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Development and Validation of Simultaneous Chromatographic method for Estimation of Metformin Hcl, Pioglitazone Hcl and Glipizide in a Combined Dosage Form by RP- HPLC

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ABSTRACT

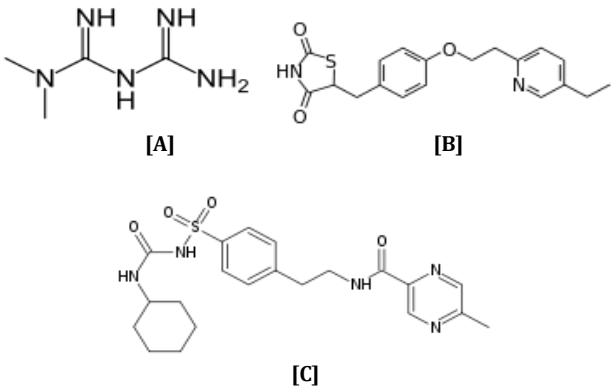
A simple, rapid, and precise reversed-phase high-performance liquid chromatographic method for simultaneous analysis of Metformin Hcl, Pioglitazone Hcl, and Glipizide in a tablet dosage form has been developed and validated. Chromatography was performed on an Inertsil C18, 250 X 4.6mm, 5µ column with 40:60 (v/v) 10 mM potassium dihydrogen phosphate buffer: methanol as mobile phase at a flow rate of 1.2 ml/min. UV detection at 240nm; Metformin Hcl, Pioglitazone Hcl, and Glipizide were eluted with retention times of 1.766, 5.316, and 9.550min, respectively. The method was validated in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the concentration ranges 5-100µg/ml for Metformin Hcl, Pioglitazone Hcl, and Glipizide. The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of the three drugs in tablets. Statistical analysis proves that the method is suitable for the analysis of Metformin Hcl, Pioglitazone Hcl and Glipizide as a bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of three drugs and also for its estimation in plasma and other biological fluids.

Key Words: *Metformin Hcl, Pioglitazone Hcl and Glipizide, RP-HPLC, Validation, Combined Dosage Forms.*

INTRODUCTION

Metformin, [MET] chemically [1,1-dimethyl biguanide hydrochloride] (**Fig. 1**) [1]. It acts by suppressing excessive hepatic glucose production and improving glucose clearance, its predominant effect is to decrease fasting plasma glucose. 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. Glipizide [GLP] is an oral rapid- and short-acting anti-diabetic drug from the sulfonylurea class. It is classified as a second generation sulfonylurea, which means that it undergoes enterohepatic circulation. Second-generation sulfonylureas are both more potent and have shorter half-lives than the first-generation sulfonylureas, it is chemically N-(4-[N-(cyclohexylcarbamyl)sulfamoyl]phenethyl)-5-methylpyrazine-2-carboxamide (**Fig. 2**) [2]. Pioglitazone hydrochloride (PIO) is chemically designated as 5-[[4-[2-(5-Ethyl-2-pyridinyl)ethoxy] phenyl]methyl]-2,4-thiazolidinedione (**Fig. 3**). It is a member of the thiazolidinedione group. The combination of Metformin Hcl, Pioglitazone Hcl, and Glipizide is used in pharmaceutical preparations. This combination, however, is not present in any official pharmacopoeia. In this respect, a method for the analysis of this combination is needed.

In the scientific literature, analysis of MET, PIO, and GLP has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of MET [3-8]. GLP [9], PIO individually [10]. And in two component formulations of PIO and MET have been analyzed in combination by [11-18]. Simultaneous HPLC analysis of MET with GLP in combinations with other drugs have also been reported [19].



[A] **[B]**

[C]

Fig. 1: Structures of Metformin (A), Pioglitazone (B), and Glipizide (C)

Because no chromatographic method for simultaneous analysis of MET, PIO, and GLP in a combined dosage form has yet been reported, it was essential to develop a chromatographic method for simultaneous estimation of all the three drugs in a tablet formulation. The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH norm [20-22].

