Original Article

Development and validation of the liquid chromatographic method for simultaneous estimation of metformin, pioglitazone, and glimepiride in pharmaceutical dosage forms

Vinay Pandit, Roopa S. Pai, Kshama Devi[1], Gurinder Singh, Satya Narayana[2], Sarasija Suresh[3]

Department of Pharmaceutics, [1]Quality Improvement cell, [2]Quality Assurance, Al-Ameen College of Pharmacy, Bangalore, Karnataka, [3]Department of Pharmaceutical Technology (Formulations), NIPER, Mohali, Punjab, India

Address for correpondence:
Dr. Sarasija Suresh, Department of Pharmaceutical Technology (Formulation), National Institute of Pharmaceutical Education and Research, SAS Nagar, Mohali, Punjab 160 062, India.
E-mail: ssuresh@niper.ac.in

Access this article online

Website: www.phmethods.org

DOI: 10.4103/2229-4708.97707

Quick response code

Abstract

Introduction: A simple, precise, and accurate HPLC method for simultaneous estimation of metformin hydrochloride (MET), pioglitazone hydrochloride (PIO), and glimepiride (GLIMP) was developed and validated. **Materials and Methods:** Chromatographic separation of the drugs was performed by using a Phenomenex-ODS-3 (C-18) column (250 × 4.60 mm, 5 µm) with a mobile phase consisting of methanol:acetonitrile:15 mM potassium dihydrogen phosphate (pH 4) in the proportion of 40:35:25 (v/v) at a flow rate of 1 ml/min. Detection was carried out using a UV-SPD-10AVP detector at 240 nm. **Results:** The retention time for MET, PIO, and GLIMP were 2.85 ± 0.03 min, 4.52 ± 0.03 min, and 7.08 ± 0.02min, respectively. Parameters such as linearity (0.2–50 µg/ml for MET, 0.2–30 µg/ml for PIO, and GLIMP, respectively), precision (intra-day % RSD was 1.01–3.24 and inter-day % RSD was 1.54–4.09 for MET; intra-day % RSD was 1.03–2.09 and inter-day % RSD was 2.26–3.10 for PIO; and intra-day% RSD was 1.00–3.15 and inter-day % RSD was 1.58–3.07 for GLIMP), accuracy (99.66 ± 0.14 for MET, 98.46 ± 0.40 for PIO, and 98.62 ± 0.39 for GLIMP), specificity and robustness were calculated in accordance with ICH guidelines. **Conclusions:** The method was proved to be simple, rapid, precise, accurate, and cost effective.

Key words: Glimepiride, ICH guidelines, metformin hydrochloride, pioglitazone hydrochloride, simultaneous

INTRODUCTION

Diabetes is a lifelong (chronic) disease in which there are high levels of sugar in the blood. The diabetes is classified into three major types namely, type I, II, and gestational diabetes. Type II diabetes constitutes 90% of the diabetic population. The combinational therapy for type II diabetes[1,2] is frequently prescribed when monotherapy fails. The combination of metformin (MET), pioglitazone (PIO), and glimepiride (GLIMP) is approved by FDA for treatment of type II diabetes.[3]

MET, PIO and GLIMP are chemically known as *N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride, 5-[4-[2-(5-ethyl-2-pyridinyl)ethoxy]benzyl] thiazolidine-2,4-dione hydrochloride, and 3-ethyl-4-methyl-*N*-(4-[*N*-((1*R*,4*R*r)-4-methylcyclohexylcarbamoyl) sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide respectively[Figure 1]. MET improves hyperglycemia primarily through its suppression of hepatic glucose production (hepatic gluconeogenesis).[4] PIO act through PPARγ, a member of the nuclear receptor superfamily of ligand-activated transcription factors.[5] Once activated, PPARγ forms a heterodimer with another nuclear receptor, the retinoid-X receptor. This heterodimer then binds to specific DNA sequences and regulates the transcriptional activity of target genes that play a role in the metabolism of glucose and lipids.[6,7] The mechanism of action[8] of GLIMP in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning

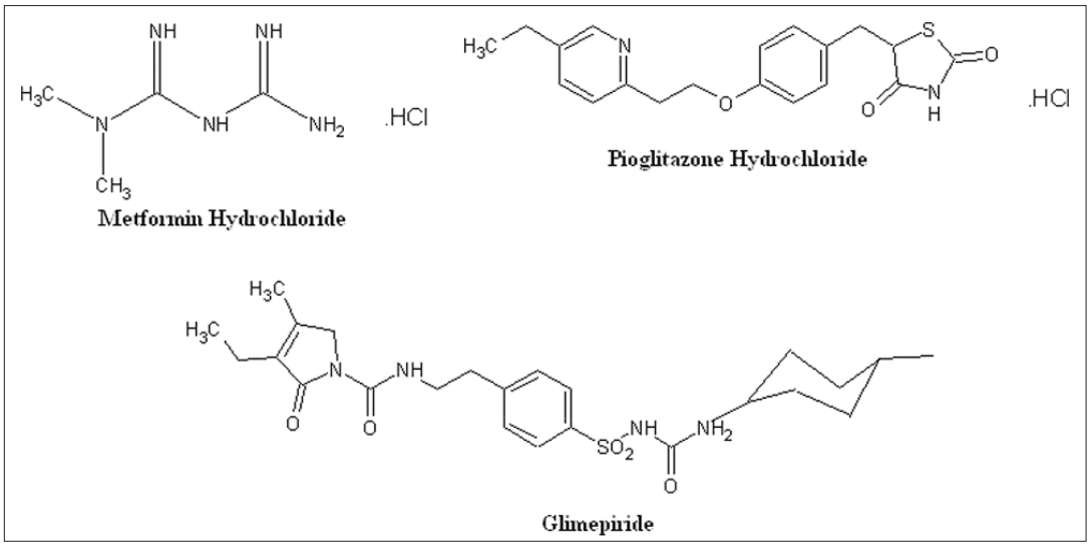

Figure 1: Structures of three anti-diabetic drugs

pancreatic β-cells, and increasing the sensitivity of peripheral tissues to insulin.

As per the literature, various methods are available for the estimation of these three drugs individually or in combination of two drugs in a pharmaceutical dosage form and also from biological samples. Very few methods are available for simultaneous estimation of all the three drugs together in a tablet dosage form.[9] This paper describes a simple, precise, and accurate HPLC method for simultaneous estimation of MET, PIO, and GLIMP.

MATERIALS AND METHODS

Materials

MET was obtained as a gift sample from Micro Labs, India, PIO and GLIMP was obtained as a gift sample from Hetero Labs, India. Methanol and acetonitrile (HPLC grade) were purchased from Merck, India. All other chemicals and reagents employed were of analytical grade and were purchased from S.D. Fine Chemicals, India. The chromatograph system Shimadzu LC 10 AT VP pumps equipped with a manual rheodyne injector of an injection volume of 50 µl and variable wavelength UV-Visibile-SPD-10AVP detector was used.

Methods

Preparation of standard solution

The stock solution for MET, PIO, and GLIMP was prepared by dissolving 50 mg of each drug in methanol HPLC grade and the volume was made up to 50 ml in order to get a final concentration of 1 mg/ml. From this solution, working standard solutions 100 µg/ml were prepared.

Chromatographic conditions

The mobile phase consisted of methanol:acetonitrile: 15 mM potassium dihydrogen phosphate (pH 4) in the proportion of 40:35:25 (v/v). The mobile phase was filtered through a 0.22 µm membrane and degassed. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1 ml/min and the injection volume was 50 µl. The column temperature was maintained at room temperature. The samples were analyzed at 240 nm.

Preparation of calibration curve

Separate standard calibration curves were plotted for each component namely, MET, PIO, and GLIMP. The concentrations were in the range of 0.2–50 µg/ml for MET and 0.2–30 µg/ml for PIO and GLIMP, respectively, were made in 10 ml volumetric flasks. The volume was adjusted with the mobile phase. The calibration curve was plotted with concentration (µg/ml) as the *x*-axis *versus* peak area (mV s) of the respective drug as the *y*-axis.

