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Analytical Method Development and Validation for Simultaneous Estimation of Metformin, Pioglitazone and Glimepiride in Combined Tablet Dosage Form by using RP-HPLC

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A B S T R A C T

Objective: To develop and validate a suitable method for the simultaneous estimation of Metformin, Pioglitazone and Glimepiride in Tablet dosage form by using RP-HPLC method. **Methods:** The chromatographic separation was performed on ZORBAX SB-PHENYL, 250X 4.6mm. The mobile phase was prepared by mixing Ammonium acetate and methanol in the ratio of (90:10%) v/v that run isocratically at the flow rate of 1.0ml/min. and detection of all the eluents is carried out by UV Detector. **Results:** The Rt of Metformin, Pioglitazone and Glimepiride were found to be 4.754 min., 6.390 min. and 9.948 min. respectively. Method was found to be linear over the range of 250-750 µg/ml for Metformin HCl, 7.5-22.5 µg/ml for Pioglitazone HCl and 0.5-1.5 µg/ml for Glimepiride. Percentage recoveries of Metformin, Pioglitazone and Glimepiride were obtained in the range of 101%, 100% and 99.66% respectively. The limit of detection of Metformin HCl, Pioglitazone HCl and Glimepiride is 2.203µg/ml, 0.828µg/ml and 0.007µg/ml respectively. The limit of Quantification of Metformin HCl, Pioglitazone HCl and Glimepiride is 7.345µg/ml, 0.2760µg/ml and 0.024µg/ml respectively. **Conclusion:** A new sensitive, simple, and stability indicating high performance layer chromatographic (HPLC) method has been developed and validated for determination of Metformin, Pioglitazone and Glimepiride. The proposed method can may be used for routine determination of Metformin, Pioglitazone and Glimepiride stability.

Keywords: Metformin, Pioglitazone and Glimepiride, RP-HPLC, UV Detector.

A R T I C L E I N F O

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1. Introduction

Metformin hydrochloride (MET) is chemically known as 1,1-dimethyl biguanide hydrochloride (Fig. 1) is an anti-diabetic drug from the biguanide class of oral antihyperglycemic agents, and used as the first line agent for the treatment of non insulin-dependent diabetes mellitus (Type II) particularly in obese patients. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by Metformin of AMP-activated protein kinase (AMPK) [1], a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for Metformin inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. It also causes improvements in endothelial dysfunction, hemostasis and oxidative stress, insulin resistance, lipid profiles, and fat redistribution [2]. Recent clinical trials suggest that Metformin, in addition to its efficacy in treating Type –II diabetes, may also have therapeutic potential in other conditions including diabetic nephropathy, cardiovascular diseases, polycystic ovary disease [3] and the prevention or treatment of cancer [4].

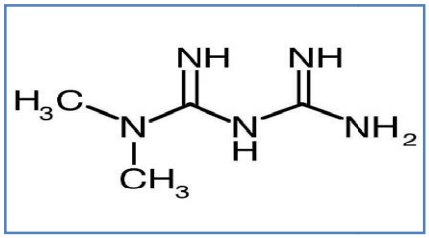

Figure 1: Chemical structure of Metformin

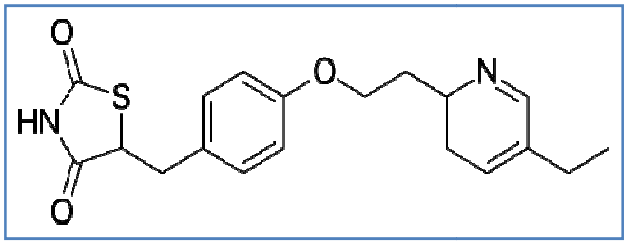

Figure 2: Chemical structure of Pioglitazone

Pioglitazone HCl is chemically known as 5-({4-[2-(5-ethylpyridin-2-yl) ethoxy] phenyl} methyl)-1, 3-thiazolidine-2, 4-Dione (Fig. 2). Pioglitazone (brand name Actos) is a prescription drug of the thiazolidinedione (TZD) class with hypoglycemic (antihyperglycemic, antidiabetic) action to treat diabetes. Pioglitazone is used to lower blood glucose levels in the treatment of diabetes mellitus type 2 (T2DM) either alone or in combination with a sulfonylurea, metformin, or insulin. The main study that looked at the medication, however, found no statistically significant difference in the main cardiovascular outcomes that were looked at. The secondary outcome of death from all causes, myocardial infarction, and stroke were lower. Pioglitazone has also been used to treat non-alcoholic steatohepatitis (fatty liver), but this use is presently considered experimental. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α.It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue, and the liver. As a result, pioglitazone reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose, insulin and glycated hemoglobin in the bloodstream. Side effects include Fluid retention, Peripheral edema, CHF and Mild weight gain.

