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Original Article

Simultaneous Estimation Of Sitagliptin, Metformin Hydrochloride, Glimepiride In Bulk And Their Pharmaceutical Dosage Form By RP-HPLC Method

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ABSTRACT: *Hyperglycemia means an increased blood glucose level, also known as Diabetes Mellitus. Every year, the number of patients with this disease has been increase tremendously. A person has to administer medicine to manage the symptoms of diabetes. Therefore, the pharmaceutical dosage form to be evaluated for patient safety and efficacy. The method was developed and validated as per the ICH guideline for the simultaneous estimation of three drugs in a combined dosage form as a tablet. Sitagliptin, Metformin Hydrochloride, and Glimepiride drugs were analysed by using the RP-HPLC technique at 230 nm. During analysis, the linearity of these drugs was 12.5-62.5, 250-1250, and 0.5-2.5 µg/ml for Sitagliptin, Metformin Hydrochloride, and Glimepiride, respectively. Accuracy was obtained between 97 to 99% for all drugs. The proposed method was employed in a pharmaceutical solid dosage form for assay values found between 98 to 99.5%, respectively. Several other parameters, including LOD, LOQ, Precision, Specificity and Robustness, were also applied, and the method was considered sensitive, precise, specific and robust, which was the prime objective of method development and validation. The proposed method has value for quantitative and qualitative estimation in any pharmaceutical dosage form. Furthermore, this technique would become a basis for bioanalytical study and the impurity profile of these substances.*

KEYWORDS: *RP-HPLC, Sitagliptin, Metformin hydrochloride, Glimepiride, Analysis.*

1. INTRODUCTION

Diabetes mellitus refers to a group of diseases that impact how the body uses blood sugar (glucose). Glucose serves as a crucial energy source for the cells in our muscles and tissues, as well as the brain's primary fuel[12]. There are two main types of diabetes. In Type 1 diabetes, an autoimmune condition, the pancreas doesn't produce enough insulin. Insulin is essential for regulating blood sugar levels. People with type 1 diabetes require insulin injections or an insulin pump to manage their blood glucose. Type 2 diabetes form is more common and often develops due to insulin resistance. In type 2 diabetes, the body's cells become less responsive to insulin, leading to elevated blood sugar levels. Lifestyle changes, oral medications, and sometimes insulin injections are used to manage Type 2 diabetes.

Consistently high blood glucose levels can lead to health complications, including heart disease, nerve damage, and eye issues. Regular monitoring, a balanced diet, physical activity, and medication play crucial roles in diabetes management. Management of this disease requires medication for a longer period of time, and sometimes, physicians prescribe medicines in a combined dosage form.

Sitagliptin regulates insulin levels in the body, increasing insulin release from the pancreas and signalling the liver to reduce glucose production when blood sugar levels are high. Metformin reduces glucose absorption from the intestines, lowers liver glucose production, and improves insulin sensitivity. Glimepiride belongs to the sulfonylurea class and stimulates insulin release from the pancreas. It helps lower blood glucose levels by increasing insulin sensitivity.[1-2]

In this proposed study, Sitagliptin (SIT), Metformin Hydrochloride (MET) and Glimepiride (GLI) drugs were estimated quantitatively and qualitatively by the RP-HPLC method. As an individual dosage form, there are plentiful methods available for estimation of SIT, MET and GLI by UV Spectrophotometer, HPLC, HPTLC, GC and LC-MS/MS [3-14], but no single method is available to quantify these drugs simultaneously. Therefore, the objective was set to develop and validate an HPLC method for a combined dosage form as per the ICH guideline.

2. EXPERIMENT AND MATERIALS

2.1. MATERIALS AND INSTRUMENTS

SIT, MET and GLI reference standards provided by Sun Pharma (Vadodara, Gujarat, India). Methanol, triple-distilled water, sodium hydroxide, and hydrochloric acid were all obtained from Finar Chemicals Pvt Ltd in Ahmedabad, India. It was of HPLC quality. The Quaternary gradient LC 2030 plus HPLC apparatus was made by Shimadzu Corporation in Japan. All of

the information was recorded using lab-solution software. The weighing balance belonged to Sartorius.

