METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN, GLIMEPIRIDE AND METFORMIN IN ITS BULK AND TABLET DOSAGE FORM USING RP-HPLC

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ABSTRACT

A Novel, simple, sensitive, and economic reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for simultaneous estimation of Sitagliptin phosphate monohydrate, Glimepiride and Metformin hydrochloride in bulk and tablet dosage form. The chromatographic separation was achieved on the Agilent C18 column (250mm×4.6mm, 5µm) by using 0.01M Ammonium Dihydrogen Orthophosphate and 0.05M Sodium Dihydrogen Orthophosphate as to Acetonitrile in the ratio of (60:40) at a flow rate 1mL/min. The detection was carried out at wavelength 257nm. The retention time of Metformin hydrochloride, Sitagliptin phosphate monohydrate and Glimepiride and were found to be 2.35 min, 4.65 min and 7.77 min respectively. The developed method was validated according to ICH Q2 (R2) guideline. The linearity was found at the concentration range 400-600 µg/mL for Metformin hydrochloride, 40-60 µg/mL for Sitagliptin phosphate monohydrate and 0.8-1.2 µg/mL for Glimepiride with a correlation coefficient of 0.999, 0.998 and 0.9998. % Recoveries were found in the 98-102% range for Sitagliptin phosphate monohydrate, Glimepiride and Metformin hydrochloride. The % RSD was found less than 2%. The Detection limit and Quantitation limit were found to be 11.65 µg/mL and 35.32 µg/ml. for Metformin hydrochloride, 0.728 µg/mL and 2.208 µg/mL for Sitagliptin phosphate monohydrate and 0.0138 µg/mL and 0.0418 µg/mL for Glimepiride respectively. The developed method is robust, reproducible, and can be used for routine quality control in bulk and pharmaceutical dosage forms in the pharmaceutical industry.

Keywords: Sitagliptin Phosphate Monohydrate, Glimepiride, Metformin Hydrochloride, RP-HPLC, Method development, Validation.

INTRODUCTION

High-performance liquid chromatography is an analytical technique that separates, identifies, or quantifies a mixture of substances based on molecular structure and composition. This is a separation technique based on a solid stationary phase and liquid mobile phase using the principle of adsorption, partition, or ion exchange depending upon the type of stationary phase used.(1) There are two types of HPLC, a) Normal phase HPLC and b) Reversed phase HPLC.(2,3) In RP-HPLC, the stationary phase is typically hydrophobic (non-polar), and the mobile phase is relatively polar, leading to the separation of compounds based on their polarity and interactions with the stationary phase.(4,5)

Sitagliptin phosphate is an oral anti hyperglycemic of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. For the treatment of type 2 diabetes, this enzyme-inhibiting medication is used either by itself or in conjunction with other oral anti-hyperglycemic medications (such metformin or a thiazolidinedione). Sitagliptin inhibits the DPP-4 enzyme in a competitive manner. The gastrointestinal hormones GLP-1 and GIP, which are released in reaction to a meal, are broken down by this enzyme. By blocking GLP-1 and GIP inactivation, they are able to boost the secretion of insulin and reduce the release of glucagon by the pancreas. This drives blood glucose levels towards normal. 7-[(3R)-3-amino-1-oxo-4-(2,4, 5- trifluorophenyl) butyl] -5,6,7, 8 tetra hydro-3-(trifluoromethyl)-1, 2, 4-triazolo [4, 3-a] pyrazine phosphate (1:1) monohydrate.(6–8)

Glimepiride (GLI) is a sulfonylurea antidiabetic agent. Chemically, it is 1-[[p-[2-(3-ethyl-4 methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4 methylcyclohexyl) urea. The molecular formula of glimepiride is C24H34N405S with a molecular mass of about 490.617 g/mol. It is a third-generation sulfonylurea derivative commonly used in the treatment of non-insulin-dependent Type 2 diabetes mellitus. Glimepiride is a second-generation Sulfonylurea agent. Glimepiride appears to work by enhancing peripheral tissues' sensitivity to insulin and inducing the release of insulin from active pancreatic beta cells. Glimepiride probably depolarizes the membrane via binding to ATP-sensitive potassium channel receptors on the surface of pancreatic cells, which lowers potassium conductance. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces secretion of insulin.(7,9)