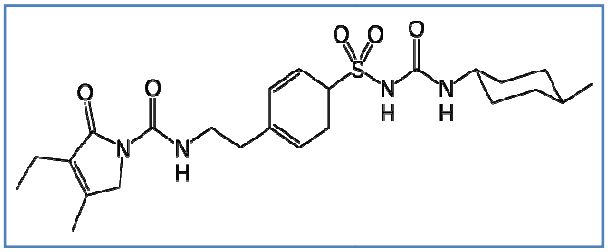

Figure 3- Structure of Glimepiride

Glimepiride is chemically known as 3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl)carbamoyl] amino}sulfonyl) phenyl] ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide.

Glimepiride (original trade name Amaryl) is an orally available medium-to-long-acting sulfonylurea antidiabetic drug. It is sometimes classified as either the first third-generation sulfonylurea, or as second-generation. The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin. Pharmacokinetics show that gastrointestinal absorption is complete, with no interference from meals. Significant absorption can occur within one hour, and distribution is throughout the body, 99.5% bound to plasma protein. Metabolism is by oxidative biotransformation. Excretion in the urine is 65%, and the remainder is excreted in the feces.Side effects include Gastrointestinal tract (GI) disturbances, occasional allergic reactions, rarely blood production disorders including thrombocytopenia, leukopenia, and hemolytic anemia.

To the best of our knowledge only 2 HPLC method of analysis has yet been reported for simultaneous analysis of Metformin hydrochloride, Pioglitazone HCl, and Glimepiride. Hence, in the present communication we would like to report a simple, economic, feasible, rapid, sensitive, and validated specific RP-HPLC method for the simultaneous estimation of Metformin hydrochloride, PIoglitazone HCl, and Glimepiride in formulation.

2. Materials and Methods

Chemicals

The Active Pharmaceutical Ingredients were obtained from Lara Drugs Pvt Ltd with a potency of Metformin Hydrochloride (99.8%), Pioglitazone Hydrochloride (99.8%) and Glimepiride (99.4%). A commercial tablet formulation Glimy MP1 from Dr. Reddy's Laboratories Ltd, (Hyderabad, India) containing 500mg of MET and 15mg of Pioglitazone HCl and 1 mg of Glimepiride was purchased from local market and used within their shelf life period. Ammonium acetate was obtained from S.D fine chem ltd, and Methanol from Rankem. All other chemicals used were of pharmaceutical or analytical grade.

Chromatographic conditions

The HPLC system (Agilent 1220 series USA) consisted of quaternary gradient system (600 Controller), in-line degasser (Agilent, model AF), VWD detector (Agilent, 2998 model) and manual sampler. Data was processed using Ez chrome software. Isocratic elution of the mobile phase Ammonium acetate: Methanol (90:10) v/v maintained at a pH 4.5 was done with the flow rate of 1 ml/min. Separation was performed on a ZORBAX SB-PHENYL, (250 x 4.6 mm i.d, 5 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the EZchrome software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 µm membrane filter and degassed by sonication before use.

Mobile phase was used as diluents. The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm. The run time was set at 15 min and a column temperature was maintained at 30°C. The volume of injection was 10 µl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluent was detected at 254 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of Metformin, Pioglitazone and Glimepiride as per ICH guidelines.

Preparation of standard stock solutions of Metformin and Telmisartan:

Standard stock solution of Metformin HCl, Pioglitazone HCl and Glimepiride is prepared by adding 1000mg of Metformin HCl, 30mg of Pioglitazone HCl & 2mg of Glimepiride to 100ml volumetric flask containing few ml of Methanol. After dissolving the solids by sonicating, make up the volume up to mark with Methanol. Transfer 10 ml of the above solution in 100 ml volumetric flask and dilute to volume with methanol, yielding a solution containing 1000µg/ml of Metformin HCl, 30µg/ml of Pioglitazone & 2µg/ml of Glimepiride.

Preparation of test Solution (Analysis in tablets):

Ten tablets of Glimy MP1 were weighed and finely powdered. A powder equivalent to the label claim was accurately weighed, transferred into a 100 ml volumetric flask containing mobile phase. The above mixture was sonicated for about 10 min. for complete mixing. This solution was filtered through Whatman No.1 filter paper. From the filtrate 5 ml of it is taken in 50 ml volumetric flasks and diluted with mobile phase up to the mark so as to get a concentration ranging from 500µg mL^{-1} each of Metformin, 15µg mL^{-1} ``Pioglitazone and 1µg $mL^{-1}$ ``Glimepiride.

3. Results and Discussion

The present research work was designed at developing a rapid, sensitive, precise and accurate HPLC method for the simultaneous estimation of Metformin, Pioglitazone and Glimepiride in pharmaceutical dosage form. A binary mixture of mixed buffer pH adjusted to 4.5 with Ammonium acetate: Methanol (90:10) v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing. A flow rate of 1.0 ml/min of the mobile phase was found to be suitable.

