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RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin, Pioglitazone and Gliclazide from Bulk and Tablet Dosage Form

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ABSTRACT

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Metformin Hydrochloride, Pioglitazone hydrochloride and Gliclazide in pure and tablet formulation. Chromatography was performed on a WATER C18 (250mm×4.6 mm, 5.0 µm) analytical column with phosphate buffer (pH adjusted to 4.2 using o-phosphoric acid): Acetonitrile in the ratio of 45:55 (v/v) as mobile phase at a flow rate of 1.0 ml/min and effluents was monitored at 228 nm. Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were eluted with retention times of 2.515 min, 5.178 min and 6.903 min respectively. The method was statistically validated as per ICH guideline for analytical method validation.

KEYWORDS

Metformin hydrochloride, Pioglitazone hydrochloride, Gliclazide, Tablet Formulation, Validation

INTRODUCTION

Metformin hydrochloride, chemically *N,N*-Dimethylimidodicarbonimidic diamide hydrochloride. It acts by suppressing excessive hepatic glucose production and improving glucose clearance, its predominant effect is to decrease fasting plasma glucose.[1] It is the most well known member of the biguanide group, regarded as the main compound in mixed therapies in patients with type-2 diabetes (non-insulin dependent), and is always used in high doses of about 500 or 850 mg.[2] Pioglitazone hydrochloride is chemically designated as 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy] phenyl]methyl]-2,4-thiazolidinedione.

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It is a member of the thiazolidinedione group. The drug used in the dose of 15, 30 or 45 mg.[3]

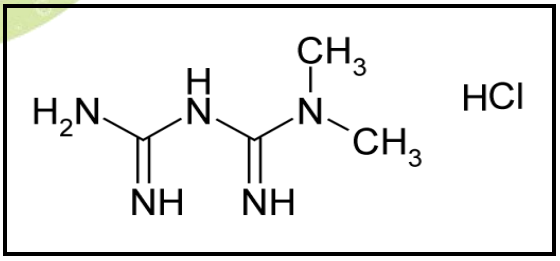

Figure 1: Chemical structure of Metformin Hydrochloride

Pioglitazone hydrochloride has been show to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues. Many patients suffering from type-2 diabetes require treatment with more than one antihyper

glycemic drug in order to achieve optimal glycemic control.

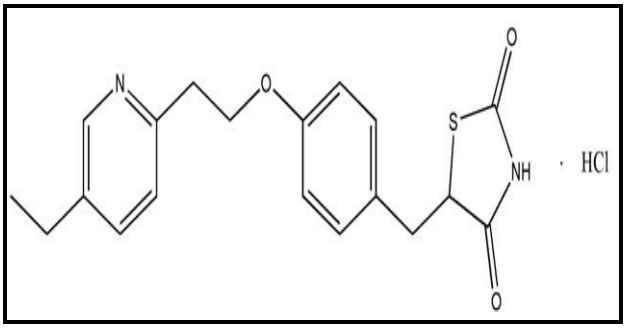

Figure 2: Chemical structure of Pioglitazone hydrochloride

Gliclazide is an oral hypoglycaemic agent which lowers the blood glucose level by stimulating the pancreatic b-cells to secrete insulin. Chemically, it is 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(p-tolylsulfonyl) urea. The drug used in the dose of 80, 60, or 30 mg.[4]

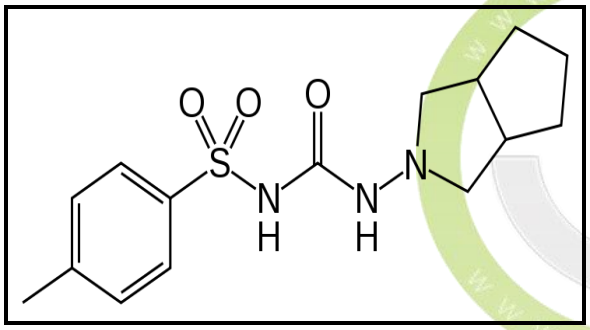

Figure 3: Chemical structure of Gliclazide

This is evident from the literature available that there are many methods HPLC, UV, Spectrophotometric method for the estimation of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide individually and in combination of two dosage form. The present paper describes a simple, sensitive, validated and economic method for the determination of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide in combination. This combination, however, is not present in any official pharmacopoeia.

