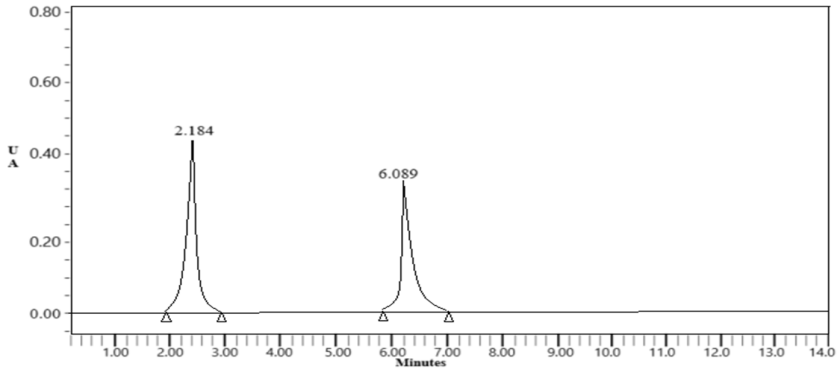

Fig 1: Optimized Chromatogram

Table 1: Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Sitagliptin	2.184	3425413	567933		1.0	5565.5
2	Metformin	6.089	1629854	517733	2.5	1.1	5355.2

Observation

From the above chromatogram it was observed that the Sitagliptin and Metformin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

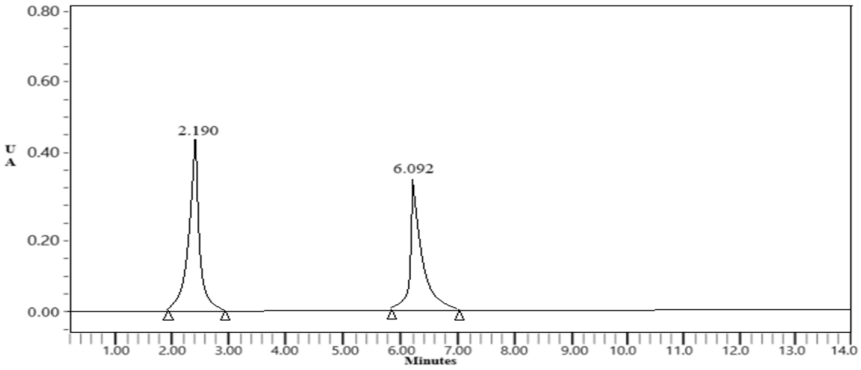

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Sitagliptin	2.190	3468547	567933		1.0	5565.5
2	Metformin	6.092	16289441	517733	2.5	1.1	5355.2

Acceptance Criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank

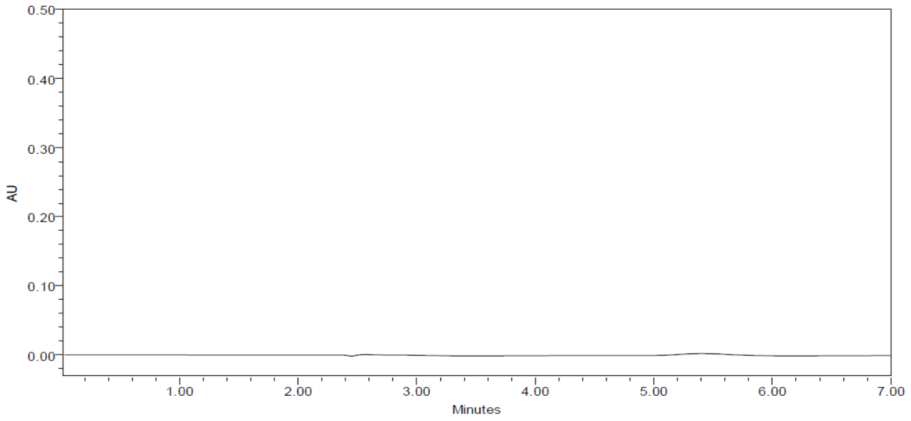

Fig 3: Chromatogram showing blank (mobile phase preparation)