Method development

After various trials, the following chromatographic conditions were finally optimized for the simultaneous estimation of Metformin and Telmisartan in a tablet dosage form. Mobile phase constitutes of Ammonium acetate: Methanol (90:10) v/v maintained at a pH 4.5. Detection wave length 254 nm flow rate 1.0 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally standard solution of the individual samples of drugs and mixed standard solutions were injected and the chromatograms were recorded.

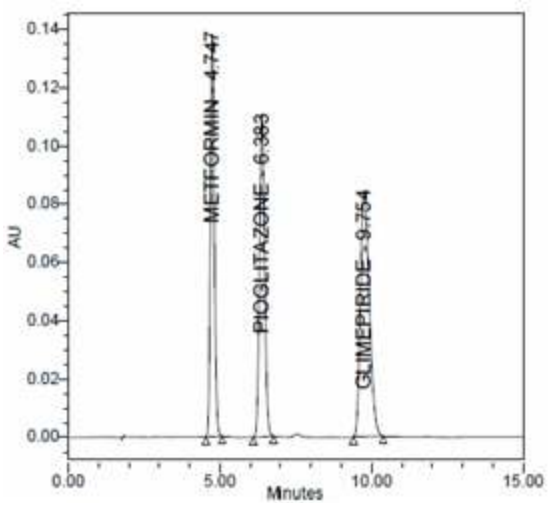

Figure 4: Typical chromatogram of Metformin, Pioglitazone and Glimepiride standard.

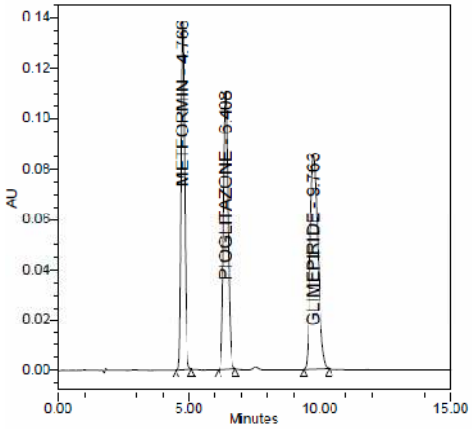

Figure 5: Typical chromatogram of Metformin, Pioglitazone and Glimepiride sample.

Method validation After development of method, validation of the method for simultaneous estimation of Metformin and Telmisartan was performed in accordance with ICH guidelines (International Conference on Harmonization (ICH) 2000) which include System suitability, Linearity, Accuracy, Precision, LOD and LOQ, Specificity and Robustness.

Linearity

Calibration graphs were constructed by plotting peak area vs concentration of Metformin, Pioglitazone and Glimepiride and the regression equations were calculated. The calibration graphs were plotted over 5 different linear concentrations in the range of 50-150% for all the drugs. Aliquots (10µl) of each solution were injected under the operating chromatographic condition described above . The Method was found to be linear over the range of 250-750 µg/ml for Metformin HCl, 7.5-22.5 µg/ml for Pioglitazone HCl and 0.5-1.5 µg/ml for Glimepiride.