Metformin Hydrochloride is a anti-diabetic drug which belongs to Biguanides drug class. The chemical formula for Metformin hydrochloride is C4H12ClN5. IUPAC name is N,N-dimethyl-imido dicarbonimidic diamide;hydrochloride. Metformin is prescribed with other medication to control high blood sugar with instruction to follow proper diet and exercise program. It is used to treat patients with type 2 diabetes. Metformin exerts its anti-diabetic action through suppression of generation of glucose in the liver.(10–12) Metformin acts on mitochondrial respiratory complex I by inhibiting its function, which results in increasing in the cellular ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) as a result of a reduction in the efficiency of ATP production. This increase in the ratio of AMP: ATP triggers the activation of AMP activated protein kinase (AMPK), which has alot of effects on energy metabolism, also down regulation of the expression of gluconeogenic genes is controlled by it. The activity of adenylate cyclase is also thought to be inhibited by increase in AMP concentration, it is an important mediator of glucagon action, therefore results in the inhibition of gluconeogenesis. The second target of metformin in mitochondria is mitochondrial glycerol 3-phosphate dehydrogenase, which plays a key role in the glycerophosphate shuttle. The activity of mitochondrial glycerol-3-phosphate dehydrogenase is also inhibited by metformin, which suppresses gluconeogenic reactions including the conversion of lactate to pyruvate.(8,13)

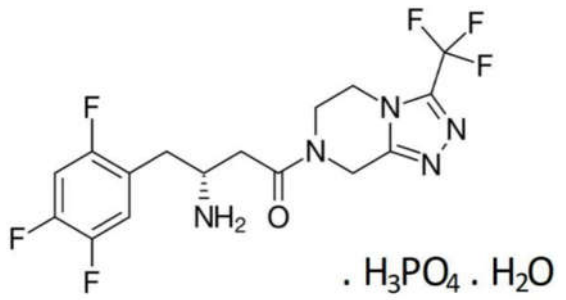

Figure 1 Chemical Structure of Sitagliptin

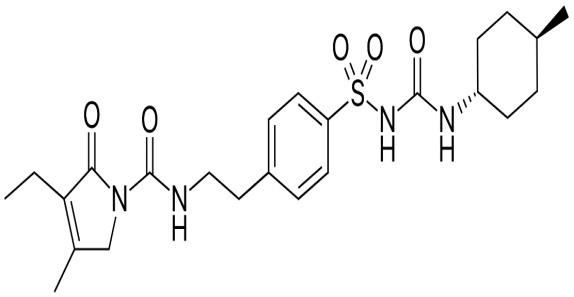

Figure 2 Chemical Structure of Glimepiride

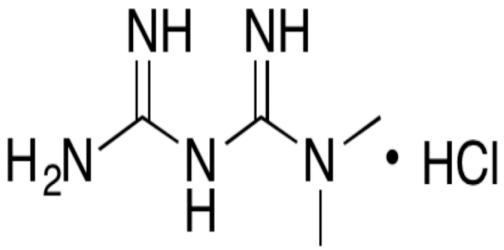

Figure 3 Chemical Structure of Metformin Hydrochloride

The literature survey reveals that several analytical method are reported for quantitative estimation of Sitagliptin, Glimepiride and Metformin alone and in combination with other anti-diabetic agents. The available methods are based on spectrophotometry, HPLC, HPTLC etc.(14–21) However, no RP-HPLC method was available for the simultaneous estimation of this combination drugs. The objective of this study was to develop a novel, simple, accurate, and robust RP-HPLC method for simultaneous estimation of Sitagliptin, Glimepiride and Metformin in its bulk and tablet dosage Form. This method was validated in accordance with ICH guideline.(22)

MATERIALS AND METHODS:

Chemicals and Reagents:

The standard API drug SITA, GLM and MET was obtained from Glenmark Pharmaceuticals, Sinnar, Nashik. Marketed tablet with brand name Istamet G- IR ® were obtained from the local pharmacy. HPLC-grade water and HPLC grade Acetonitrile were obtained from Alpha Chemika. Analytical grade Ammonium dihydrogen orthophosphate and Sodium dihydrogen orthophsophate from SD Fine - Chem Limited were used.

