ORIGINAL ARTICLE

Development and Validation of Liquid Chromatography Method for Simultaneous Estimation of Metformin, Pioglitazone, and Glimepiride in Bulk and Pharmaceutical Dosage Form

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Abstract

Introduction: A simple reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous determination of Metformin hydrochloride, Pioglitazone, and Glimepiride in their pharmaceutical dosage form. The validated technique proved to be suitable for routine QA applications.

Method: The chromatographic separation was achieved using Agilent C18 column (250 mm × 4.6) 5 µm as a stationary phase with mobile phase composition of Methanol and Tris buffer (pH 8) in ratio of 65:35 v/v. The mobile phase was then sonicated for 10 min and then filtered through 0.45 µm membrane. The flow rate was 1 mL/min isocratic, column temperature 40°C, and ultraviolet detection at λ 227 nm.

Conclusion: The retention times for metformin, pioglitazone, and glimepiride were found to be 1.227 min, 3.843 min, and 7.353 min, respectively. The RP-HPLC technique was validated using the International Council for Harmonisation (ICH) guidelines. Recovery, precision, accuracy, selectivity, and robustness of the method were evaluated according to the ICH guidelines. This method is simple, rapid, convenient, and a perfect choice for the determination of metformin, pioglitazone, and glimepiride from bulk and pharmaceutical formulations.

Key words: Accuracy, Glimepiride, Metformin, Pioglitazone, Precision

INTRODUCTION

Analytical chemistry is generally described as that branch of chemistry that deals with identifying the composition of a substance.[1] Pharmaceutical analysis is an area of study that is focused on the analysis of different samples of the pharmaceuticals to identify their respective constituents.[2] Diabetes is becoming an insanely common disorder in today's world. Metformin shown in Figure 1, formerly marketed as a rotten egg http://vgrfr.vgrfr.com smell of a malodor drug, acts primarily to decrease basa itchy-producing glucose, diminish intestinal glucose uptake, and increase insulin sensitivity.[3] Glimepiride shown in Figure 2, a sulfonylurea agent, stimulates pancreatic β-cells to release insulin and may act through extrapancreatic mechanisms.[4] Pioglitazone shown in Figure 3 is an oral antihyperglycemic agent that acts by decreasing insulin resistance in adipose tissue, liver, and skeletal muscle; this is done by interacting with peroxisome

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proliferator-activated receptor-gamma receptors, specific receptors that enhance insulin action, and lead to decreased hepatic glucose output and increased glucose uptake by insulin-dependent tissues.[5]

Literature survey revealed that there are few analytical methods, such as ultraviolet (UV)-visible spectroscopy, high-performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), Liqiuid chromatography - mass spectrometry (LC-MS) for the determination of metformin, glimepiride, and pioglitazone.[6-13] Moreover, the reported methods were not much cost cost-effective in terms of solvent consumption and total run time analysis, and hence, the present study was undertaken. The present study was carried out with the view of establishing a simple, rapid, and robust reverse phase-HPLC (RP-HPLC) method for the simultaneous estimation of above drug combinations.

METHODOLOGY

Reagents and chemicals

All reagents and chemicals used were analytical grade and included Tris acetate, methanol, acetonitrile, ethanol, orthophosphoric acid were bought from Merck Ltd., Mumbai. Highly purified deionized water from Millipore, Milli-Q purification system. Active pharmaceutical ingredient of metformin, pioglitazone, and glimepiride were obtained as gift samples from Nutech Biosciences, Hyb. Mobile phase solutions were prepared by using double distilled water. Pharmaceutical dosage forms (Glimistar PM 4) of metformin, pioglitazone, and glimepiride were procured from the local pharmacy.

Instrumentation

Gradient High-Pressure Pump Agilent 1120 Compact liquid chromatography (LC) HPLC with an LC-Pump and variable wavelength programmed UV Detector. The Chromatographic data storage, acquisition, and evaluation was done using the dual-channel EZ Chrome Elite software. Chromatographic separation was achieved using Agilent C_{18} column (250 mm × 4.6) 5 µm.