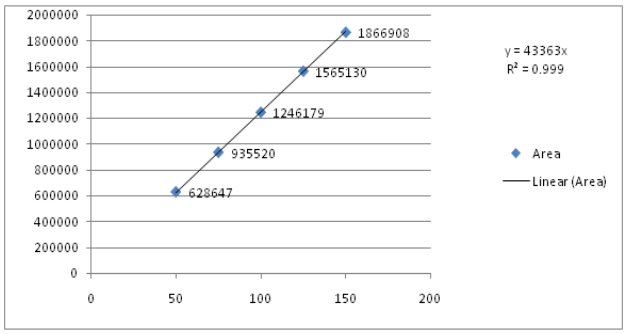

Figure 6 Calibration curve of Metformin HCl

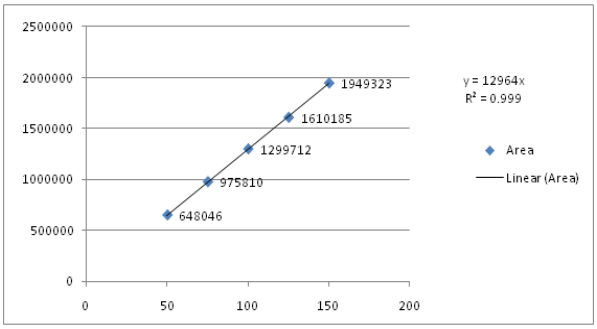

Figure 7 Calibration curve of Pioglitazone HCl

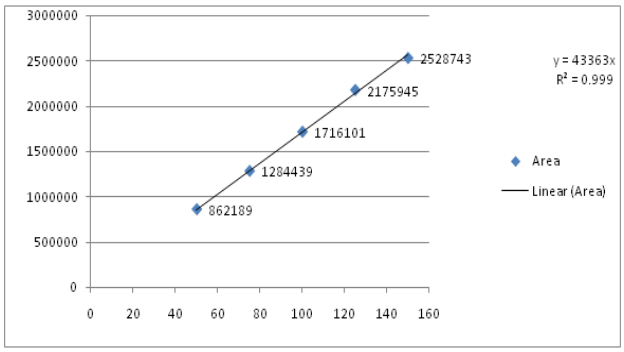

Figure 8 Calibration curve of Glimepiride

Accuracy: The accuracy of the method was established by recovery studies i.e external standard addition method. The known amount of standard was added at three different levels to pre analyzed sample. Each determination was performed in triplicate. The mean recoveries obtained were 101,100 and 99.66 for Metformin, Pioglitazone and Glimepiride respectively. The results of accuracy were tabulated in table 2.

Precision

The Method precision of the proposed method was determined by analyzing mixed standard solution of Metformin, Pioglitazone and Glimepiride at 100 %. The results are reported in terms of relative standard deviation. The % RSD values of Metformin, Pioglitazone and Glimepiride were found to be 0.26%, 0.18%, 0.14% respectively.

Limit of detection (LOD) and limit of quantitation (LOQ): The limit of detection (LOD) and limit of quantitation (LOQ) of Metformin and telmisartan were determined by calculating the signal-to noise (S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD values for Metformin, Pioglitazone and Glimepiride were found to be 2.203, 0.0828, 0.007µg/ml respectively. LOQ values for Metformin, Pioglitazone and Glimepiride were found to be 7.345, 0.2760, 0.024µ g ml^{-1} respectively.

Robustness: The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions like flow rate (0.2 mL min^{-1}), and temperature of the mobile phase (± 2^0c).

Assay of the tablet dosage form: The proposed validated method was successfully applied to determine Metformin, Pioglitazone and Glimepiride in tablet dosage form. The result obtained was comparable with corresponding labeled amounts (Table 2).

The accuracy of the proposed method was assessed by recovery studies. All solutions were prepared and analysed in triplicate. The above procedure is adopted for both the drugs and a high recovery values obtained (Table-1) indicate that the proposed method is highly accurate. The method specificity was assessed by studying the chromatograms (Figure 4,5) obtained for a mixture of the drugs and the common excipients. As none of the excipients interfered with the analytes of interest, the method was found to be suitable for analyzing the commercial formulation of these drugs.

Table 1: Summary of validation parameters

S. No	Validation Parameters	Results		
		Metformin	Pioglitazone	Glimepiride
1	Accuracy (% Recovery)	101	100	99.6
2	Precession (% RSD)	0.26%	0.18%	0.14%
3	LOD	2.203µ g ml^{-1}	0.0828µg ml^{-1}	0.007µg ml^{-1}
4	LOQ	7.345µg ml^{-1}	0.2760µg ml^{-1}	0.024µg ml^{-1}
5	Linearity	250-750 µg ml^{-1}	7.5-22.5 µg ml^{-1}	0.5-1.5 µg ml^{-1}

Table 2: Assay of the tablet dosage form

METFORMIN			PIOGLITAZONE	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	124386	1246275	1282406	1293211
Injection-2	1248393	1240666	1291899	1297355
Injection-3	1257518	1241027	1301747	1292670
Injection-4	1254496	1248739	1300525	1295978
Injection-5	1238863	1245073	1284422	1292717
Injection-6	1247556	1246622	1297206	1291207
Average Area	1248443	1244733	1293034	1293856
Tablet average weight	1040.8		1040.8	
Standard weight	500		15	
Equivalent weight	1040.8		1040.8	
Label amount	500mg		15mg	
std. purity	99.8		99.8	
Amount found in mg	**498.5**		**15**	
Assay(%purity)	**99.7**		**100**	

GLIMEPIRIDE		
	Standard Area	Sample Area
Injection-1	1709422	1720918
Injection-2	1723703	1726375
Injection-3	1732004	1725269
Injection-4	1708493	1720979
Injection-5	1715934	1721150
Injection-6	1738415	1723454
Average Area	1721328	10338145
Tablet average weight	1040.8	
Standard weight	1	
Equivalent weight	1040.8	
Label amount	1mg	
std. purity	99.4	
Amount found in mg	**1**	
Assay(%purity)	**100**	

4. Conclusion

The present results provide clear evidence that the proposed method can be successfully used for simultaneous determination of drug content in marketed formulations.

5. Acknowledgement

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