Instrumentations and chromatographic condition:

Chromatographic separation was performed using HPLC - Shimadzu, LC-2010C_{HT} equipped with solvent delivery pump, autosampler injector and a uv/visible detector. Seperation was achieved using Agilent C18 column (250mmx4.6 mm internal diameter with particle size 5µm). The mobile phase, standard solution and sample solution were degassed using an ultrasonicator. Acetonitrile and water in the ratio of 40:60 (v/v) was used as diluent to make the standard dilutions.

Preparation of standard solution:

Accurately weighed about 50mg of Sitagliptin, 1mg of Glimepiride and 500mg of Metformin in 100 ml volumetric flask, dissolved in 20mL acetonitrile and sonicated for 10 minutes and make up to volume with mobile phase which gives 500 µg/mL, 10 µg/mL and 5000 µg/mL respectively.

Preparation of working solution:

1 ml from the standard stock solution was pipette out in 10 ml volumetric flask and made up the volume with mobile phase which gives 50 µg/mL, 1 µg/mL & 500 µg/mL solution of Sitagliptin, Glimepiride & Metformin respectively.

Preparation of Sample Solution (Test Solution):

For analysis of the marketed formulation 20 tablets (Istamet G- IR) combination of Sitagliptin, Glimepiride and Metformin were weighed and crushed to a fine powder. Transfer powder containing 50mg of Sitagliptin, 1 mg of Glimepiride and 500 mg of Metformin to a 100 mL dry volumetric flask, dissolved in 20 ml of acetonitrile and sonicated for 10 minutes and make up to volume with mobile phase, filter solution through 0.45µm membrane filter. Further dilute 1 mL of above solution to 10 mL with mobile phase which gave 50 µg/mL, 1 µg/mL & 500 µg/mL solution of Sitagliptin, Glimepiride & Metformin.

System suitability

The standard solutions comprising MET 500 µg/mL, SITA 50 µg/mL and GLM 1 µg/mL were injected in six replicate injections to determine the system appropriateness parameters. All percent relative standard deviations (RSD) were calculated for resolution, retention time (Rt), USP tailing and theoretical plates.

Method Validation

The developed method for estimation of Metformin, Sitagliptin and Glimepiride was validated as per ICH Q2(R2) guidelines. Different parameters assessed were linearity, accuracy, precision, limit of detection, limit of quantification, specificity, and robustness.(22)

RESULTS AND DISCUSSION

Method optimization

A simple reverse phase high performance liquid chromatography method was developed for the determination of Sitagliptin, Glimepiride and Metformin in pure form and in pharmaceutical formulations using Agilent C18 column (250 × 4.6 mm, 5 µm particle size). Several trials were conducted in order to achieve resolution of the three medications that is to generate sharp and well-resolved peaks. The optimum mobile phase comprised of 0.01M Ammonium Dihydrogen Orthophosphate and 0.05M Sodium Dihydrogen Orthophosphate: Acetonitrile in 60:40 v/v. The mobile phase was chosen after several trials to reach the optimum stationary /mobile phase matching. The flow rate is 1.0 ml/min;injection volume 20µl and detection wavelength at 257nm. The average retention times under the conditions described ere 2.35 min, 4.65 min and 7.77 min for Metformin, Sitagliptin and Glimepiride respectively (Table 1, Figure 4).