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METERIALS AND METHODS

1. Experimental:

1.1. Apparatus:

The analysis was performed by using the analytical balance Shimadzu Libror, pH meter Control Dynamics, the HPLC used is of Younglin with UV detector. Column used in HPLC is Inertsil C18, 250 X 4.6mm, 5µ (isocratic). The mobile phase consists of A & B with mixture of Buffer and Methanol which are degassed in a sonicator for about 10minutes the injection volume is 20mL and the ultra violet detection was at 230nm.

1.2. Reagents and solutions:

Pure samples of Metformin Hcl, Pioglitazone Hcl, Glipizide and other reagents such as Methanol and water used were of HPLC and milli-q grade. All other chemicals like glacial acetic acid used were of AR grade. Optimized chromatographic conditions are listed in **Table.1**.

1.3. Standard solution preparation:

Accurately weigh about 5mg of Metformin Hcl, Pioglitazone Hcl and Glipizide and transfer it into a 100ml volumetric flask. Add 50ml of diluent and kept in an ultrasonic bath until it dissolved completely. Make up to the mark with the mobile phase and mix. This yielded solution of 50µg/ml concentration. This reference standard solution was analyzed using the HPLC instrument conditions mentioned.

Validation experiments were performed to demonstrate System suitability, precision, linearity, Accuracy study of analytical solution and robustness.

2. Method validation:

The method was validated according to the ICH guidelines [17]. The following validation characteristics were addressed: linearity, accuracy, precision, and specificity, limits of detection and quantitation and robustness.

2.1. Linearity and range:

The Linearity of detector response is established by plotting a graph to concentration versus area of Metformin Hcl, Pioglitazone Hcl and Glipizide standards respectively and determining the correlation coefficient. A series of solutions of Metformin Hcl, Pioglitazone Hcl and Glipizide standards in the concentration ranging from about 5 to 100µg/ml level of the target concentration (50µg/ml of Metformin Hcl, Pioglitazone Hcl and Glipizide) were prepared and injected into the HPLC system.

2.2. 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. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument.

2.3. 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. By applying the visual evaluation method, LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQ was considered as the lowest concentration of analytes in standards that can be reproducibly measured with acceptable accuracy and precision.

2.4. Robustness and system suitability:

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.1 ml/min), mobile phase composition (methanol±5 ml).These chromatographic variations were evaluated for resolution between MET, PIO, GLP.

2.5. System suitability:

The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between MET, PIO, and GLP peaks were defined.

2.6. Accuracy:

Accuracy of the method was carried out by applying the method to drug sample (MET, PIO, and GLP combination tablets) to which known amounts of MET, PIO, and GLP standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and 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 (%) were calculated.

2.7. Analysis of marketed formulation:

The marketed formulation was assayed as described above. The peak areas were measured at 240nm, and concentrations in the samples were determined using multilevel calibration developed on the same LC system under the same conditions using linear regression analyzed for MET, PIO, and GLP in the same way as described earlier.

RESULTS AND DISCUSSION

1. Method development and optimization:

MET, PIO, GLP standards having concentration 50µg/ml was scanned in UV- region between 200- 400 nm. λmax of MET, PIO, GLP Isobesthic point was found to be at 240nm. The MET, PIO, GLP peaks in the sample was identified by comparing with the MET, PIO, GLP standards and the Retention time was found to be .766, 5.316, and 9.550mins, respectively. The estimation MET, PIO, GLP were carried out by RP-HPLC using Mobile phase having a composition volumes of 40 volumes of buffer (0.05M KH_2PO_4) and 60 volumes of Methanol. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Inertsil C18, (250 X 4.6mm, 5µ particle size). Flow rate of Mobile phase was 1.2ml/min.

2. Validation:

2.1. Linearity:

Linearity was evaluated by analysis of working standard solutions of MET, PIO, and GLP of five different concentrations. The range of linearity was from 5-100µg/ml for MET, PIO, and GLP. The regression data obtained are represented in **Table 2** & **Fig. 5-7**. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration of each drug.

2.2. Precision:

The results of the repeatability and intermediate precision experiments are shown in **Table 2**. The developed method was found to be precise, with RSD values for repeatability and intermediate precision.