Analysis of tablets

To determine the content of MET, PIO, and GLIMP in the tablet dosage form; ten tablets containing 500 mg MET, 15 mg PIO, and 1 mg GLIMP were weighed; average weight was determined and was finely powdered. An accurately weighed sample of powdered tablets was extracted with methanol in a 100 ml volumetric flask, and 50 ml of methanol was added to the same. The flask was sonicated for 10 min, and the volume was made up to the mark with methanol. The above solution was filtered using Whatman filter paper (#1). The obtained filtrate (1 ml) was transferred into a 10 ml volumetric flask, and the

Table 1: Intra-day and inter-day precision and accuracy of pioglitazone

Conc. (µg/ml)	Recovered concentration (µg/ml)		Relative error (%)		RSD (%)	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.2	0.195 ± 0.003	0.193 ± 0.006	2.56	3.62	1.51	3.10
2	1.941 ± 0.02	1.927 ± 0.05	3.03	3.78	1.03	2.59
10	9.791 ± 0.101	9.713 ± 0.220	2.14	2.95	1.03	2.26
30	29.271 ± 0.610	28.981 ± 0.85	2.49	3.51	2.09	2.93

The symbol ± indicates mean SD; RSD, relative standard deviation.

Table 2: HPLC data for metformin, pioglitazone, and glimepiride

Parameter	MET	PIO	GLIMP
Retention time (min)	2.85 ± 0.03	4.52 ± 0.03	7.08 ± 0.02
Linearity range (µg/ml)	0.20–50	0.20–30	0.20–30
R^2 value	0.9983	0.9978	0.9971
Equation for linearity	38.72*x* + 32.72	96.01*x* + 69.98	101.1*x* + 29.84
Plate count	5817 ± 103	4987 ± 209	3833 ± 193
Tailing factor	0.98 ± 0.03	0.99 ± 0.08	1.21 ± 0.03
Asymmetry (10%)	0.93 ± 0.23	1.03 ± 0.21	1.05 ± 0.30
Capacity factor	2.81 ± 0.33	1.77 ± 0.48	3.02 ± 0.43
Resolution	–	4.2	3.6
LOD (µg/ml)	0.04	0.05	0.08
LOQ (µg/ml)	0.12	0.19	0.18

The symbol ± indicates mean SD

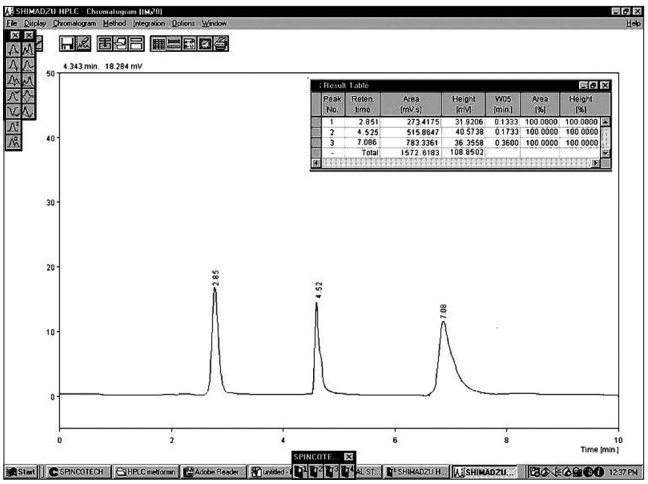

Figure 2: A typical chromatogram of showing peaks for metformin (2.85 min), pioglitazone (4.52 min), and glimepiride (7.08 min)

volume was made up to the mark with the mobile phase to obtain 50 µg/ml of MET, 15 µg/ml of PIO, and 1 µg/ml of GLIMP. The solution was sonicated for 10 min and injected under above chromatographic conditions and the peak area was measured. The assay procedure was repeated in triplicate, and the percentage of drug found in formulation was calculated. The results were shown in Table 1.

