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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE MONOHYDRATE, METFOIRMIN HYDROCHLORIDE AND GLIMEPIRIDE IN TABLET DOSAGE FORM USING RP-HPLC

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ABSTRACT

The aim of the current study was to develop a method that could be used for quantitation of Metformin hydrochloride, sitagliptin and glimepiride in a tablet dosage form. The HPLC system used in this study consisted of Inertsil C8 (250 x 4.6 mm, 5 µm particle size), and the mobile phase was composed of methanol and buffer, pH 5 in a gradient elution mode. The flow rate was set at 1.0 mL/min, and the detection was performed at a wavelength of 215 nm. The method was validated per ICH guidelines, assessing key analytical parameters such as specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness. Linearity was established within the concentration range of sitagliptin phosphate monohydrate 25-75 µg/mL, metformin hydrochloride 500-1500µg/mL and glimepiride 1-3 µg/mL, with a recovery rate ranging for sitagliptin phosphate monohydtrate from 98 to 100.3%, metformin hydrochloride range from 99-101.2% and glimepiride range from98-101.3%. The developed RP-HPLC method demonstrated excellent specificity, accuracy, precision, and robustness, making it well-suited for routine quality control analysis of Tablet Dosage form.

INTRODUCTION

Diabetes mellitus (DM) It is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia.

SYMPTOMS

1. Feeling Very Thirsty
2. Needing To Urinate More Often Than Usual
3. Blurred Vision
4. Feeling Tired
5. Losing Weight Unintentionally
6. Slow Healing Sores

Causes and Risk Factors:

1. Overweight / Obesity
2. Low physical activity
3. High fat & cholesterol level
4. High blood pressure
5. Smoking & Alcohol
6. Have a family history of diabetes
7. Lead an inactive lifestyleCauses

TREATMENT

A. Insulin sensitizers

- Biguanides: metformin
- Thiazolidinediones: Pioglitazone, Rosiglitazone

B. Insulin Secretagogues

- Sulfonylureas: Tolbutamide, Chlorpropamide, Glimepiride, Glipizide
- Meglitinide: Repaglinide Nateglinide

C. α-Glucosidase Inhibitors

- Acarbose
- Miglitol

D. Dipeptidyl Peptidase IV Inhibitors • Sitagliptin • Vildagliptin • Saxagliptin • Linagliptin

E. Sodium-Glucose Co-Transporter Inhibitors • Canagliflozin • Dapagliflozin

Experimental work

In the present research, an effort was made to develop and validate a stability-indicating RP-HPLC method for the estimation of Sitagliptin phosphate monohydrate, metformin hydrochloride and glimepiride in tablet dosage form.

Procurement of drug

Sitagliptin phosphate monohydrate, metformin hydrochloride and glimepiride API was received as gift a sample zydus lifescience Ahmedabad, Gujarat

REAGENTS AND CHEMICALS

The list of Reagents and chemicals used in method development was shown in Table

Reagents/Samples	Supplier
HPLC grade Methanol 99%	Rankem
AR Grade Triethylamine	Merck
HPLC grade Orthophosphoric Acid	Thermo Fisher Scientific
HPLC grade Water	Spectrochem
Metformin Hydrochloride (Working Standard)	Zydus Healthcare
Glimepiride (Working Standard)	Zydus Healthcare
Sitagliptin (Working Standard)	Zydus Healthcare
SitaKite-GM2 Tablets (50 mg Sitagliptin, 1000 mg Metformin HCl, 2 mg Glimepiride)	Biofast / Local Pharmacy

List of Reagents and Chemicals

INSTRUMENTS AND APPARATUS

List of Instruments

Sr. No.	Name of Equipment/ Instrument	Manufacturer
1	UV Spectrophotometer	Shimadzu UV-1800 double-beam UV- VIS spectrophotometer Software: UV Probe
2	HPLC	Make: Shimadzu Model:- LC 2010 CHT Injector: 100µL fixed loop. Detector: UV Detector Software: LC Solution
3	Analytical balance	Sartorius Analytical Balance Accuracy: 0.001 g
4	pH Meter	Lab India Digital pH Meter
5	Melting Point Apparatus	Lab Junction Melting Point Apparatus
6	Ultrasonic Bath	Athena Technology
7	Hot Air Oven	Patel Scientific
8	FT-IR	Agilent Cary 630 FTIR Spectrometer Software: MicroLab Expert
9	Micropipette	Eppendorf

List of instrument

Apparatus

Components	Description
Volumetric flask	Borosilicate glass
Pipette	Borosilicate glass
Measuring cylinder	Borosilicate glass
Beaker	Borosilicate glass

PRELIMINARY WORK

Drug identification

The identification of the sitagliptin phosphate monohydrate,metformin hydrochloride and glimepiride standard API for experimental work was carried out to confirm its identity, standard, quality, and purity. This was achieved through melting point determination and IR analysis.