MATERIALS AND METHODS

Materials, Reagents and Chemicals

Metformine hydrochloride was received as gift sample from Jenburkt Pharmaceuticals Ltd., Mumbai, Pioglitazone hydrochloride was received from Indian Pharmacopoeia Commission (IPC) Ghaziabad, India and Gliclazide was from BRD Medilabs, Baddi, H.P, India as gift sample. Orthophosphoric acid AR grade, HPLC grade Acetonitrile, methanol and double distilled water used were from Rankem, Mumbai. The pharmaceutical dosage form containing 500 mg Metformine (Glycomet-USV Pharmaceuticals Ltd.), 15 mg Pioglitazone (Glitaris 15- Eris Lifesciences Pvt. Ltd.) and 60 mg Gliclazide (Reclide-XR 60- Dr. Reddy's Laboratories Ltd.) purchased from a local drug store.

Equipment

The development and validation of the assay was performed on a LC2010 Shimadzu HPLC (Kyoto Japan), provided with high speed auto sampler, column oven, degasser and UV detector. LC10 solution software was used for data acquisition.

Experimental

Chromatographic Conditions

The column used was WATER C18 (250mm×4.6 mm, 5.0 µm) analytical column with phosphate buffer (pH adjusted to 4.2 using o-phosphoric acid): Acetonitrile in the ratio of 45:55 (v/v) as mobile phase at a flow rate of 1.0 ml/min and effluents was monitored at 228 nm. The mobile phase and samples was filtered using 0.45 µm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at 40°C.

Preparation of Stock Solutions

The standard stock solutions were prepared with methanol to give the final concentration of 1000 µg/ml. The working standard solutions of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10ml with methanol to get concentrations of 25-500 µg/ml of Metformin hydrochloride, 1-10 µg/ml of Pioglitazone hydrochloride and 4-40 µg/ml of Gliclazide.

Table 1: Determination of Metformin, Pioglitazone and Gliclazide in Tablet dosage form

Drug	Label claim (mg)	Amount found (mg)	% Purity	SD	%RSD
MET	500	497.33	99.46	0.0439	0.0441
PIO	15	14.82	98.83	0.2719	0.2751
GLIC	60	59.56	99.26	0.0526	0.0530

Preparation of Sample Solutions

20 tablets of Glycomet (USV Pharmaceuticals Ltd.) containing 500 mg of Metformin, 20 tablets of Glitaris 15 (Eris Lifesciences Pvt. Ltd.) containing 15 mg Pioglitazone and 20 tablets of Reclide-XR 60 (Dr. Reddy's Laboratories Ltd.) containing 60 mg Gliclazide were weighed accurately and powdered individually using pestle-mortar. Powder equivalent to 500 mg of Metformin, 15 mg of Pioglitazone and 60 mg of Gliclazide was weighed respectively and transferred to a standard volumetric flask and dissolved in methanol. The mixture was sonicated for 15 minutes to dissolve drugs and then volume was made up to the mark with methanol. The solution was finally filtered to collect the filtrate containing extracted Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide, which was diluted appropriately with methanol to obtain the final concentration of 500 µg/ml of Metformin hydrochloride, 15 µg/ml of Pioglitazone hydrochloride and 60 µg/ml of Gliclazide.

Preparation of Calibration Curve

From the mixed standard stock solution, aliquots are made with diluents to concentration of 25-500 µg/ml of Metformin hydrochloride, 1-10 µg/ml of Pioglitazone hydrochloride and 4-40 µg/ml of Gliclazide. The solution of (20 µL) was injected into column. All measurements were repeated three times for each concentration. The calibration curves were plotted against mean area under curve (AUC) Vs concentration.