Validation

The method was validated for the following characteristics: linearity, accuracy, precision, specificity, limit of detection, limit of quantitation, robustness, and ruggedness as per ICH guidelines.[10]

RESULTS AND DISCUSSION

The development of an analytical method for the determination of triple drugs by the RP-HPLC method has received considerable attention in recent years because of their importance in quality control of drugs and drug products in bulk dosage forms. The mobile phase containing methanol, acetonitrile, and phosphate buffer (pH 4.0 with glacial acetic acid) in the proportion of 40:35:25 (v/v) was selected because it was found to give peaks with minimum tailing (<2). With the above-mentioned composition of the mobile phase, a sharp peak was achieved with reasonable short run time within 10 min. The criteria employed for assessing the suitability of above said solvent system were cost, time required for analysis, solvent noise, preparatory steps involved in the use of the same solvent system for the extraction of the drug from formulation excipient matrix for the estimation of drug content. The resolution of peaks were good (>2) and the plate count was ranging between 3833 ± 193 and 5817 ± 103 indicating the suitability of the method [Table 2]. A typical chromatogram of the test solution is shown in Figure 2.

Specificity

Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, degradants, or excipients. A volume of 50 µl of working placebo sample solution was injected, and the chromatogram was recorded. No peaks were found at the retention time of 2.85 ± 0.03, 4.52 ± 0.03, and 7.08 ± 0.02 min. Hence, the proposed method was specific for MET, PIO, and GLIMP.

Limit of detection and limit of quantitation

The limit of detection (LoD) and limit of quantitation (LoQ) were determined by examining the signal-to-noise ratio. The results were tabulated in Table 2.

Table 3: Intra-day and inter-day precision and accuracy of Metformin

Conc. (µg/ml)	Recovered concentration (µg/ml)		Relative error (%)		RSD (%)	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.2	0.197 ± 0.002	0.194 ± 0.003	1.52	3.09	1.01	1.54
2	1.929 ± 0.03	1.906 ± 0.005	3.68	4.93	1.55	2.62
10	9.899 ± 0.402	9.816 ± 0.003	1.02	1.87	3.24	4.09
50	48.991 ± 1.210	48.121 ± 1.63	2.05	3.90	2.46	3.32

The symbol ± indicates mean SD; RSD, relative standard deviation

Table 4: Intra-day and inter-day precision and accuracy of glimepiride

Conc. (µg/ml)	Recovered concentration (µg/ml)		Relative error (%)		RSD (%)	
	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
0.2	0.198 ± 0.002	0.192 ± 0.003	1.01	3.36	1.00	1.58
2	1.979 ± 0.03	1.951 ± 0.06	1.06	2.51	1.51	3.07
10	9.890 ± 0.312	9.780 ± 0.311	1.11	2.24	3.15	3.17
30	29.681 ± 0.910	29.309 ± 0.671	1.07	2.35	3.06	2.28

The symbol ± indicates mean SD; RSD, relative standard deviation.

Table 5: Accuracy of the developed HPLC method

Drug	Test spiked (%)	Recovery (%)	RSD (%)
Metformin	80	98.95	1.12
	100	99.35	0.86
	120	103.21	1.01
Pioglitazone	80	97.58	0.98
	100	98.63	1.23
	120	102.22	1.05
Glimpiride	80	98.61	1.21
	100	99.56	0.94
	120	100.2	1.31

Table 6: Assay of the marketed tablet dosage form

Formulation	Labelled claim (mg)	Amount found (mg), mean ± SD	Assay	% RSD
Metformin	500	499.75 ± 0.98	99.66 ± 0.14	0.196
Glimepiride	1	0.99 ± 0.008	98.46 ± 0.40	0.808
Pioglitazone	15	14.98 ± 0.04	98.62 ± 0.39	0.267

The symbol ± indicates mean SD; RSD, relative standard deviation.