Procedure for Solution Preparation **Standard Solution Preparation:**

Buffer Preparation (Reference IP 2022, monograph of metformin and glimepiride tablets, page 2877): Dissolved 0.5 g sodium dihydrogen phosphate in 500 ml of water, adjusted to pH 2.1 with orthophosphoric acid.

Mobile phase preparation:

Buffer: Methanol were used in a gradient program setting.

Diluent:

Buffer: Methanol 50:50

Metformin Stock solution (10,000 ppm):

About 250 mg of metformin hydrochloride working standard was accurately weighed and transferred into 25 ml volumetric flask. To this, 10 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Sitagliptin stock solution (500 ppm):

About 50 mg of linagliptin working standard was accurately weighed and transferred into 100 ml volumetric flask. To this, 100 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Glimepiride stock solution (20 ppm):

About 5 mg of glimepiride working standard was accurately weighed and transferred into 250 ml volumetric flask. To this, 0.5 mL 0.1 N NaOH added to solubilize the content. 100 ml of methanol was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Mix standard solution (1000 ppm metformin + 50 ppm linagliptin+ 2 ppm glimepiride):

Pipette out 1 ml of each stock solution into 10 ml volumetric flask. Make volume upto the mark with diluent to get mixed standard solution.

Sample preparation:

Five tablets of Istamet G 50/1000/2 were taken and make fine powder. Powder equivalent to one tablet was weighed and transferred to 100 mL volumetric flask. To this approximately 50 mL methanol and 0.5 mL 0.1 N NaOH added and sonicated for 10 minutes and vortex for 5 minutes. The volume was made upto the mark with methanol and filtered through Whatmann 0.45 µ syringe filter. Further aliquot of 1 ml of the clear filtrate was taken into 10 ml volumetric flask and diluted upto the mark with diluent to get a final concentration of 1000 ppm metformin + 50 ppm sitagliptin+ 2 ppm glimepiride.

Mobile Phase Preparation

Method Development and Optimization of Chromatographic Conditions

The objective of the present study was to develop and validate a stability-indicating RP-HPLC method for the estimation of sita,met and glime in tablet dosage form. During method development, chromatographic parameters such as mobile phase composition, flow rate, detection wavelength, analytical column, and column temperature were optimized to enhance the efficiency of the chromatographic system.

Method Development and Optimization of Chromatographic Conditions

The objective of the present study was to develop and validate a stability-indicating RP-HPLC method for the estimation of sitagliptin phosphate monohydrate ,metformin hydrochloride and glimepiride in tablet dosage form. During method development, chromatographic parameters such as mobile phase composition, flow rate, detection wavelength, analytical column, and column temperature were optimized to enhance the efficiency of the chromatographic system.

HPLC analytical columns, including InertsilC8 (250 mm × 4.6 mm × 5 µm), were evaluated during method development. System suitability parameters such as retention time, theoretical plates, tailing factor, and resolution were assessed. Based on these parameters, the InertsilC8 (250 mm × 4.6 mm × 5 µm) column was selected as the final choice. Various mobile phase combinations, including methanol, acetonitrile, and buffer in different proportions, were tested to achieve optimal separation

VALIDATION OF RP-HPLC METHOD

The proposed RP-HPLC method was validated in accordance with the ICH Q2 (R2) guidelines. The validation method evaluated a number of parameters, including system suitability, precision, accuracy, specificity, linearity, range, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability in the analytical solution. Each parameter was assessed to ensure the technique for estimating the dosage of relugolix in tablet form was accurate, repeatable, and reliable.

System Suitability Study

To evaluate system compatibility, a mixed standard solution of drug was injected into the HPLC system in six replicates (n=6). In order to make sure the system is suitable for the desired analysis, system suitability testing is an essential part of developing chromatographic methods. Resolution, tailing factor, and theoretical plates were among the system appropriateness factors assessed.

Specificity

A chromatogram of the blank was taken in order to guarantee the specificity of the created RP-HPLC technique. By making sure that the analyte peak was clearly separated from any possible interfering peaks, the method's specificity was verified. Additionally, the spectra of the sample solution and standard solutions of drug were compared in order to assess specificity. To compare retention duration and resolution parameters, a representative chromatogram of the standard and sample solutions was examined to make sure that no excipient or impurity interference was detected. This evaluation confirmed that additional formulation ingredients do not interfere with the method's ability to identify the drug.

Linearity and Range (n = 3)

The linearity of the well-established RP-HPLC method was evaluated by applying least squares regression on the calibration curve. The method showed a linear response for in the

concentration range. Several volumes of the standard stock solution were diluted into volumetric flasks to generate calibration standards, and methanol was added to the final volume. A graph was plotted with concentration on the x-axis and peak area on the y-axis. The correlation coefficient (R2), slope, and intercept were calculated to verify that the procedure responded linearly within the measured range.

