Development And Validation Of RP-HPLC Method For Simultaneous Quantification Of Three Anti-Diabetic Agents In Pharmaceutical Formulation

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Abstract
The simultaneous determination of sitagliptin, metformin, and glimepiride in pharmaceutical preparation has been accomplished through the development and validation of a novel, straightforward, quick, and sensitive HPLC approach. Using an isocratic mobile phase made of 0.01M phosphate buffer: acetonitrile (40:60), separation was accomplished on a 250mm x 4.6mm, 5µm particle, C18 column. The pH of the mobile phase was set at 5.6 with 50% orthophosphoric acid. A flow rate of 1 mL/min was used for the analysis. All chemicals were detected using UV absorbance at 230 nm, and the analytes were eluted in less than ten minutes. According to ICH criteria, the approach was validated. Over the concentration range of 10-70 µg mL-1 for MET, 5-35 µg mL-1 for SITA, and 1-30 µg mL-1 for GLI beyond, the linearity, accuracy, and precision of the method were satisfactory to good. The lower detection limit was found to be 0.67, 0.36, and 0.96µg mL-1 for, Metformin, Sitagliptin, and Glimepiride individually. The developed method is successfully used for the routine analysis of formulations for quality control and stability valuation.

Keywords: simultaneous, formulation, validation, Metformin, Sitagliptin, Glimepiride

Introduction

High-performance or high–pressure or liquid chromatography is mainly used in the pharmaceutical industry for the valuation of a large diversity of samples. It is the method of choice to examine the purity of new chemical entities, monitor changes in synthetic procedures or scale-ups, evaluate new formulations, and carry out quality control/assurance of the ultimate drug product [1].

Metformin Hydrochloride (MET) chemically described as 1-carbamimidamido-N,N-dimethyl. methanimid amide hydrochloride is a biguanide antihyperglycemic agent and first-line pharmacotherapy used in the management of type II diabetes. It is considered an antihyperglycemic drug because it lowers blood glucose concentration in type II diabetes without causing hypoglycemia. It is commonly described as an “insulin sensitizer”[1].Sitagliptin phosphate monohydrate (SITA)chemically(3*R*)-3-amino.-1-[3-(trifluoromethyl)-6,8-dihydro-5*H*-[1,2,4]triazolo.[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one is inhibits DPP-4 which leads to increased levels of glucagon–like peptides-1(GLP-1) and glucose-dependent insulinotropic polypeptides (GIP), decreased levels of glucagon, and a stronger insulin response to glucose[2]. Glimepiride(GLI) chemically described 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulphonyl]-3-*trans*-(4methylcyclohexyl)urea[2]. It is a direct secretagogue, indirectly it also increases insulin secretion in response to fuels such as glucose[3].

A review of the literature showed that whereas many articles have described various HPLC and UV techniques for quantifying these three chemicals separately and in combination with other medications, very few HPLC techniques have been applied for simultaneous measurement. GLI, MET, and SITA in the formulation. Nagunath ***et. al.,*** concluded Stability indicating Method Development and validation for Simultaneous estimation of Sitagliptin Phosphate and metformin HCl in tablets by HPLC.(2018), Ambadas, R. R.,***et. al.,*** estimated Metformin by UV Spectrophotometric Method in Pharmaceutical Formulation (2014),Rageeb,M.M., ***et al.,*** developed “Stability Indicating RP-HPLC Method for Determination of Saxagliptin and Dapagliflozin in Bulk and Tablet Dosage Forms” (2020), Mante,G.V.,***e.t al.,*** developed method for Dapagliflozin from its Tablet Formulation by UV-Spectrophotometry(2017).Joshi,R.,P.,***et al.,***“Developed and Validated of UV-Spectrophotometric Method for Estimation of Metformin Hydrochloride and Pioglitazone in Tablet Dosage Form” (2019).Jani,B., ***et al.,***“concluded UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture” (2015)[4-9].

Experiment:

Chemical and Reagents:

Working reference standards of Metformin (MET), Sitagliptin(SITA), and Glimepiride(GLI), were gifted by Biogenetic Drugs Pvt Ltd, Baddi, Himanchal. Water, methanol, orthophosphoric acid, HPLC-grade acetonitrile,

and di-sodium hydrogen orthophosphate were acquired from Rankem, Ranbaxy Fine Chemical Limited, located in New Delhi, India. Before use, the injection solution and mobile phase were filtered via a 0.45µm membrane.