Table 7: Robustness of the developed HPLC method

Parameter	Modification	Retention time			Asymmetry		
		MET	PIO	GLM	MET	PIO	GLM
Flow rate	0.8	2.95	4.98	8.20	0.51	1.35	1.26
	0.9	2.88	4.61	7.51	0.92	1.32	1.21
	1.0	2.85	4.52	7.08	1.02	1.03	1.00
	1.1	2.83	4.52	7.09	2.63	1.11	1.23
	1.2	2.81	4.48	7.09	2.01	0.98	1.03
Changes in pH	3.5	2.90	4.58	7.12	0.89	1.02	0.92
	4.0	2.85	4.52	7.08	0.99	0.98	0.99
	4.5	2.82	4.55	7.15	1.03	1.32	1.09
Changes in organic phase	70	3.06	5.06	8.69	1.02	2.34	1.33
	75	2.85	4.52	7.08	0.93	1.02	1.03
	80	2.83	4.49	7.02	0.88	1.00	1.34

Linearity

The linearity of calibration curves in pure solution was checked over the concentration range of 0.2–50 µg/ml for MET, 0.2–30 µg/ml for PIO, and 0.2–30 µg/ml for GLIMP through the HPLC method [Table 2].

Precision

The precision assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at the same concentration and during the same day. The intermediate precision was studied by comparing the assays on five different days. Four sample solutions were prepared and assayed [Tables 3–4].

Accuracy

Accuracy was determined by percentage recovery studies. The reference standard of the drug was spiked at 80%, 100%, and 120% levels to the formulation and recovery studies were carried out in three replicates using HPLC methods. The percentage recovery and % relative standard deviation were calculated, and the results were presented in Tables 5 and 6.

Robustness

The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the pH (3.5–4.5) and in the percentage of the organic phase (70–80%) in the mobile phase and changes in the flow rate (0.8–1.2 ml/min). The effect on retention time and asymmetry of the peak was studied [Table 7].

CONCLUSION

The validated HPLC method employed here proved to be simple, rapid, precise, accurate and cost effective. The specificity experiment showed that there was no interference from the excipients. The low LoD and LoQ values proved the method to be sensitive. The proposed method can be applied for routine analysis for the estimation of bulk drugs and pharmaceutical dosage forms.

REFERENCES

1. Bell DS., Ovalle F. Long-term efficacy of triple oral therapy for type 2 diabetes mellitus. Endocr Pract 2002;8:271-5.
2. Burke J. Combination treatment with insulin and oral agents in type 2 diabetes mellitus. Br J Diabetes Vasc Dis 2004;4:71-6.
3. Meshram DM, Langade DG, Kinagi SB, Naikwadi AA, Morye V, Chopra D. Evaluation of efficacy and safety of fixed dose combination of glimepiride 2 mg pluspioglitazone 15 mg plus metformin SR 500 mg in the management of patients with type-2 diabetes mellitus. J Indian Med Assoc 2005;103:447-50.
4. Pandit V, Pai RS, Yadav V, Devi K, Surekha BB, Inamdar MN, Suresh S. Pharmacokinetic and pharmacodynamic evaluation of floating microspheres of metformin hydrochloride. Drug Dev Indl Pharm, Ahead of Print: Pages 1-11. Posted online on February 28, 2012. (doi:10.3109/03639045.2012.662503)
5. Derosa G, Salvadeo SA. Glimepiride-pioglitazone Hydrochloride in the Treatment of Type 2 Diabetes. Clin Med Ther 2009;1:835-45.
6. Waugh J, Keating GM, Plosker GL, Easthope S, Robinson DM. Pioglitazone: A Review of its Use in Type 2 Diabetes Mellitus. Drugs 2006;66:85-109.
7. Pandit V, Gorantla R, Devi K, Pai RS, Sarasija S. Preparation and characterization of pioglitazone cyclodextrin inclusion complexes. J Young Pharm 2011;3:267-74.
8. Langtry HD, Balfour JA. Glimepiride. A review of its use in the management of type 2 diabetes mellitus. Drugs 1998;55:563-84.
9. Jain D, Jain S, Jain D, Amin M. Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in Tablet Formulation**.** J Chromat Sci 2008;46:501-4.
10. ICH, Q2 (R1) Validation of Analytical Procedure, Test and Methodology, International Conference.

How to cite this article: 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.
Source of Support: Nil, **Conflict of Interest:** None declared.