Table 1 Optimized Conditions

Column	Agilent C18 column
Column Dimension	4.6mm×250mm, 5µm
Mobile Phase	0.01M Ammonium Dihydrogen Orthophosphate and 0.05M Sodium Dihydrogen Orthophosphate:Acetonitrile (60:40)
Flow rate	1mL/min
Wavelength	257 nm
Injection Volume	20 µL
Detector	UV Detector
Column temperature	30 °C
Run Time	10 min

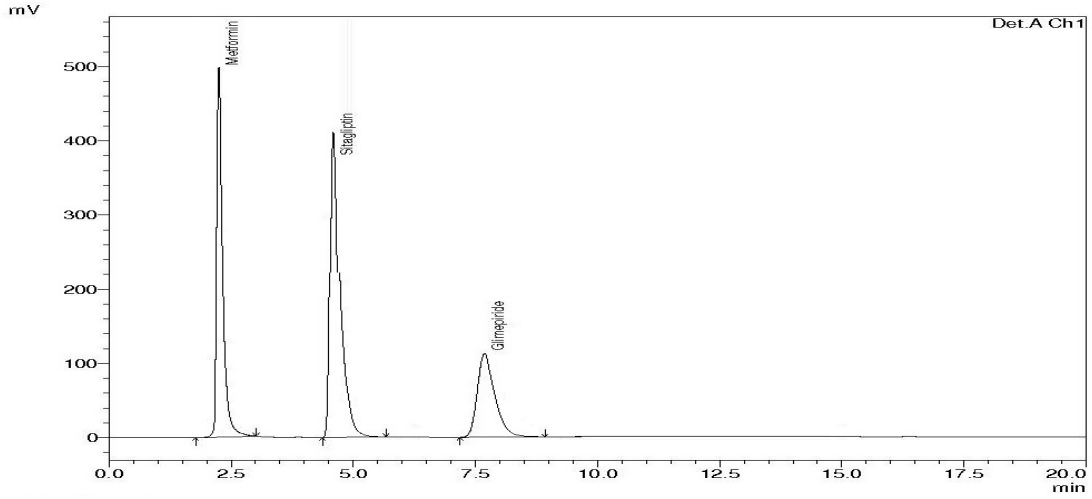

Figure 4 Optimized Chromatogram

System suitability

The outcomes of the system suitability test are represented in Table 2.

Table 2 System suitability results for the developed HPLC method

Parameters	Metformin	Sitagliptin	Glimepiride
Retention time (min)	2.35	4.65	7.77
Peak Area	4228376	2175996	604474
USP Tailing	1.34	1.46	1.47
Theoretical Plate	5644	5162	6102
Resolution	0.00	8.196	14.352

Acceptance Criteria:

USP Tailing: NMT 2

Theoretical Plate: NLT 2000

Resolution: NLT 2

Method Validation

Linearity

A significant linear relationship was attained among the concentration and peak areas of MET, SITA and GLM over the ranges 400 - 600 µg/mL, 40 – 60 µg/mL and 0.8 – 1.2 µg/mL respectively, under optimized chromatographic conditions with the correlation coefficient R2 = 0.999 for MET, R2 = 0.998 for SITA and R2 = 0.9998 for GLM. The analytical outcomes for linearity with slope and intercept are depicted in (Table 3) and The linearity graph for MET, SITA and GLM described in Figure 5, 6, 7.

Table 3 The results of Linearity parameters for MET, SITA and GLM

Parameters	**MET**	**SITA**	**GLM**
Linearity (µg/mL)	400 – 600	40 – 60	0.8 – 1.2
Slope	8635.2	45260	586688
Intercept	- 7451.8	-177631	19120
Correlation coefficient	0.999	0.998	0.9998