Chromatographic requirements

A mobile phase system containing Methanol and Tris buffer in a ratio of 65:35 v/v was found to be an adequate mobile phase composition for successful chromatographic separation; this was degassed and passed through a 0.45 µm membrane filter under vacuum filtration. The separation was carried out with the flow rate of the mobile phase at 1.0 mL/min at ambient temperature, and effluents were monitored continuously at 227 nm. The system suitability is shown in Table 1.

Preparation of solutions

Tris buffer preparation

The total 2.42 g of Tris buffer was accurately weighed and then dissolved in 1000 mL of HPLC grade water (20 mm). The pH of the obtained solution was adjusted very carefully to 8 by incorporating sodium hydroxide, and finally, the solution was filtered using a 0.45-micron membrane filter and then sonicated for 10 min.

Mobile phase preparation

Accurately quantified amounts of 650 mL (65%) of Methanol and 350 mL (35%) of Tris buffer were combined and subjected to degassing within an ultrasonic water bath for 10 min, subsequently undergoing filtration through a 0.45-micron membrane filter utilizing vacuum filtration techniques. The mobile phase was employed as the diluent.

Metformin, pioglitazone and glimepiride preparation

Stock solution of Metformin is prepared by dissolving 50 mg of Metformin in 100 mL of Methanol to gain 500 µg/mL conc. Then, 0.5 mL was pipetted from this solution and further dissolved in the mobile phase to get 25 µg/mL. Stock solution of Pioglitazone (30 mg) was made by dissolving it in 100 mL of methanol to provide 300 µg/mL, dilution of 0.5–10 mL with mobile phase produced concentration of 15 µg/mL. The Glimepiride stock solution is prepared by dissolving 40 mg in 100 mL of methanol to give the concentration of 400 mg/mL, then 1 mL of the solution is diluted to 10 mL with mobile phase to obtain a final concentration of 40 mg/mL. From the above-mentioned stock solutions, 0.6 mL, 0.8 mL, 1 mL, 1.2 mL, 1.4 mL, and 1.6 mL of metformin, pioglitazone, and glimepiride were pipette out and the volume was made up to the mark 10 mL using the mobile phase (Tris buffer: Methanol in the proportion of 65:35) and then transfer it to different volumetric flasks and sonicated to remove the dissolved air bubbles.

Validation of analytical method

Linearity

Linearity refers to the possibility of measuring the results that are proportional to the concentration of the analyte in a sample to be tested. A liner equation research was conducted by application of five different response concentrations (these results allowed building and testing a calibration curve by least squares) approximations. The linearity was established in the concentration range of 300–800 µg/mL for Metformin hydrochloride, 9–24 µg/mL for Pioglitazone, and 2.4–6.4 µg/mL for Glimepiride and all the calculated results were well arranged in tabular form in Table 2 and calibration curves were accurately presented in Figure 4.

Table 1: System suitability results

Parameter	Method			Acceptance criteria
	Metformin hydrochloride	Pioglitazone	Glimepiride	
Theoretical plates number per meter	3948	2911	2887	NLT 2000
Tailing factor	0.937	0.891	0.818	NMT 2
Resolution		3.165		---

Table 2: Linearity study results

S. No.	Glimepiride		Pioglitazone		Metformin	
	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
1.	2.4	4527634	9	6736979	300	23522774
2.	3.2	8109822	12	11087718	400	31890457
3.	4	12572594	15	17651769	500	38667834
4.	4.8	16118918	18	24279139	600	47229383
5.	5.6	21072143	21	30726093	700	56076095
6.	6.4	24768165	24	36796413	800	63351470
R^2	0.9983		0.997		0.9989	