System Suitability

Table 3: Results of system suitability for Sitagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sitagliptin	2.191	3569412	567917	5568.0	1.0
2	Sitagliptin	2.186	3465125	517719	6359.2	1.1
3	Sitagliptin	2.189	3598154	567933	5565.5	1.0
4	Sitagliptin	2.190	3586491	517733	5355.2	1.1
5	Sitagliptin	2.184	3582694	567917	6348.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

Acceptance Criteria:

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

Table 4: Results of method precession for Metformin

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	6.090	3582264	567917	5568.0	1.0	2.5
2	Metformin	6.095	3586491	517719	5359.2	1.1	2.5
3	Metformin	6.091	3598154	567933	5565.5	1.0	2.5
4	Metformin	6.092	3564125	517733	5355.2	1.1	2.5
5	Metformin	6.089	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

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.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Assay (Standard)

Table 5: Peak results for assay standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Sitagliptin	2.184	3425681	567917		1.0	5568.0	1
2	Metformin	6.089	16235984	517719	2.5	1.1	5359.2	1
3	Sitagliptin	2.190	3425413	567933		1.0	5565.5	2
4	Metformin	6.092	16298543	517733	2.5	1.1	5355.2	2
5	Sitagliptin	2.189	3465423	567933		1.0	5545.5	3
6	Metformin	6.091	16260213	517733	2.5	1.1	5352.1	3

Assay (Sample)

Table 6: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Sitagliptin	2.186	3469821	567917		1.0	6568.0	1
2	Metformin	6.091	16259845	517719	2.5	1.1	5359.2	1
3	Sitagliptin	2.191	3468547	567933		1.0	5565.5	2
4	Metformin	6.090	16287531	517733	2.5	1.1	5355.2	2
5	Sitagliptin	2.189	3468143	567813		1.0	5391.1	3
6	Metformin	6.091	16282431	517623	2.5	1.1	5564.0	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Sitagliptin and Metformin in pharmaceutical dosage form was found to be 100.1%.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY

Sitagliptin

Concentration µg/ml	Average Peak Area
0	0
5	1010252
10	2049374
15	3072706
20	3921068
25	4952813

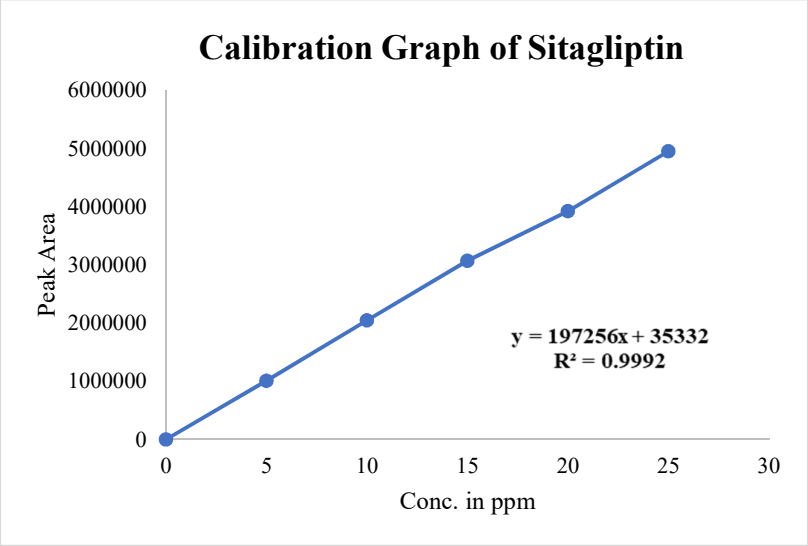

Fig 4: Calibration Graph for Sitagliptin

LINEARITY PLOT

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 35332. These values meet the validation criteria.

