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Research Article

SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE, PIOGLITAZONE HYDROCHLORIDE AND GLICLAZIDE BY VALIDATED RP-HPLC METHOD IN SOLID DOSAGE FORM

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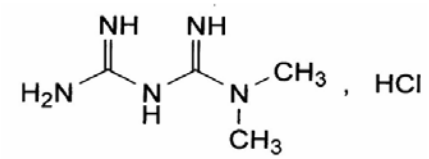
ABSTRACTun 2012

A simple, rapid and precise reversed-phase high-performance liquid chromatographic method for simultaneous estimation of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide in a dosage form has been developed and validated. Chromatography was performed on a Lichrosphere (R) RP -18e (250mm×4.6 mm, 5.0µ m) column with 0.05 mM sodium dihydrogen ortho-phosphate monohydrate buffer (pH- 5): acetonitrile in the ratio of 55:45 (v/v) as mobile phase at a flow rate of 1.0 ml/min and effluents was monitored at 230 nm. Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were eluted with retention times of 2.119 min, 10.494 min and 8.603 min respectively. The developed method was validated in terms of specificity, precision, accuracy, LOD and LOQ as per ICH guidelines. Limit of detection and Limits of quantitation was found to be 3.54696 µg/ml and 10.748386 µg/ml for Metformin hydrochloride, 0.054051 µg/ml and 0.16379 µg/ml for Pioglitazone hydrochloride and 0.775066 µg/ml and 2.34864 µg/ml for Gliclazide.

Keywords: Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide

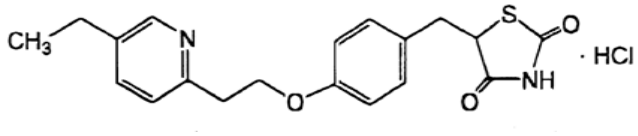
INTRODUCTION

Metformin hydrochloride, chemically [1,1-dimethyl biguanide 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, and is always used in high doses of about 500 or 850 mg.2



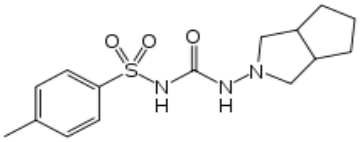
Metformin hydrochloride

Pioglitazone hydrochloride is chemically designated as 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-thiazolidinedione. It is a member of the thiazolidinedione group. The drug used in the dose of 15, 30 or 45 mg.[3]



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]



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

Reagents

Metformin hydrochloride, Pioglitazone hydrochloride were received as gift sample from Abhilasha Lab., Punjab, GMH Laboratories, Baddi and Gliclazide from Kwality Pharmaceuticals Pvt. Ltd., Amritsar. Methanol, HPLC Water and Acetonitrile (HPLC grade from Merck). Sodium dihydrogen orthophosphate monohydrate from Merck Chemicals, Mumbai, India was used throughout the analysis

Instrumentation

The HPLC system consisted of a Waters 600E Controller with manual injecting facility programmed at 20 µl capacity per injection was used. The detector consisted of a Waters 2998 PDA Detector model operated at a wavelength of 230 nm. The Empower software was used.

Chromatographic conditions

The column used was Lichrosphere (R) RP -18e (250mm×4.6 mm, 5.0 µm). Different mobile phases were tested in order to find the best conditions for separation of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide. The mobile phase contained 0.05 mM sodium dihydrogen ortho-phosphate monohydrate buffer (pH- 5.0): acetonitrile in the ratio of 55:45 (v/v) and the flow rate was maintained at 1.0 ml/min. UV detection was carried out at 230 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 ambient temperature.

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 mobile phase to get concentrations of 50- 350 µg/ml of Metformin hydrochloride, 1.5- 10.5 µg/ml of Pioglitazone hydrochloride and 6- 42 µg/ml of Gliclazide.

Preparation of sample solutions

20 tablets were weighed accurately and powdered using pestle-mortar. The quantity of the powder equivalent to amount was taken and dissolved in 100ml of methanol. The solution was finally filtered to collect the filtrate containing extracted Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide, which was diluted appropriately with mobile phase to obtain the final concentration 500 µg/ml, 15 µg/ml and 60 µg/ml.