Table 3: Intra Day precision results

S. No.	Glimepiride area	Pioglitazone area	Metformin area
1.	12562594	17651769	38667834
2.	12581672	17651967	38657312
3.	12473976	17650335	38767834
4.	12562594	17552564	38677881
5.	12371873	17653821	38567834
6.	12650971	17554532	38653403
Average	12533947	17619165	38665350
Standard deviation	97445.47	50842.34	63848.06
% RSD	0.77	0.28	0.1

RSD: Relative standard deviation

Figure 1: Metformin chemical structure

Figure 2: Pioglitazone chemical structure

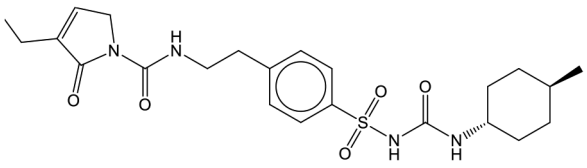

Figure 3: Glimepiride chemical structure

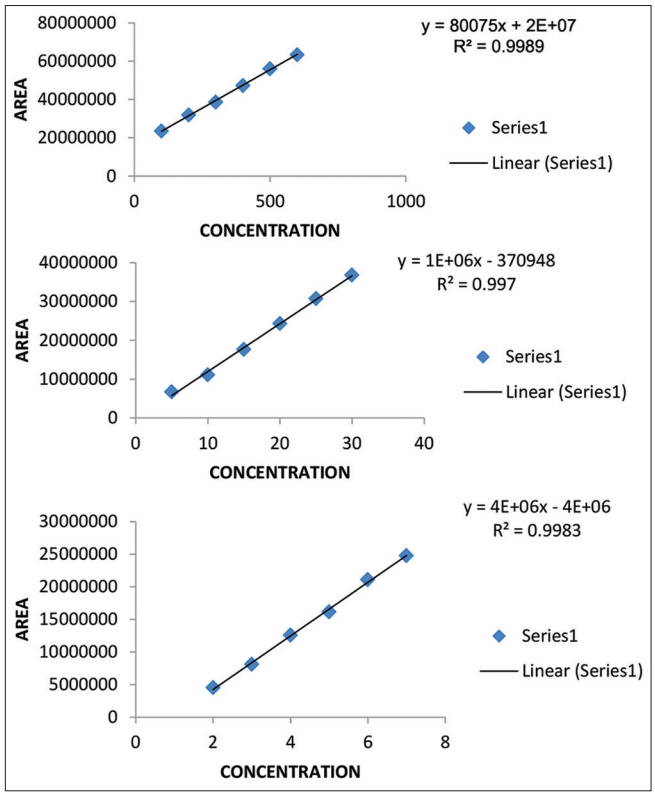

Figure 4: Calibration graphs of metformin, pioglitazone and glimepiride

Accuracy

Accuracy was established by the determination of intra- and inter-day variation in peak areas for three consecutive days. The peak area of the variation was calculated as % Relative Standard Deviation, and the results are shown in tabular form