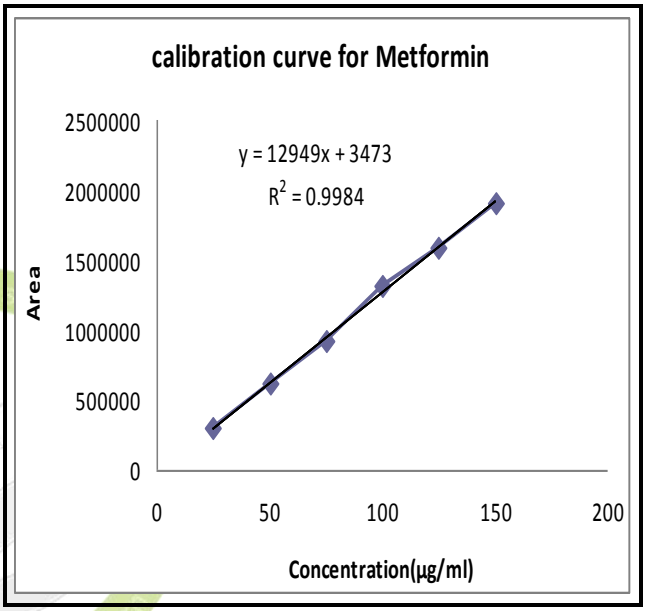

Figure 4: Calibration curve for Metformin

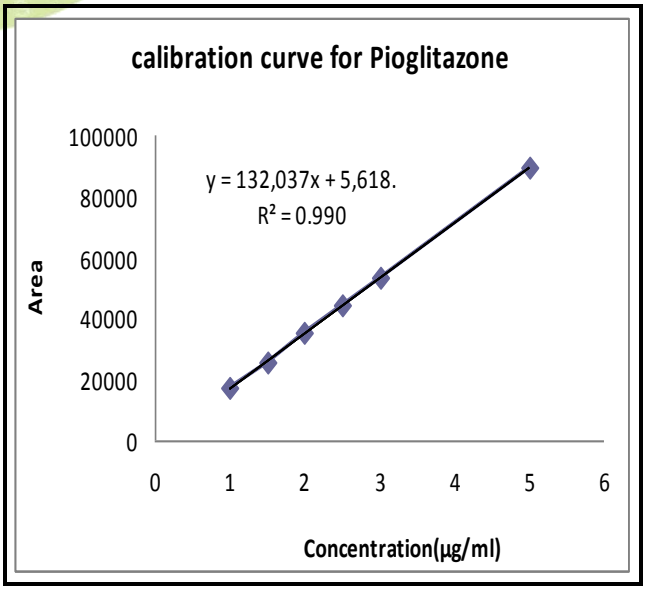

Figure 5: Calibration curve for Pioglitazone

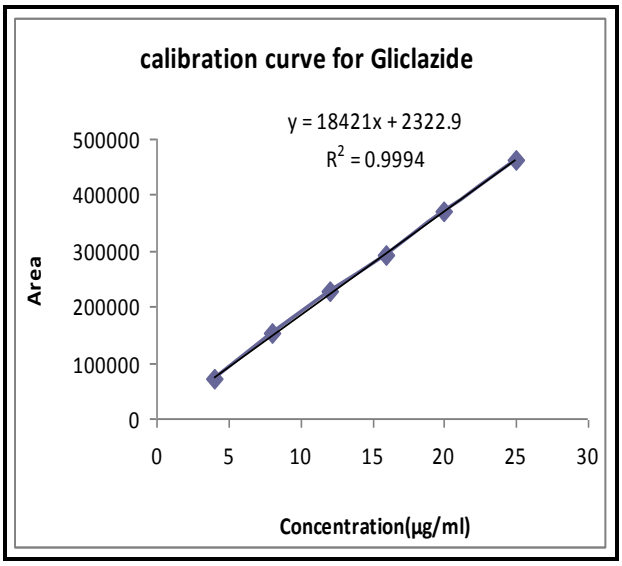

Figure 6: Calibration curve for Gliclazide

RESULTS AND DISCUSSION

Method Development and Optimization[5-8]

Present study indicates the suitability of reversed-phase column procedure for the simultaneous analysis of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide in dosage form. Different ratios of Buffer: ACN were experimented to optimize the mobile phase. Finally a mixture of Buffer: ACN in the ratio of 45:55 % at the flow rate of 1.0ml/min was used for the elution of these drugs. Buffer used was Sodium dihydrogen orthophosphate monohydrate. Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were eluted with retention times of 2.515 min, 5.178 min and 6.903 min respectively.

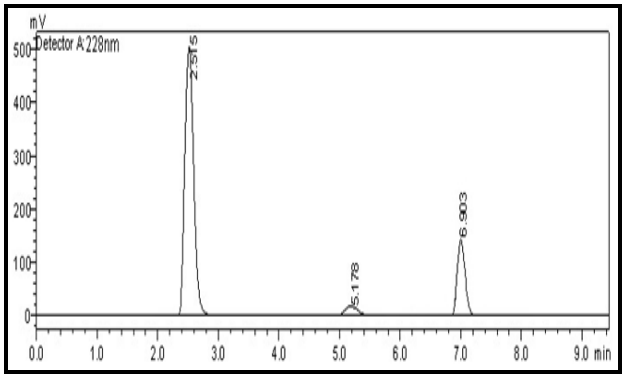

Figure 7: Typical Chromatogram of Metformin, Pioglitazone and Gliclazide in Mix Standard

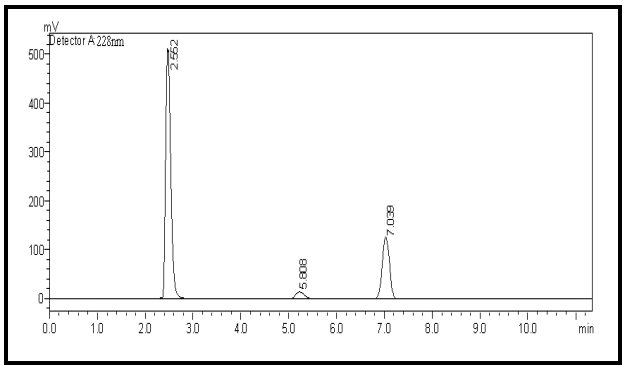

Figure 8: Typical Chromatogram of Metformin, Pioglitazone and Gliclazide in Pharmaceutical Dosage Form