2.3. LOD and LOQ:

The LOD and LOQ values were found to be and 0.15 and 0.45 µg/ml for MET, 0.03 and 0.1 µg/ml for PIO, and 0.04 and 0.12 µg/ml for GLP.

2.4. System suitability:

System suitability parameters such as the number of theoretical plates, Resolution and peak tailing are determined. The results obtained are shown in **Table 2**.

2.5. Robustness of the method:

To ensure the insensitivity of the developed HPLC method to minor changes in the experimental conditions, it is important to demonstrate its robustness. None of the alterations caused a significant change in resolution between MET, PIO, and GLP, peak

area, % RSD, Tailing Factor and Theoretical Plates as shown in **Table 3**.

2.6. Recovery studies:

Good recoveries of the MET, PIO, and GLP were obtained at various added concentrations for the tablets as shown in **Table 1**.

2.7. Analysis of a commercial formulation:

Experimental results of the amount of MET, PIO, and GLP in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets.

Table No. 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Younglin with UV detector)
Column	Inertsil C18, 250 X 4.6mm, 5µ is suitable
Mobile Phase*	Buffer: Acetonitrile (40:60v/v)
Flow rate	1.2ml/min
Detection	PDA at 240nm
Injection volume	20µl
Column Temperature	Ambient
Runtime	12 mins

Table No. 2: System suitability parameters of MET, PIO, and GLP

Parameter	MET	PIO	GLP
Calibration range (µg/ml)	5-100	5-100	5-100
Theoretical plates	2730.7	3339.0	4551.6
Resolution	0	13.2284	9.0824
Tailing factor	1.102	1.084	1.012
Correlation Coefficient(r^2)	0.9997	0.9991	0.9998
% Recovery	100.90 - 99.30%	99.21-100.89%	98.68-99.92%
Assay %	97.40%	98.03%	98.50%
System Suitability %RSD	0.82%	0.58%	0.91%

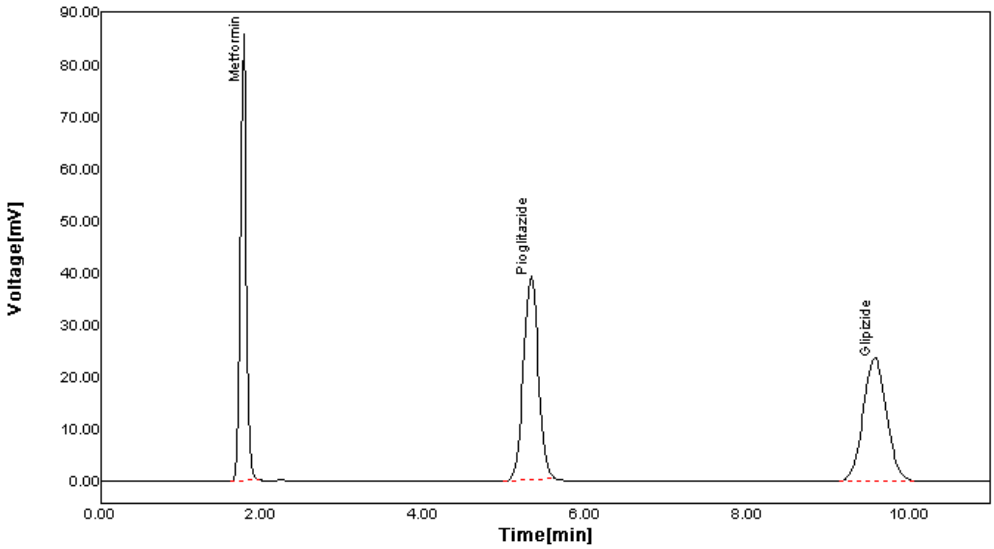

Fig. 4: Standard Chromatogram of MET, PIO, and GLP

Table No. 3: Linearity of MET, PIO, and GLP

Conc.s	Peak Area of MET	Peak Area of PIO	Peak Area of GLP
5	51974	54573	52165
10	101564	112936	103862
25	226874	257528	238178
50	452392	497947	473184
75	679367	728963	709015
100	908634	968986	956876