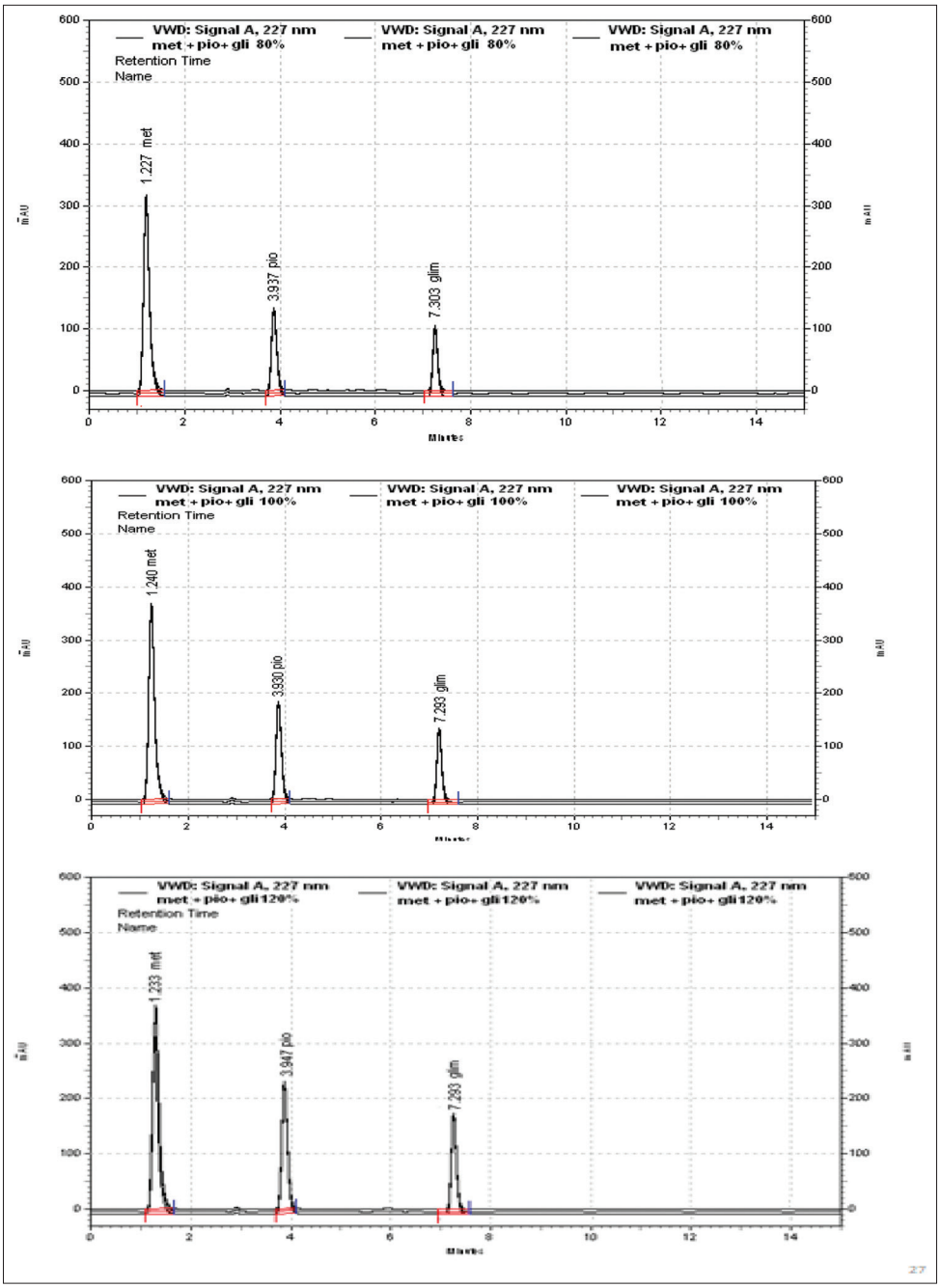

Figure 5: Chromatograms for accuracy

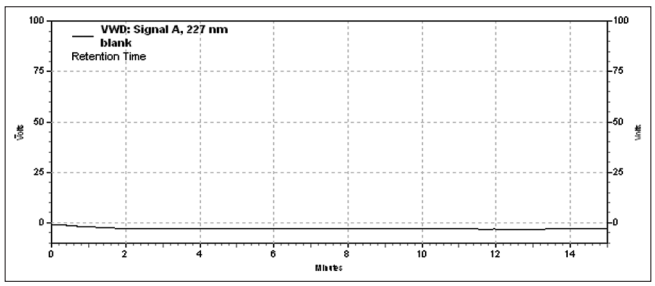

Figure 6: Chromatogram representing blank run

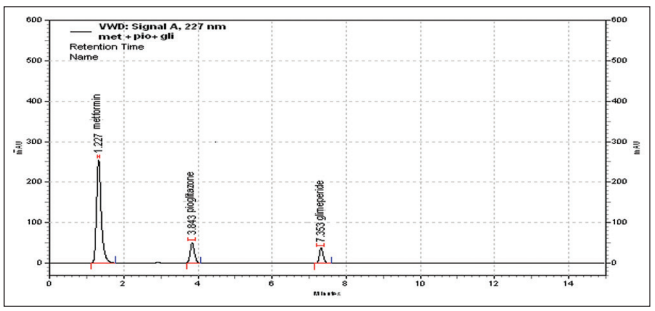

Figure 7: Chromatogram for optimised method

and in Figure 5. The formulation was commercially available in the form of Glimistar PM 4, which is manufactured by Mankind Pharma Ltd, contains Glimepiride at 4 mg, Pioglitazone at 15 mg, and Metformin at 500 mg.

Table 4: Inter-Day precision results

S. No.	Glimepiride area	Pioglitazone area	Metformin area
1.	12574931	17649543	38663478
2.	12567653	17657352	37569456
3.	12670634	17568120	38761038
4.	12761673	17563694	37667954
5.	12671445	17657495	38653792
6.	12589362	17741769	36654080
Average	12639283	17639662	37994966
Standard deviation	75842.91	66398.52	843065.5
RSD (%)	0.6	0.37	0.22

RSD: Relative standard deviation

Table 5: LOD and LOQ results

Sample Name	LOD	LOQ
Metformin	1.36 µg/mL	4.55 µg/mL
Pioglitazone	0.19 µg/mL	0.644 µg/mL
Glimepiride	0.029 µg/mL	0.079 µg/mL

LOD: Limit of detection, LOQ: Limit of quantification

Specificity

The specificity of the analytical method was ascertained through the resolution of two distinct peaks, utilizing parameters such as retention time, resolution, and tailing factor. Blank Chromatogram is shown in Figure 6.

Robustness and ruggedness

Robustness studies were carried out to determine the changes in flow rate and mobile phase composition. Robustness was also tested by preparing and injecting the drug solutions in different conditions.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were estimated by different procedures and based on measured responses and slopes at the signal-to-noise ratio per regulatory requirements, and results are systematically presented in a tabulated manner in Table 5.