2.2. CHROMATOGRAPHIC CONDITION

The following chromatographic conditions were used to estimate pharmaceuticals. The column is the heart of separation in HPLC. The Kromasil 100 C18 column was employed, with dimensions of 150 mm x 4.6 mm and particle size of 5 µm. The temperature of the column oven was set to 40 °C. Several attempts for mobile phase selection were conducted before settling on Methanol: 50mM phosphate buffer pH 3.00 ± 0.02: Acetonitrile (30:40:30 %v/v/v) to provide the optimum chromatographic parameters, such as resolution, tailing factor, theoretical plates, and asymmetric factor. The flow rate and isobestic wavelength were both set to 0.8 ml/min and 230 nm.

2.3. PREPARATION OF MOBILE PHASE

The mobile phase was made by combining methanol, phosphate buffer and acetonitrile as per ratio given in the chromatographic condition. The prepared mobile phase was degassed and filtered using a nylon membrane filter with a 0.45 µm diameter.

2.4. PREPARATION OF SOLUTIONS

10 mg of SIT, MET and GLI were precisely weighed and then added to a 10 ml volumetric flask. The drugs were dissolved in the mobile phase to make a solution with a concentration of 1000 µg/ml (stock solution). Aliquots of the solutions were taken to prepare a working solution of 100 µg/ml for the respective drugs.

3. METHOD VALIDATION

The proposed method was validated with several experimental parameters, such as linearity, precision, accuracy, specificity, LOQ, LOD, and robustness.

3.1. LINEARITY

Weight accurately 10 mg SIT and 1 mg MET and GLI each and transfer into 50 ml and 10 ml volumetric flasks, respectively. The volume made up to the mark with diluent to get final concentrations of SIT (12.5-62.5µg/ml), MET (250-1250 µg/ml) and GLI (0.5-2.5µg/ml).[8]

3.2. SYSTEM SUTABILITY (REPEATABILITY)

To attain repeatability, a solution containing both medications at a concentration of 37.5µg/mL (SIT),750µg/mL (MET) and 1.5 µg/mL (GLI) was injected six times onto the HPLC system. Each time, the area under the curve and the tailing factor were recorded and used to determine the relative standard deviation.

3.3. ACCURACY

This parameter was calculated using the standard addition approach. The drug solutions were spiked at three different doses (80,100, and 120%) in a sample solution of 25 µg/ml for SIT, 500 µg/ml for MET and 1 µg/ml for GLI of three medications for recovery analysis. The mean recovery, as well as the SD and RSD, were computed.

3.4. PRECISION: (REPRODUCIBILITY)

Interday and intraday studies were carried out in order to express within-laboratory variances on different days of analysis. The precision of the proposed investigation was assessed at different concentrations for linearity and the range of respective drugs. All samples were collected in duplicate.[9]

3.5. LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The LOD and LOQ of the drugs were calculated using the following equations as per the ICH guideline.
LOD = 3.3 X N/S and
LOQ = 10 X N/S.
Where N is the standard deviation of the peak areas of the drug, and S is the slope of the corresponding calibration curve.

3.6. ROBUSTNESS

It was designed by deliberately adjusting key parameters such as mobile phase ratio, wavelength, flow rate, and % RSD.

3.7. SPECIFICITY

The specificity of an analytical method shows that the analytical method can measure the analyte of interest accurately and precisely without interference from blank and placebo. A specificity research was carried out to demonstrate that the presence of excipients had no effect on the procedure.

4. RESULTS AND DISCUSSION [5]

4.1. LINEARITY

Peak area and concentrations of SIT, MET and GLI in the range of 12.5-62.5 µg/mL, 250-1250µg/mL, and 0.5-2.5 µg/mL, respectively, were found to have a linear relationship. The calibration curve revealed that the correlation coefficient 0.9996,0.9991 and 0.9995 for SIT, MET and GLI, respectively. (Figure:1)