Chromatographic Conditions:

SPD-10AV vp SHIMADZU using UV vis detector equipped with LC-10ADvp Shimadzu pump. Rheodyne injector with a 20µl loop. Separation and quantitation were achieved on a reversed-phase Princeton C18 analytical column (250 x 4.6mm, 5µm particle size) using mobile phase 0.01M Na2HPO4 buffer: acetonitrile with N2000 chromatography data system software. The pH of the mobile phase was adjusted at 5.6 with 50% orthophosphoric acid. The analysis was performed at a flow rate of 1 mL/min. The system was equilibrated with the mobile phase before injection. The column effluent was monitored on a UV detector set at 230nm

Preparation of Stock and Standard Solutions:

The drugs were dissolved in the solvents and diluted to the appropriate concentration to create standard stock solutions. The solvent system was the mobile phase. A 100 mL volumetric flask was filled with precisely weighed 100 mg of MET, SITA, and GLI, which were then dissolved in the mobile phase.

Preparation of Pharmaceutical Formulations for Assay:

The developed method was effectively applied to investigate MET, SITA, and GLI-marketed tablet formulations. The amounts recovered were stated as percent of the label claim. Analysis of marketed tablets Sitazit GM forte Tab, (Batch no. SZIT470, Glanmark Pharmaceuticals Ltd.) was carried out using enhanced mobile phase and HPLC conditions. A illustrative chromatogram is shown in Figure 1. The regular % drug contents of tablets obtained by the proposed method for MET, SITA, and GLI were noted to be 98.89 %, 99.28 %, and 100.25 %, respectively. Commercial Pharmaceutical Preparation: Sitazit GM forte(1000mg Metformin HCl, 100mg Sitagliptin phosphate monohydrate, and 1mg Glimepiride) a tablet from Glanmark Ltd., India were assayed.

Results and Discussion:

Method Development and Optimization: For the separation, MET, SITA, and GLI on a C18 column, chromatographic conditions were optimized with respect to pH, mobile phase, wavelength, and flow rate. Two organic solvents (methanol and acetonitrile) and pH values ranging from 6.7 to 2.75 (adjusted by the addition of orthophosphoric acid) were investigated throughout the method's optimization. Investigations using varying percentages of acetonitrile (20–70%) showed variations in solute retention with respect to the composition of the mobile phase, followed standard patterns, and showed faster elution of all substances studied. Acetonitrile 60% was selected as the best percentage because it strikes a balance between decision-making and research time. A pH of 5.6 was used to promote faster elution and better drug peak shape, which reduced the total analysis time. For MET, SITA, and GLI measurements by HPLC, the concomitant effects of flow rate, mobile phase pH, and optimum eluent composition were examined. A C18 column (250 x 4.6 mm, 5 µm particle size), a mobile phase of 0.01M Na2HPO4 buffer: acetonitrile (40:60) with pH 5.6, a flow rate of 1 mLmin-1, and a 20 µl injection volume at room temperature were the conditions that were ultimately chosen. Figures 1 and 2 depict a typical chromatogram of commercially available formulations and conventional medications, respectively.

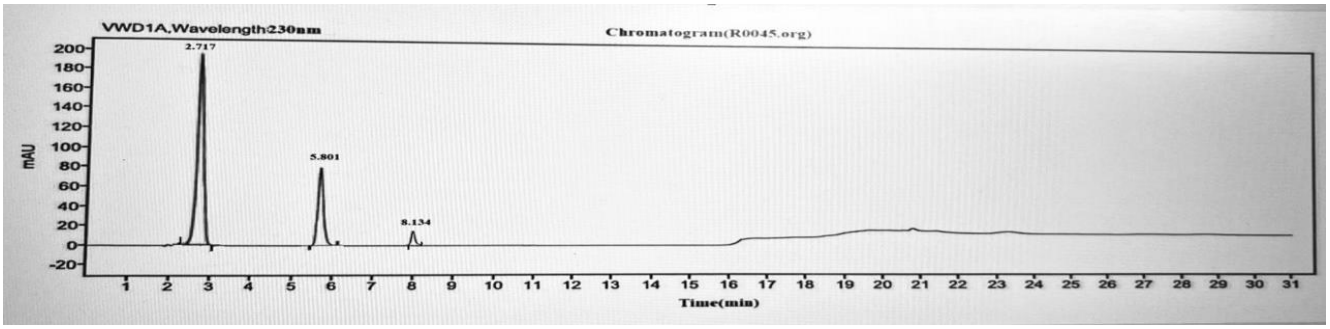

Fig:1 Chromatogram of a standard solution of Metformin, Sitagliptin, and Glimepiride