RESULTS AND DISCUSSION

System suitability

System suitability parameters were meticulously evaluated, demonstrating distinct separation, thereby indicating the absence of interferences.

Linearity and range

Linearity ranges were studied among concentrations of 300–800 µg/mL, 9–24 µg/mL, and 2.4–6.4 µg/mL for metformin hydrochloride, pioglitazone, and glimepiride, respectively. The linearity between the concentrations and the peak area was illustrated in a graph for the linear regression analysis. Regression coefficients (R^2) were determined as 0.9989 for Metformin, 0.997 for Pioglitazone, and 0.9983 for Glimepiride, which were found to be in good agreement with the permissible limits proposed by the ICH.

Precision

The accuracy was assessed by evaluating both intra-day and inter-day variability for a collection (6 replicates) of pharmaceutical solutions to examine the response which are shown in Tables 3 and 4.

Accuracy

The accuracy of the assay was determined by standard addition procedure at various levels, i.e., 80%, 100% and 120%. The preparations were made in triplicates and finally analyzed as per the procedure. Recoveries of metformin, pioglitazone, and glimepiride on spiking of matrix blank at three levels were found to be 98.2–100.9%, 99.9–100.4%, and 99.6–99.7%, respectively, suggestive of few interferences from matrix components.

Strength

The precision of an analytical method is, as outlined by ICH guidelines, its capacity to remain unaffected by small variations in chromatographic conditions. The robustness in the present study had been checked by changing the flow rate of the mobile phase ±0.1 mL/min and varying the pH of the mobile phase ±1, and no significant changes were found it is shown in Figure 7.