Preparation of Calibration curve

From the mixed standard stock solution, aliquots are made with diluents to concentration of 50- 350 µg/ml of Metformin hydrochloride, 1.5- 10.5 µg/ml of Pioglitazone hydrochloride and 6-42 µg/ml of Gliclazide. The solution of (20 µL) was injected into column with the help of Hemilton syringe. All measurements were repeated three times for each concentration. The calibration curves were plotted against mean area under curve (AUC) Vs concentration.

Optimization of HPLC Method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide respectively. The mixed standard stock solution (500 µg/ml of Metformin hydrochloride, 15 µg/ml of Pioglitazone hydrochloride and 60 µg/ml of Gliclazide) injected in HPLC. Different ratios of Buffer: ACN were experimented to optimize the mobile phase. Finally a mixture of Buffer: ACN in the ratio of 55:45 % at the flow rate of 1.0ml/min was used for the elution of these drugs, Buffer used was Sodium dihydrogen orthophosphate monohydrate (pH 5.0).

RESULTS AND DISCUSSION

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable HPLC method for the analysis of Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide in fixed dose combined dosage form. Different ratios of Buffer: ACN were experimented to optimize the mobile phase. Finally a mixture of Buffer: ACN in the ratio of 55:45 % at the flow rate of 1.0ml/min was used for the elution of these drugs, Buffer used was Sodium dihydrogen orthophosphate monohydrate (pH 5.0). Metformin hydrochloride, Pioglitazone hydrochloride and Gliclazide were eluted with retention times of 2.119 min, 10.494 min and 8.603 min respectively.

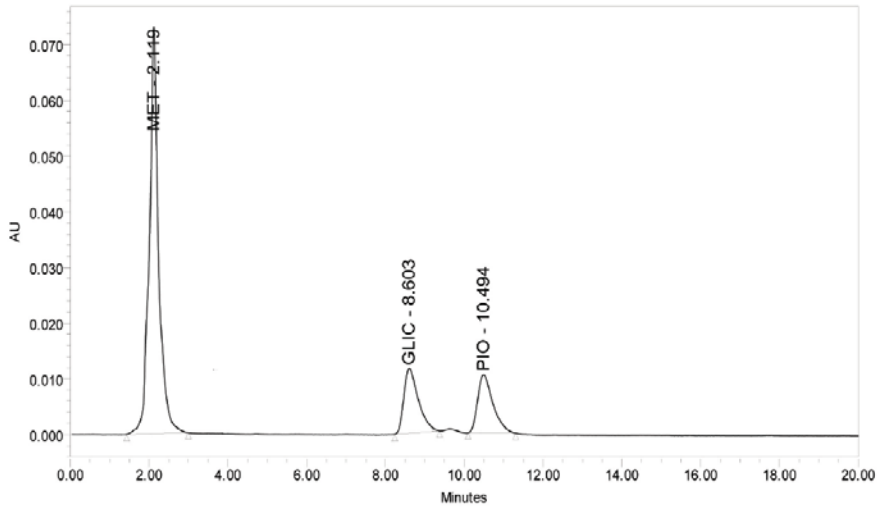


Fig. 1: A typical chromatogram showing the peaks of Metformin hydrochloride (2.119 min), Pioglitazone hydrochloride (10.494 min) and Gliclazide (8.603) in mix standard