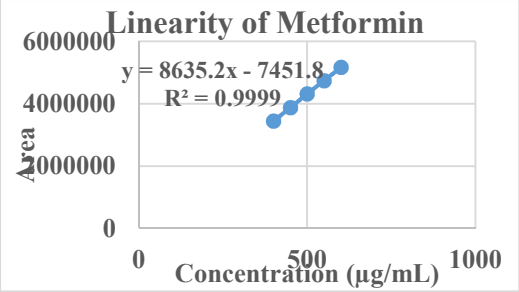

Figure 5 Linearity graph for MET

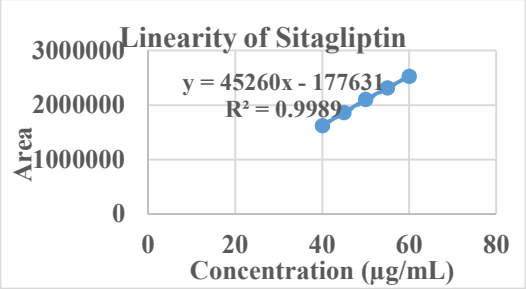

Figure 6 Linearity graph for SITA

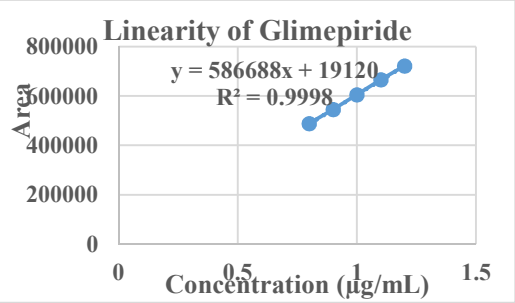

Figure 7 Linearity graph for GLM

Precision

Summarizes the intraday and interday fluctuation in precision analysis included in Table. The method's repeatability is indicated by the low RSD value (less than-2 %). These results show that the approach has a high level of precision and repeatability. (Table 4)

Table 4 Precision Results for MET, SITA and GLM

Analyte	Intraday Precision		Interday Precision		Repeatability	
	Peak area ± SD	% RSD	Peak area ± SD	% RSD	Peak area ± SD	% RSD
MET	4218301 ± 2403.32	0.05	4401877 ± 54320.96	1.23	4226266 ± 30500.18	0.72
SITA	2153816 ± 7716.26	0.35	2230438 ± 18946.25	0.84	2144129 ± 9997.46	0.46
GLM	605070 ± 101.23	0.01	616016 ± 1659.92	0.26	594170 ± 2454.66	0.41

Accuracy

The accuracy of the method was determined by recovery experiments which were carried out and the relative standard deviation and recovery percentages were computed. The data showed that standard drug recoveries were accurate. (Table 5).

Table 5 Accuracy results for MET, SITA and GLM

Analyte	Accuracy Level	% Mean recovery	SD	% RSD
MET	80 %	100.02	0.412	0.412
	100 %	99.95	0.083	0.083
	120 %	99.65	0.288	0.289
SITA	80 %	99.90	0.175	0.175
	100 %	99.70	0.345	0.346
	120 %	100.10	0.745	0.744
GLM	80 %	99.76	1.229	0.232
	100 %	99.58	0.301	0.302
	120 %	100.45	0.570	0.567

Limit of Detection & Limit of Quantifications:

The results of limit of detection and limit of quantification are depicted in (Table 6)

Table 6 Result of Limit of Detection and Limit of Quantifications

Parameter	Metformin	Sitagliptin	Glimepiride
Detection limit (DL)	11.65 µg/mL	0.728 µg/mL	0.0138 µg/mL
Quantitation limit (QL)	35.32 µg/mL	2.208 µg/mL	0.0418 µg/mL