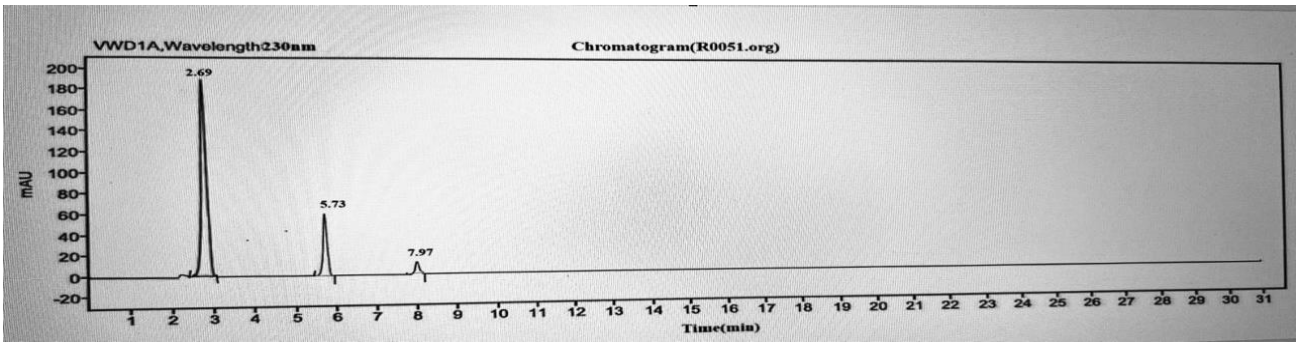

Fig :2 Chromatogram of tablet solution of Metformin, Sitagliptin, and Glimepiride

Linearity and Range:

Seven distinct concentrations were used to create the calibration curves, which ranged from 10–70 µg mL-1 for MET, 5–35 µg mL-1 for SITA, and 3–13 µg mL-1 for GLI. After injecting each solution three times, the peak mean area was plotted against the drug concentration to create a regression equation. The linear regression equation provided the best fit for the calibration curve, and the regression coefficient values (r2) for MET, SITA, and GLI were determined to be 0.9999, 0.9999, and 0.9991 respectively, suggesting a high degree of linearity for all medications.The MET, SITA, and GLI calibration curves are shown in Figure 3. MET, SITA, and GLI were determined to have linear regression equations of y = 150820x - 490054, y = 264140x + 32724, and y = 29123x + 174701, respectively. Table 1: Linearity parameters for the simultaneous estimation of MET, SITA and GLI.

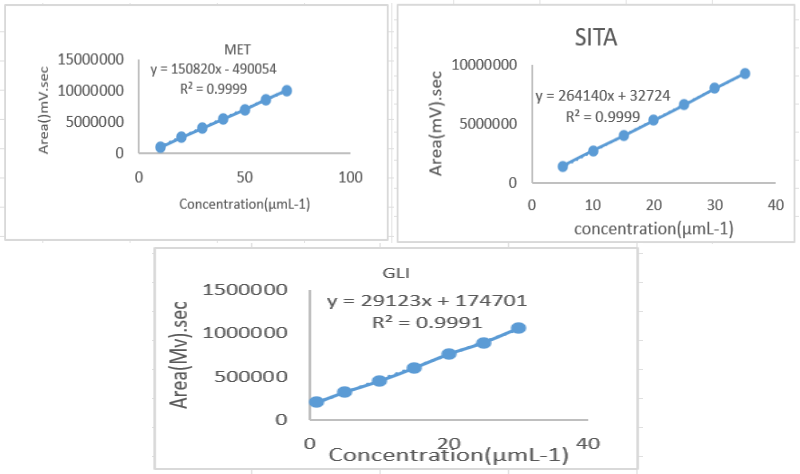

Figure:3-Calibration curves MET, SITA, and GLI

Parameters	**Drug**		
	MET	SITA	GLI
Range	10-70 µg.mL^{-1}	5-35 µg.mL^{-1}	3-13 µg.mL^{-1}
Regression coefficient (R2)	0.9999	0.9999	0.9991
Slope (m)	150820	264140	29123
Intercept (c)	460054	32724	174701

Table 1: Linearity parameters for the simultaneous estimation of MET, SITA and GLI

Accuracy and Recovery:

It was established by using the analytical approach on a synthetic combination of excipients that had been supplemented with known amounts of the medications, which corresponded to 80%, 100%, and 120% of the label claim [12]. Recovery and RSD were computed after three analyses of each concentration. The results showed that this strategy had a good level of accuracy. Pharmaceutical formulations contain excipients that do not impede the analysis of the substances they contain. The data of recovery studies are compiled in table 2.