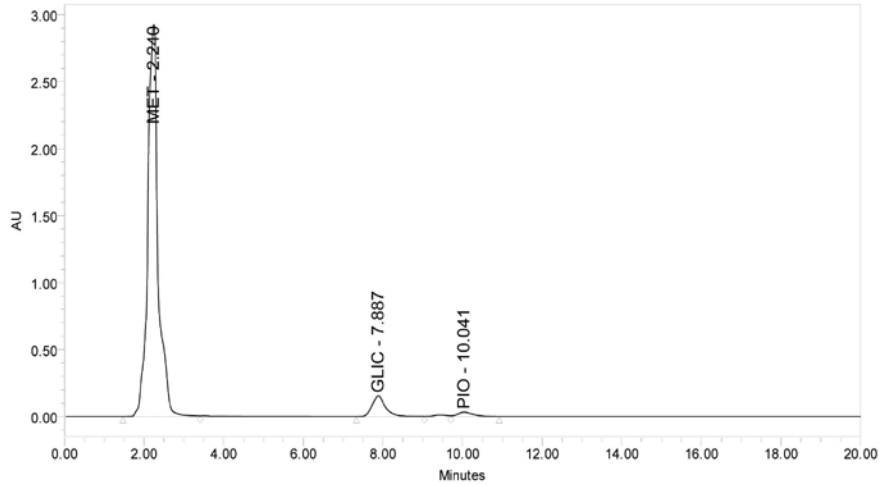


Fig. 2: A typical chromatogram showing the peaks of Metformin hydrochloride (2.240 min), Pioglitazone hydrochloride (10.041 min) and Gliclazide (7.887) in pharmaceutical dosage forms

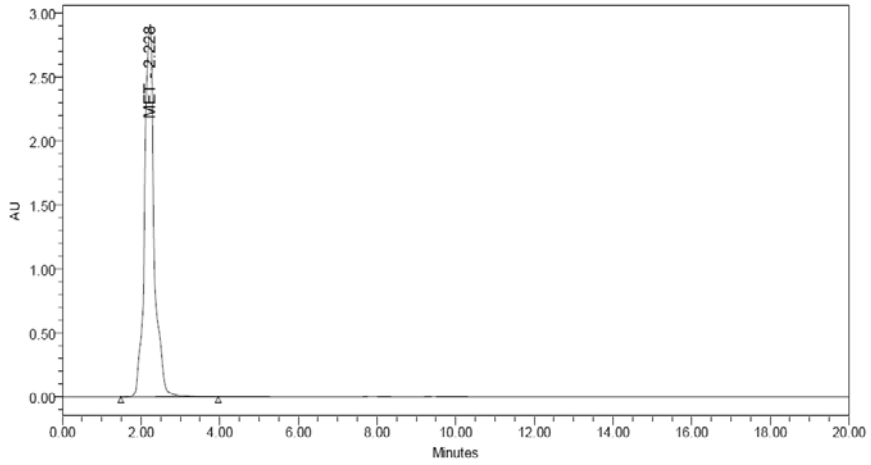


Fig. 3: A typical chromatogram showing the peak of Metformin hydrochloride (2.228 min)

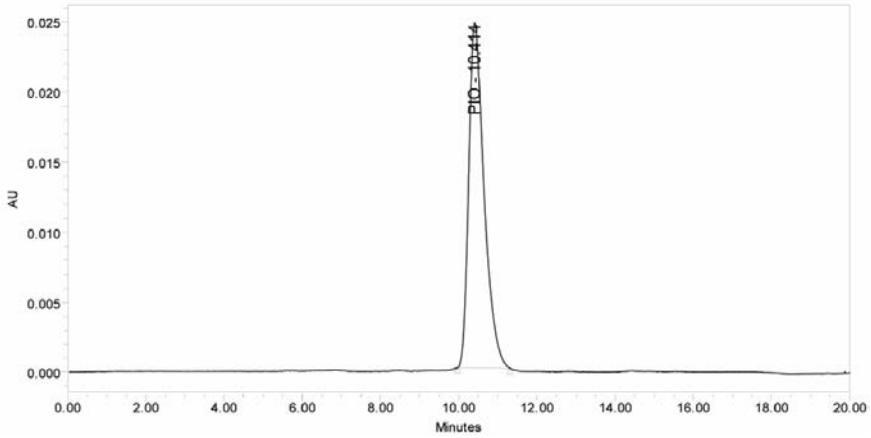


Fig. 4: A typical chromatogram showing the peak of Pioglitazone hydrochloride (10.414 min)