Specificity

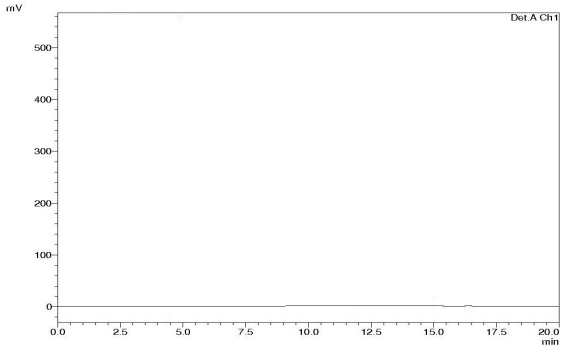

Figure 8 Chromatogram of Blank

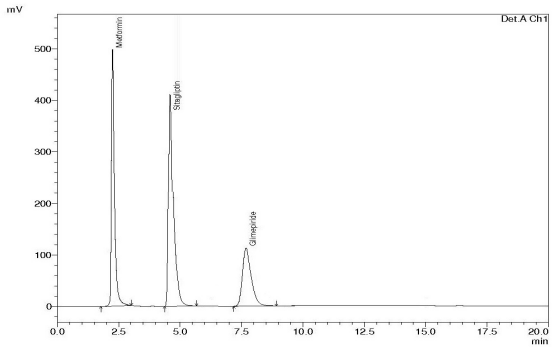

Figure 9 Chromatogram of Standard

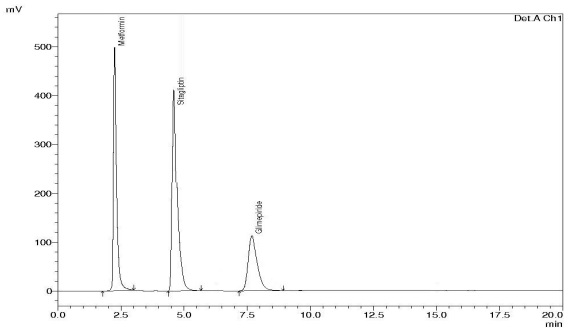

Figure 10 Chromatogram of Test

There is no interference from Blank with the main peak of the Metformin, Sitagliptin and Glimepiride in the Standard & Test sample solution. (Figure 8, 9, 10)

Robustness

The robustness of the method was studied by changes in the method like change in flow rate (±0.1 ml/min of set value i.e. 0.9 ml/min and 1.1 ml/min), detection are evaluated (Table 7 & Figure 11, 12) and change in wavelength (±5 nm of set value i.e. 252 nm and 262 nm) (Table 8 Figure 13, 14)