LOD and LOQ

LOD and LOQ were also calculated according to the ICH guidelines, and were expressed in terms of the signal-to-noise ratio. When determining the LOD and LOQ a S/N ratio of 3:1 and 10:1 was used, respectively. LOD and LOQ of the analytical method were found to be 1.36 µg/mL, 4.55 µg/mL for Metformin, 0.19 µg/mL, 0.644 µg/mL for Pioglitazone and 0.029 µg/mL, 0.079 µg/mL for Glimepiride, respectively.

CONCLUSION

The proposed method is characterized by its accuracy, precision, rapidness and sensitivity, selectivity. Inexpensive

solvents are used for the separation of the mixture of the drugs, whereas the washing of the instrument is carried out with the same solvent, that makes a method more economical for estimation of the above-mentioned combination of drugs in bulk and tablet forms both. Validation of the developed method was done as per ICH Guidelines. No other substance peak was observed to be interfered with the ingredients indicating the purity of the compounds. As a result, the proposed method is deemed to be suitable for routine analytical determination of the drugs in bulk and pharmaceutical dosage forms and is also useful in drug monitoring and bioavailability studies.

REFERENCES

1. Sharma BK. Instrumental Methods of Chemical Analysis. 28th ed. Meerut: Goel Publishing House; 2012. p. 286-385.
2. Conner KA. A Textbook of Pharmaceutical Analysis. 3rd ed. New Delhi: CBS Publishers and Distributors Pvt Ltd.; 2001. p. 3-6.
3. Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, *et al*. Metformin: A review of its potential indications. Drug Des Devel Ther 2017;11:2421-9.
4. Langtry HD, Balfour JA. Glimepiride. A review of its use in the management of type 2 diabetes mellitus. Drugs 2012;55:563-84.
5. Al Majed A, Bakheit AH, Abdel Aziz HA. Pioglitazone. Profiles Drug Subst Excip Relat Methodol 2016;41:379-438.
6. Upendra M, Nirupa G. RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: Glimepiride, pioglitazone and metformin and its pharmaceutical dosage forms. J Chem 2013;2013:726235.
7. Jain D, Jain S, Jain D, Amin M. Simultaneous estimation of metformin hydrochloride, pioglitazone hydrochloride, and glimepiride by RP-HPLC in tablet formulation. J Chromatogr Sci 2008;46:501-4.
8. Pandit V, Pai RS, Devi K, Singh G, Narayana S, Suresh S. Development and validation of the liquid chromatographic method for simultaneous estimation of metformin, pioglitazone, and glimepiride in pharmaceutical dosage forms. Pharm Methods 2012;3:9-13.
9. Nataraj KS, Rao AS, Nagamani KV, Divya P, Renukachandrasekhar B. Analytical method development and validation of metformin, pioglitazone and glimepiride by RP-HPLC in tablet dosage forms. Pharma Innov 2019;8:266-73.
10. Pandit V, Pai R, Singh G, Devi K, Narayana S, Suresh S. Development and validation of the liquid chromatographic method for simultaneous estimation of metformin, pioglitazone, and glimepiride in pharmaceutical dosage forms. Pharm Methods 2012;3:9-13.
11. Sebaiy MM, El-Adl SM, Baraka MM, Hassan AA. Rapid RP-HPLC method for simultaneous estimation of metformin, pioglitazone, and glimepiride in human plasma. Acta Chromatogr 2020;32:57-63.
12. Shaikh SS, Dighe NS. Simultaneous estimation of pioglitazone, glimepiride and metformin hydrochloride in bulk and tablet dosage form by UV, RP-HPLC method. Int J Pharm Chem Anal 2021;8:91-9.
13. Suchitra M, Sunitha D, Parthiban C, Sidhartha B, Madhavi C. Method development and validation of metformin, glimepiride and pioglitazone in tablet dosage form by RP-HPLC. Int Res J Pharm 2013;4:250-4.

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