Metformin

Concentration µg/ml	Average Peak Area
0	0
10	8040807
20	14318417
30	21087985
40	27913928
50	34584741

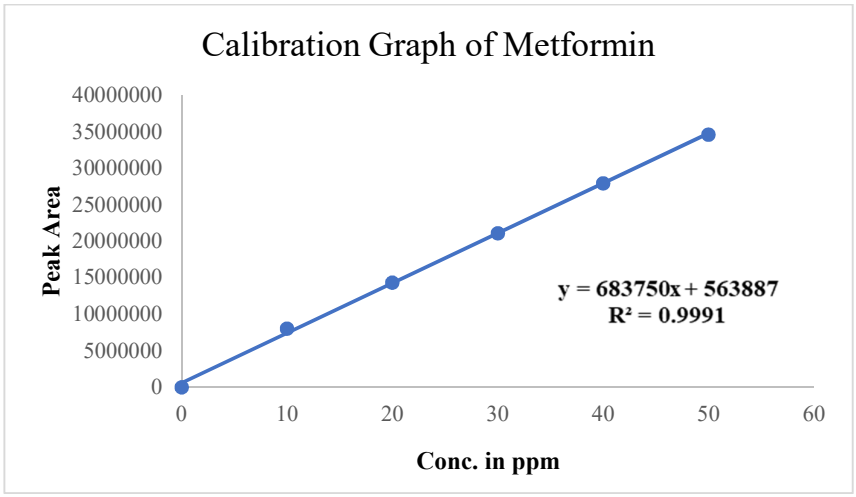

Fig 5: Calibration Graph for Metformin

LINEARITY PLOT

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 56388. These values meet the validation criteria.

PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 7: Results of repeatability for Sitagliptin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Sitagliptin	2.191	3569412	567917	5568.0	1.0
2	Sitagliptin	2.189	3465125	517719	5359.2	1.1
3	Sitagliptin	2.186	3598154	567933	5565.5	1.0
4	Sitagliptin	2.191	3586491	517733	5355.2	1.1
5	Sitagliptin	2.189	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

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.

Table 8: Results of method precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	6.090	3582264	567917	5568.0	1.0	2.5
2	Metformin	6.091	3586491	517719	5359.2	1.1	2.5
3	Metformin	6.095	3598154	567933	5565.5	1.0	2.5
4	Metformin	6.090	3564125	517733	5355.2	1.1	2.5
5	Metformin	6.091	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

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 precision

Day 1:

Table 9: Results of Intermediate precision for Sitagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sitagliptin	2.189	3481579	567917	5568.0	1.0
2	Sitagliptin	2.191	3458121	517719	5359.2	1.1
3	Sitagliptin	2.186	3426081	567933	5565.5	1.0
4	Sitagliptin	2.189	3465712	517733	5355.2	1.1

5	Sitagliptin	2.191	3451476	567917	5568.0	1.0
6	Sitagliptin	2.189	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.

Table 10: Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	6.091	15481579	567917	5568.0	1.0	2.5
2	Metformin	6.090	15369852	517719	5359.2	1.1	2.5
3	Metformin	6.095	15248454	567933	5565.5	1.0	2.5
4	Metformin	6.091	15874692	517733	5355.2	1.1	2.5
5	Metformin	6.090	15236547	567933	5568.0	1.0	2.5
6	Metformin	6.091	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

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.

Day 2:

Table 11: Results of Intermediate precision Day 2 for Sitagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Sitagliptin	2.189	3481579	567917	5568.0	1.0
2	Sitagliptin	2.191	3458121	517719	5359.2	1.1
3	Sitagliptin	2.189	3426081	567933	5565.5	1.0
4	Sitagliptin	2.186	3465712	517733	5355.2	1.1
5	Sitagliptin	2.191	3451476	567917	5568.0	1.0
6	Sitagliptin	2.189	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.

Table 12: Results of Intermediate precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Metformin	6.091	15481579	567917	5568.0	1.0	2.5
2	Metformin	6.090	15369852	517719	5359.2	1.1	2.5

3	Metformin	6.095	15248454	567933	5565.5	1.0	2.5
4	Metformin	6.091	15874692	517733	5355.2	1.1	2.5
5	Metformin	6.090	15236547	567933	5568.0	1.0	2.5
6	Metformin	6.091	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

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

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

Table 13: The accuracy results for Sitagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	15	15.2	101.9	100.9%
100%	3035883	30	30.4	101.4	
150%	4451005	45	44.7	99.4	

Table 14: The accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	30	30.07	100.2	99.6%
100%	2096069	60	59.6	99.4	
150%	3112684	90	89.3	99.3	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

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 quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Sitagliptin:

=1.9µg/ml

Metformin:

=2.60µg/ml

LIMIT OF QUANTITATION

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\mathbf{LOQ=10\times\sigma/S}$$

Where

σ = Standard deviation of the response
S = Slope of the calibration curve
Result:
Losartan:
= 3.9µg/ml
Metformin:
= 6.5µg/ml

ROBUSTNESS

Table 15: Results for Robustness

Sitagliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	2.184	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	2.190	5868.8	1.2
Less aqueous phase	3175485	2.191	5836.2	1.2
More aqueous phase	3365431	2.189	5282.6	1.1

Acceptance Criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Metformin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	6.089	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	6.092	5989.2	1.1
Less aqueous phase	1973724	6.090	5387.2	1.1
More aqueous phase	2102838	6.091	5938.1	1.1

Acceptance Criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

A simple, accurate, and precise Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Sitagliptin and Metformin in bulk and pharmaceutical dosage forms. The optimized chromatographic conditions included the use of a Waters HPLC system with an autosampler and PDA detector (996 model), employing an Altima C18 column (4.6 × 150 mm, 5 µm) maintained at 35°C. The mobile phase consisted of Methanol and Acetonitrile in a 50:50 (v/v/v) ratio, with a flow rate of 1.0 mL/min. Detection was carried out at 260 nm using a 10 µL injection volume, and the total run time was 14 minutes. Under these conditions, both Sitagliptin and Metformin were well resolved with sharp, symmetric peaks and satisfactory retention times. The method was validated according to ICH guidelines, showing excellent linearity, accuracy, precision, specificity, and sensitivity. It was also successfully applied for the analysis of marketed tablet formulations without interference from excipients. In conclusion, the developed RP-HPLC method is reliable, efficient, and suitable for routine quality control analysis of Sitagliptin and Metformin in both bulk and tablet dosage forms.

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REFERENCES

1. Khan MC, Reddy NK, Ravindra G, Reddy KVSRK, Dubey PK. Development and validation of a stability indicating HPLC method for simultaneous determination of four novel fluoroquinolone dimers as potential antibacterial agents. J Pharmaceut Biomed Anal, 59, 2012, 162–166.
2. Blanchet B, Sabourea C, Benichou AS, Billemont B, Taieb SR, Alain D. Development and validation of an HPLC-UVvisible method for sunitinibquantifcation in human plasma. ClinChimActa, 404, 2009, 134–139.
3. FDA Guidance for Industry. Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER), 2000.
4. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modeling in HPLC method development. J Adv Res, 3, 2012, 53–63.
5. Ferrarini A, Huidobro AL, Pellati F, Barbas C. Development and validation of a HPLC method for the determination of sertraline and three non-chiral related impurities. J Pharmaceut Biomed Anal, 53, 2010, 122–129.
6. Collier JW, Shah RB, Bryant AR, Habib MJ, Khan MA, Faustino PJ. Development and application of a validated HPLC method for the analysis of dissolution samples of levothyroxine sodium drug products. J Pharmaceut Biomed Anal, 54, 2011, 433–438.
7. Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. Phrama Tech, 24, 2000, 1- 14. 280 Santhosh G.et al. / Vol 4 / Issue 4 / 2014 / 274-280.
8. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. LcGc N. Am, 75, 2002, 49-50.
9. Synder LR, Kirkland JJ, Glajach JLX. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643- 712.
10. Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab, 37, 2005, 22-27.
11. Debebe Z, Nekhai S, Ashenaf M, David BL, Kalinowski DS, RG Victor, Byrnes WM, Richardson DR, Karla PK. Development of a sensitive HPLC method to measure invitro permeability of E- and Z-isomeric forms of thiosemicarbazones in Caco-2 monolayers. J Chromatogram B, 906, 2012, 25–32.
12. www.agilent.com/chem/store (Accessed on 18/5/2013)
13. Dolan JW. Peak tailing and resolution. LcGc N. Am, 20, 2002, 430-436.
14. Qiang Fu, Shou M, Chien D, Markovich R, Rustum AM. Development and validation of a stability-indicating RP-HPLC method for assay of betamethasone and estimation of its related compounds. J Pharmaceut Biomed Anal, 51, 2010, 617– 625.