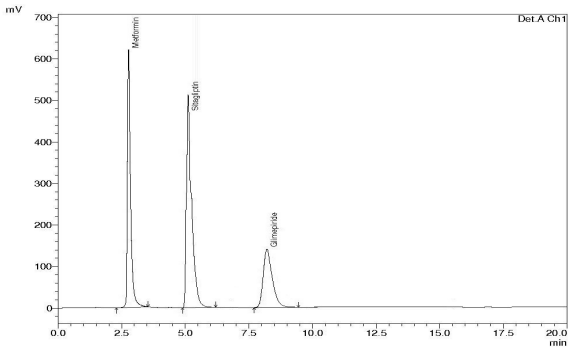

Figure 11 Change in Flow rate 0.9 mL/min

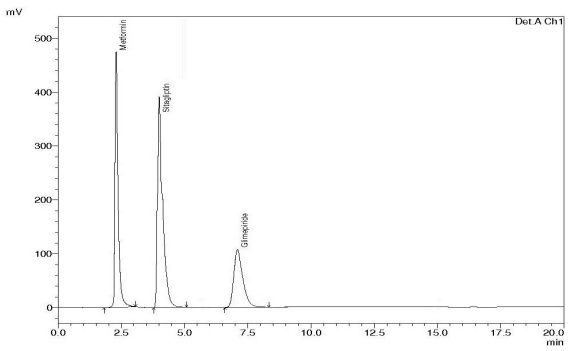

Figure 12 Change in Flow rate 1.1 mL/min

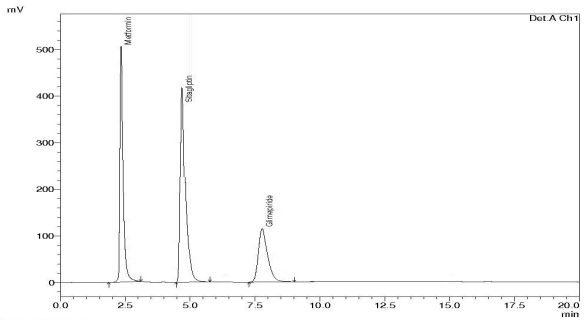

Figure 13 Change in Wavelength 252 nm

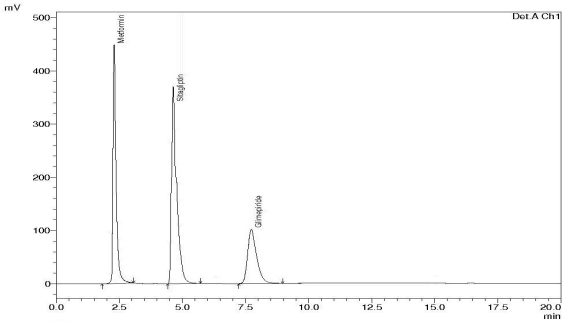

Figure 14 Change in Wavelength 262 nm

Table 7 Result of Robustness by Change in Flow rate

Parameter	Variation	Area		
		MET	SITA	GLM
Flow rate	0.9 mL/min	5073200	2465352	620478
	Mean	5081471.618	2464843.626	623741.2526
	SD	7178.395294	548.0915374	3641.856715
	%RSD	**0.141266071**	**0.022236361**	**0.583872992**
	1.1 mL/min	3796531	2277556	608541
	Mean	3796398.998	2281956.636	605224.5638
	SD	146.7378615	4799.771661	3045.377043
	%RSD	**0.003865185**	**0.210335796**	**0.503181335**

Table 8 Result of Robustness by Change in Wavelength

Parameter	Variation	Area		
		MET	SITA	GLM
Wavelength	255 nm	3942466	2328043	608831
	Mean	3942373.666	2326962.176	610362.7356
	SD	106.6036272	1048.171901	3204.648239
	%RSD	**0.002704047**	**0.045044647**	**0.525039956**
	259 nm	4322278	2331340	622059
	Mean	4321929.325	2329532.25	623341.8906
	SD	331.9944779	1706.927454	1440.67623
	%RSD	**0.007681627**	**0.073273399**	**0.231121356**

Assay

The results of assay calculated in Table by comparing area of std and area test. (Table 9)

Table 9 Results of Assay for MET, SITA and GLM

Sample Name	Area of Std.	Area of Test	Conc. of Std. (µg/mL)	Found Conc. (µg/mL)	% Assay
MET	4210843	4196839	500	498.3371501	**99.66743001**
SITA	2242170	2225274	50	49.62322215	**99.24644429**
GLM	545582	544402	1	0.997837172	**99.7837172**

CONCLUSION

The literature survey reveals that various analytical methods were reported to estimate Sitagliptin phosphate monohydrate, Glimepiride and Metformin hydrochloride individually or combined with other drugs. In the present work, a novel, simple, sensitive, and acceptable RP-HPLC method for simultaneous estimation of Sitagliptin phosphate monohydrate, Glimepiride and Metformin hydrochloride in bulk and tablet dosage form was developed and validated. In the developed RP-HPLC method, the stationary phase was used as the Agilent C18 column (4.6mm×250mm, 5µm), and the mobile phase was used as 0.01M Ammonium Dihydrogen Orthophosphate and 0.05M Sodium Dihydrogen Orthophosphate and Acetonitrile in the ratio of (60:40) at a flow rate 1mL/min with a detection wavelength of 257 nm by using a UV-Visible detector. The retention time of Metformin hydrochloride, Sitagliptin phosphate monohydrate and Glimepiride and were found to be 2.35 min, 4.65 min and 7.77 min respectively. The developed RP-HPLC method was validated by using ICH Q2 (R2) guidelines and various validation parameters were performed. The linearity was found at the concentration range 400-600 µg/mL for Metformin hydrochloride, 40-60 µg/mL for Sitagliptin phosphate monohydrate and 0.8-1.2 µg/mL for Glimepiride with a correlation coefficient of 0.999, 0.998 and 0.9998. The % recoveries were obtained at a range of 98-102% which was acceptable. The %RSD was less than 2%, demonstrating the methods appreciable. The developed method was robust, reproducible, and can be used for routine quality control in bulk and pharmaceutical dosage forms in the pharmaceutical industry.

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