Validation[9]

Linearity

Linearity of the method was studied by injecting the mixed standard solutions in the concentration range of 25-500 µg/ml of Metformin hydrochloride, 1-10 µg/ml of Pioglitazone hydrochloride and 4-40 µg/ml of Gliclazide injected six times into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Accuracy

The accuracy of the method was carried out by applying the method to drug sample (Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide combination tablets) to which known amounts of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was analyzed by running chromatograms in optimized mobile phase. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%), SD was calculated.

Precision

The precision was studied both intra-day and inter-day. Six replicate sample solutions were prepared from the stock solution for study of intra-day precision.

Table 2: Recovery Studies of Metformin, Pioglitazone and Gliclazide.

Drug	Amount taken (µg/ml)	Amount added (%)	% Recovery	Average % recovery	SD
MET	500	80%	99.57	97.73	0.15
		100%	99.84		0.08
		120%	99.80		0.16
PIO	15	80%	99.65	100.38	0.91
		100%	101.23		0.54
		120%	100.26		0.51
GLIC	60	80%	100.91	100.60	0.10
		100%	100.75		0.20
		120%	100.16		0.06

Table 3: Results of Intraday Precision, Interday Precision, LOD and LOQ

Drug	Intraday Precision %RSD	Interday Precision %RSD			LOD (µg)	LOQ (µg)
		Day 1	Day 2	Day 3		
MET	0.1410	0.1388	0.1186	0.1213	0.3064	0.9286
PIO	0.1065	0.0492	0.0260	0.0559	0.0076	0.0232
GLIC	0.8068	0.3321	0.4693	0.1499	0.0423	0.1283

The concentration of the three drugs were measured three times on the same day at intervals of 1 h. In the inter-day study the drug concentration were measured on three different days. Results are shown in table 3. The %RSD of inter-day and intra-day precision obtained was less than 1% for all days. From the data obtained, the developed HPLC method was found to be precise and accurate.

Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) for the procedure were performed on samples containing very low concentrations of analytes under the ICH guidelines.

Based on the Standard Deviation of the Response and the Slope the LOD and LOQ were determined. LOD and LOQ were calculated by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank and S is the slope of the calibration plot. The results are reported in Table 3.

Selectivity and Specificity

The specificity of the method was assessed by comparing chromatograms obtained from drug standard with that obtained from tablet solutions. The retention times of the drug standards and the drugs from sample solutions were same, so the method was specific.

Table 4: Summary of the accepted system suitability requirements

Parameter	MET	PIO	GLIC	Accepted limit
Retention time	2.515	5.178	6.903	-
Tailing factor (Tf)	1.09	1.23	1.15	≤ 2.0
Resolution (Rs)	-	2.89	3.29	≥2.0
Number of theoretical plates (N)	2752	2425	3135	≥2000
Capacity factor (k')	1.19	1.75	1.94	≥1.0

Table: 5 Robustness testing of the three active ingredients of MET, PIO and GLIC

Parameter	Modification	%Recovery ± SD (n=6)		
		MET	PIO	GLIC
Flow rate (1 ml/min)	± 0.1	100.18±0.42	100.68±1.32	99.81±0.15
Mobile phase composition Buffer : Acetonitrile 45:55 (v/v)	± 1	99.56±0.60	99.98±1.26	99.05±0.39
Wave length (228nm)	± 1	99.20±1.42	100.98±1.36	99.07±0.54
Injection volume (20 µl)	± 1	99.92±0.49	100.23±1.43	99.44±0.55
Column temperature (40°C)	± 2	98.91±1.37	100.15±1.46	99.09±0.58

The method was also specific and selective because there was no intereference from excipients in the tablets.

System Suitability

The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide peaks were defined.

Robustness

Predetermined variations were performed under the experimental conditions of the RP-HPLC method to assess its robustness. The variations imposed on the chromatographic method are summarized in Table 5. The modifications include different mobile phase flow rates of (± 0.1ml/min) and different column temperatures in the range (± 2°C).

Different mobile phase composition (in the range of ± 1 of the nominal value) and wavelength variation (± 1 nm) were also investigated. The % RSD values showed no significant change in the final assay results of each of the ingredients using variations.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for the Simultaneous estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Gliclazide in solid dosage form. This method will help in further analysis Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide in combined formulation.

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