Precision

Precision was examined at three levels: repeatability, intraday (intermediate precision), and interday (reproducibility). The consistency of the analytical method was assessed by calculating the relative standard deviation (% RSD) from replicate measurements under each condition.

Repeatability

A standard solution of sitagliptin phosphate monohydrate ,metformin hydrochloride and glimepiride was injected six times (n = 6) under the same chromatographic conditions in order to evaluate repeatability. To assess the method's short-term repeatability, the percentage RSD of the peak regions was computed.

Intermediate Precision

Intraday Precision:

Six replicate injections of sitagliptin,metformin and glimepiride sample solution were carried out using the suggested RP-HPLC method in order to evaluate same-day variability. To verify method reliability over the course of a single day, the percentage RSD of peak areas was computed.

Interday Precision:

Sample solutions containing sitagliptin,metformin and glimepiride were examined over three days in order to assess between-day variance. To confirm the method's repeatability over time, the percentage RSD was calculated.

Limit of Detection and Limit of Quantitation

The calibration curve was used to determine the LOD and LOQ independently. The standard deviation (SD) of the intercept was computed after the calibration curve was repeated three times. The following formula was then used to determine LOD and LOQ:

$LOD = 3.3 * \sigma/S$ $LOQ = 10 * \sigma/S$

Where, σ= Standard deviation of Y-intercepts, S = Mean slope of calibration curve.

Accuracy

The usual addition procedure was used to make sure the suggested methodology was appropriate and dependable. Three levels of standard RELU solutions (50, 100, and 150%) were added to the sample solution that had already been analyzed. Aliquots of the working standard solution were spiked independently and diluted with diluent to the appropriate levels in a 100 ml volumetric flask containing 1 ml of the pre-analyzed sample solution of drug. Concn of drug was then determined by analyzing the resulting solutions.

Robustness

An analytical procedure's robustness indicates its dependability under typical operating conditions and is a measure of its ability to withstand minor but intentional alterations in the technique parameters. Robustness was tested (n=3) by adjusting a number of parameters, including temperature, mobile phase flow rate, Finding The wavelength Changes were made to the mobile phase flow rate (± 0.2 ml/min), the detection wavelength (± 2 nm), and the temperature(±5°C).

Assay of Formulation (n=3)

Five tablets were weighed, and the average weight was calculated. The tablets were then finely powdered using a mortar and pestle. A quantity of powder equivalent to drug was accurately transferred to a 100 mL volumetric flask.

Sample Preparation Procedure:

- **Dilution & Sonication:** diluent was added to the flask, and the mixture was sonicated for 15 minutes to ensure complete dissolution.
- **Final Volume Adjustment:** The solution was diluted to the mark with the diluent.
- **Settling & Filtration:** The solution was allowed to settle for 5 minutes, then filtered through a 0.45-micron syringe filter to obtain a clear filtrate.
- **Final Sample Preparation:** 1 mL of the clear filtrate was further diluted to 10 mL using diluent, and this solution was used for the assay.
- The prepared sample solution was analyzed using the validated RP-HPLC method for the estimation of relugolix in tablet dosage form.

SUMMARY AND CONCLUSION

METHOD VALIDATION SUMMARY of RP-HPLC

Parameters	Sitagliptin	Metformin	Glimepiride
Linearity Range (µg/ml)	25-75	500-1500	1-3
Regression equation, y = mx + c	Y=7084.4x+5047.4	Y=8302.3x+96668	Y=267842x+128840
CorrelationCoefficient (R^2)	0.9988	0.9994	0.9981
Detection limit (µg/ml)	2.8 µg/ml	3.29 µg/ml	0.11 µg/ml
Quantitation limit (µg/ml)	8.6 µg/ml	9.98 µg/ml	0.33 µg/ml
Repeatability (% RSD	0.66	0.42	0.74
Intra-day, % RSD	0.35-1.39	0.24-0.75	0.10-0.50
Inter-day, % RSD	0.51-1.88	0.31-0.80	0.33-0.83
Accuracy (%recovery % RSD	98.9-100.3	99.8-101.2	98.1-101.3
Robustness	The system suitability parameters were found well within the acceptance criteria as per system suitability.		
Change in Temperature (+5 ℃ and -5 ℃) Change in Flow rate (+1 ml/min and -1 ml/min) Change in Organic Phase (+ 2 nm and -2 nm)	All are Within the Range		
Label amount found % label claim ± SD	101.7±0.010	100.0±5.0	100.7±0.020

Summary of RP-HPLC Validation Parameters of sitagliptin phosphate monohydrate, metformin hydrochloride and glimepiride

CONCLUSION

From above observations, it can be concluded that developed Stability indicating method and validation of Sitagliptin,metformin and glimepiride in tablets by RP-HPLC is, specific, linear, accurate, precise and robust. Thus above developed RP-HPLC method can be applied for routine analysis.

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