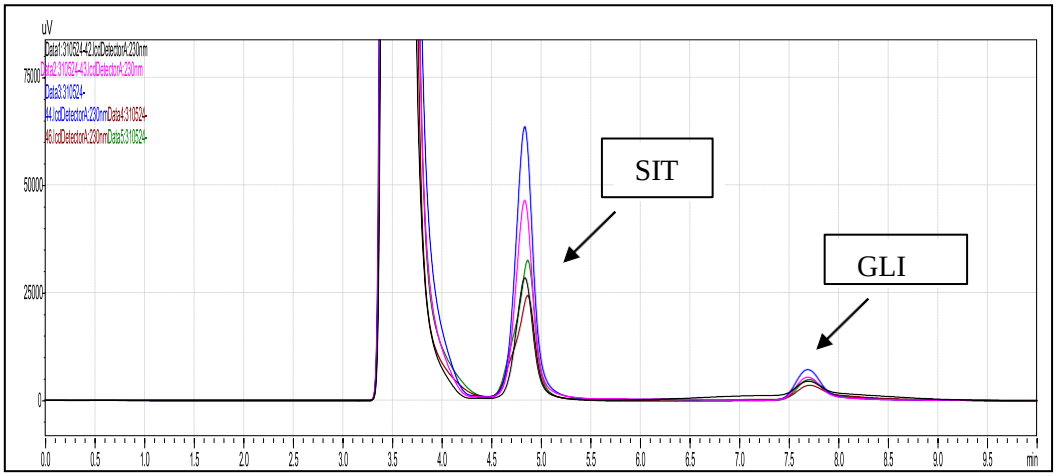

FIGURE I Chromatogram of MET(3.4 min), SIT (4.8 min) and GLI (7.7 min)

4.2. SYSTEM SUITABILITY

The relative standard deviation of the resulting area under the curve was calculated and found to be 1.08% for SIT, 1.2 % for MET and 1.4 % for GLI, respectively. All of the results were within an acceptable range, less than 2%, indicating that the approach was reproducible.

4.3. ACCURACY

The recovery experiment was carried out by the standard addition method. Values of % mean recovery of all three drugs were found to be 99 – 101 %.

4.4. PRECISION

The intra-day and inter-day precision % RSD was obtained between 1.1 – 1.2 for the ball drugs.

4.5. LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limits of detection for SIT, MET and GLI were 5.41 µg/ml, 0.41 and 122.4 µg/ml, respectively, while the limits of quantification for SIT, MET and GLI were 16.42 µg/ml, 1.2 and 371.1 µg/ml, respectively. All data was calculated using a formula.

4.6. SPECIFICITY

Specificity was determined by injecting blank and combined standard solutions. HPLC chromatograms were compared to check for interference.

4.7. ROBUSTNESS

On deliberate, minor changes of chromatographic parameters include wavelength, mobile phase and flow rate. % RSD was between 0.05 and 0.91 for both drugs.

ASSAY: The prepared solutions from the synthetic mixture were injected into the HPLC apparatus. The result was within an acceptable range. There was no evidence of excipient interference with peaks of interest. The proposed approach would be used to conduct quantitative and qualitative analyses of the medications mentioned.

TABLE 1 Summary of validation parameters

System Suitability parameters			
Parameters	**SITA**	**MET**	**GLIME**
ValidationParameters			
Specificity	specific	Specific	Specific
Linearity(n=3)	12.5-62.5µg/ml	250-1250µg/ml	0.5-2.5µg/ml
Repeatability(%RSD)	1.08	1.2	1.4

Intradayprecision(%RSD)	1.5	1.4	1.1
Interdayprecision(%RSD)	1.3	1.3	1.2
Accuracy(%Recovery)	99.00	99.94	101.0
Robustness	Robust	Robust	Robust
LOD(µg/mL)	5.41	0.41	122.4
LOQ(µg/mL)	16.42	1.2	371.1
%Assay	99.6	99.2	99.5

5. CONCLUSION

The proposed HPLC method was developed and validated for the simultaneous estimation of SIT, MET and GLI in pharmaceutical dosage form. The method was linear, accurate, precise and robust. However, even with the LC-MS/MS method for impurity profile generation for antidiabetic drugs, is available and comprehensive impurity profile is mandatory for regulatory approval to ensure quality, safety and efficacy of formulations.[14] Analysts may make a basis for such sensitive and superior techniques. The proposed method is to be extended for bioanalysis too, and to meet the IPQC requirement.

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