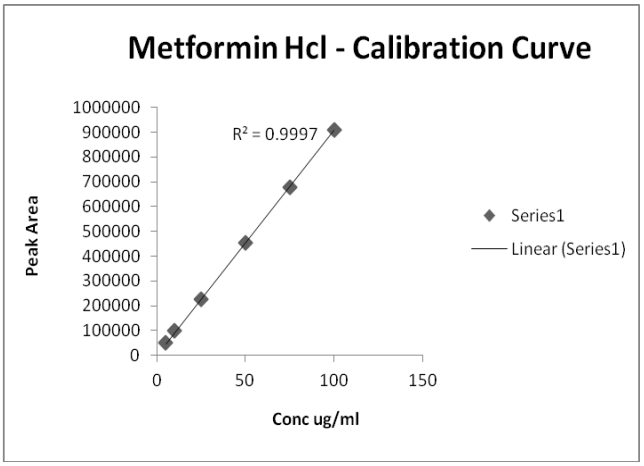

Fig. 5: Regression analysis of the calibration curve for MET

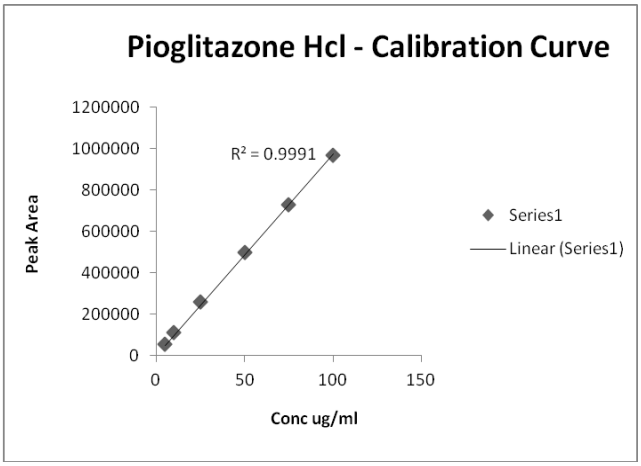

Fig. 6: Regression analysis of the calibration curve for PIO

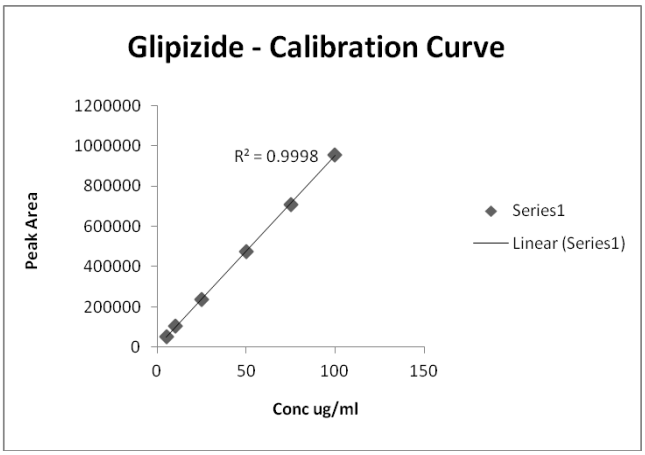

Fig. 7: Regression analysis of the calibration curve for GLP

Table No. 4: Robustness Parameters of MET, PIO, and GLP

Parameters	Adjusted To	% RSD (MET)	% RSD (PIO)	% RSD (GLP)
Flow Rate as per Method (1.2ml/min)	1.1 ml/min	0.66	0.39	0.82
	As it is	0.12	0.83	0.76
	1.3ml/min	0.66	0.48	0.92
Mobile Phase Comp. (Buffer:Methanol) 40:60	Buffer:Methanol (35:65)	0.23	0.53	0.65
	As it is	0.52	0.34	0.46
	Buffer:Methanol (45:55)	0.34	0.36	0.58

CONCLUSION

The new HPLC method described in this paper provides a simple, convenient and reproducible approach for the simultaneous identification and quantification that can be used to determine Metformin Hcl, Pioglitazone Hcl, and Glipizide in routine quality control.

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