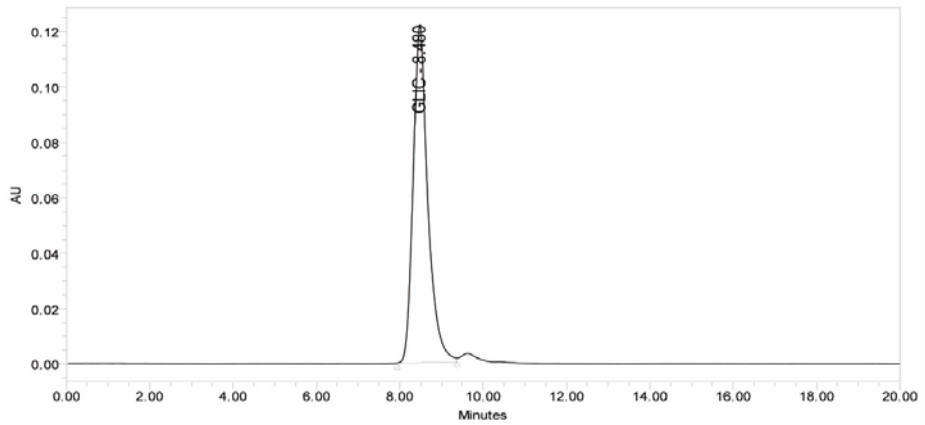


Fig. 5: A typical chromatogram showing the peak of Gliclazide (8.480 min)

Table 1: Results from assay in tablet formulation

Drug	Label claim (mg per tablet, n=6)	Amount found (mg)	% Purity	SD	% RSD
MET	500	497.957	98.37	0.146363	0.148778
PIO	15	14.180	98.67	0.075830	0.076885
GLIC	60	58.834	98.40	0.0805536	0.0818579

Each mean value is the result from three replicate analysis

Method validation

The method was validated according to the ICH Guidelines. The following validation characteristics were addressed: linearity, accuracy, precision, and specificity, limits of detection and quantitation.

Linearity and range

Linearity of the method was studied by injecting the mixed standard solutions in the concentration range of 50-350 µg/ml for Metformin hydrochloride, 1.5-10.5 µg/ml for Pioglitazone hydrochloride and 6-60 µg/ml for 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.

Precision

The precision of the proposed method was evaluated by carrying out six independent assays of test sample. RSD (%) of six assay values obtained was calculated.

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.

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

Accuracy

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 (%), RSD (%) was calculated.

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.

Table 2: Results for Intraday Precision, Interday Precision, LOD and LOQ

rug	Intraday Precision %RSD	Interday Precision %RSD			LOD	LOQ
		Day 1	Day 2	Day 3		
MET	0.2184357	0.562985	0.235882	0.782714	3.5469676	10.748386
PIO	0.2587827	0.0587171	0.4378775	0.2305139	0.0540513	0.1637919
GLIC	0.6728594	0.2653044	0.575267	0.6405603	0.775066	2.3486848

Each mean value is the result from three replicate analysis

%RSD= (SD/Mean) x 100

Table 3: Results for Accuracy

Drug	Amount taken (µg/ml)	Amount added(%)	% Recovery	SD	% RSD
MET	500	80	99.7333	0.55581	0.5573
		100	99.05666	0.02081	0.02101
		120	99.24666	0.025166	0.02535
PIO	15	80	100.7033	0.210079	0.208612
		100	99.91	0.045826	0.045867
		120	100.7263	0.201991	0.200534
GLIC	60	80	100.8	0.10535	0.10452
		100	99.26667	0.087369	0.088014
		120	99.92	0.829277	0.829941

Each mean value is the result from three replicate analysis

Table 4: Summary of System Suitability Parameters

S. No.	System suitability parameters	Metformin Hydrochloride	Gliclazide	Pioglitazone Hydrochloride	ICH limits
1.	Retention time	2.119	8.603	10.494	-
2.	Resolution	-	11.349640	2.605616	>2
3.	Asymmetry factor	1.02	1.54	1.50	< 2
4.	Tailing factor	1.02	1.54	1.50	< 2
5.	Capacity factor	1.1194	7.6028	9.4940	1-10
6.	Plate count	910.72	2559.22	3531.11	>2000

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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