Table 2: Results of recovery tests for the drugs under study by proposed method.

label claim (Percentage)	Metformin		Sitagliptin		Glimepiride	
	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
80%	100.19	0.16	101.08	0.19	100.75	0.16
100%	100.29	0.37	100.8	0.17	101.03	0.36
120%	100.31	1.21	100.31	0.10	101.12	1.20

Precision:

The degree of agreement between individual tests when the analytical technique is applied repeatedly to multiple sampling of a homogenous sample is expressed by the precision of the procedure. There are two levels of precision that are taken into consideration: intraday repeatability and interday intermediate precision. By analyzing the sample in duplicate on a single day, intraday precision was ascertained. Three consecutive days were used to measure inter-day precision, and the relative standard deviation (RSD) was used to represent the results [11]. The intra-day precision RSD values ranged from 0.0.33% to 0.96%, whereas the inter-day precision RSD values ranged from 0.24% to 0.67%.

The data of repeatability and intermediate precision are presented in table 2.

Table 2: Results of the precision (intra- and inter- days) for standard solution.

Real Concentration ($\mu g.mL^{-1}$)	**Measured Concentration ($\mu g.mL^{-1}$), RSD (%)**	
	Intra-day	Inter-day
MET		
12	12.17, ±0.61	12.11, ±0.52
24	24.29,± 0.75	24.26, ±0.44
36	36.34, ±0.55	36.09, ±0.65
SITA		
12	11.97, ±0.41	12.11, ±0.31
24	23.72, ±0.70	24.05, ±0.51
36	36.12, ±0.34	36.28, ±0.50
GLI		
12	12.11, ±0.31	12.23, ±0.41
24	24.17, ±0.63	24.26, ±0.60
36	36.13, ±0.47	36.14, ±0.78

Limits of Detection and Limit of Quantitation

The lowest concentration of the analyte in a sample that could be detected under the specified experimental conditions was known as the limit of detection (LOD), and the lowest concentration of the active ingredients in a sample that could be identified with a sufficient degree of precision and accuracy was known as the limit of quantitation (LOQ). The method based on the response's standard deviation (SD) and slope (m) was employed to establish the detection and quantitation limits in accordance with the International Conference on Harmonization's (ICH) recommendation [10]. The formula for calculating LOD is LOD = 3.3 (SD/m), and the formula for calculating LOQ is LOQ = 10 (SD/m). The LOD values for MET, SITA, and GLI were determined to be 0.678µgmL-1, 0.361µgmL-1, and 0.960µgmL-1, respectively. LOQ values were discovered to be 2.056µgmL-1 for MET, 1.09µgmL-1 for SITA, and 2.91µgmL-1 for GLI.The values of LOD and LOQ are summarized in Table 3

Table 3: The values of LOD and LOQ of M,S and G

Drug	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
MET	0.678	2.056
SITA	0.361	1.09
GLI	0.960	2.91

Assay of Pharmaceutical Dosage Form:

MET, SITA, and GLI in their dose forms were successfully determined using the suggested approach. The calibration curve approach was used to determine the concentration of each chemical. For every chemical, satisfactory findings that were in good agreement with label claims were obtained, as shown in table 4. The results show that the drug's content matches that on the label, confirming the suggested method's high accuracy. In fact, the test percentage ranged from 98.89 to 100.25% in relation to the label claims, indicating that the dosage form estimation was accurate.

Table 4: Assay results of active ingredients in a commercial sample

Drug	Label Claim (mg/tab)	Mean (µV.sec)	Conc. Found (mg/tab)	% Assay
MET	1000	99567790	988.9	98.89%
SITA	100	27617560	99.18	99.18%
GLI	1	41975.2	1.0025	100.25%

System Suitability:
To make sure the system is operating properly during the analysis, system suitability parameters need to be verified [13]. Standard working solutions were analyzed to determine system suitability data for the procedure. Table 5 shows the results of the determinations of the resolution, capacity factor, tailing factor, theoretical plates, retention volume, and asymmetry factor. It is clear from the table that the column efficiency is sufficient, with peaks that are clearly separated from the void volume and from one another.

Table 5: System suitability data

Parameter for System Suitability	MET	SITA	GLI	Acceptance Criteria
Resolution	-	6.24	7.61	Not more than5.0
Tailing factor	1.02	1.13	1.17	Not more than 2.0
Peak asymmetry	1.20	1.07	0.80	Not more than2.0
Theoretical plates	8750	7830	5588	Not less than 4000
Retention volume	2.717	5.801	8.134	-
% RSD	0.46	0.31	0.32	Not more than2.0

Conclusion:
In less than ten minutes, an isocratic HPLC method has been devised to determine the dose forms of Metformin, Sitagliptin, and Glimepiride in tablet form. The approach is more straightforward and precise. Validation of experimental results demonstrated the method's accuracy and dependability, making it suitable for routine tablet analysis for stability assessment and quality control.

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