



Research Article

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SIMULTANEOUS ESTIMATION OF PIOGLITAZONE HCL AND GLIMEPIRIDE BY RP-HPLC METHOD

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ABSTRACT

The main objective of the present work is to develop a new simple RP-HPLC method for simultaneous estimation of Pioglitazone HCl and Glimepiride. A series of mobile phases were tried, among the various mobile phases 0.05 M Disodium hydrogen phosphate Buffer (pH 3 ± 0.2) Methanol and Acetonitrile in the ratio of 45:30:25 was found to be an ideal mobile phase since it gave a good resolution and peak shapes with perfect optimization. The flow rate was found to be optimized at 1 ml/min. The linearity and range was found to be in the range of 30-150 $\mu\text{g/ml}$ for Pioglitazone HCl and for Glimepiride 4-20 $\mu\text{g/ml}$. The correlation coefficient of Pioglitazone HCl and for Glimepiride was found to be 0.9982 and 0.9978 respectively, which indicates a perfect correlation. The developed method was validated for accuracy, precision, and system suitability. The percentage recovery of Pioglitazone HCl and for Glimepiride was found to be 99.77% and 100.06% respectively. The good percentage recovery of the sample clearly indicates the reproducibility and accuracy of the developed method. Similarly the % RSD value for precision was also found to be within the acceptable limit.

KEY WORDS: Pioglitazone HCl ,Glimepiride and RP-HPLC

INTRODUCTION:

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action. 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 lipidic, muscular tissues and in 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 haemoglobin in the bloodstream. Although not clinically significant, pioglitazone decreases the level of triglycerides and increases that of high-density lipoproteins (HDL) without changing low-density lipoproteins (LDL) and total cholesterol in patients with disorders of the lipid metabolism, although statins are the drug of choice for this. Glimepiride is a medium-to-long acting sulfonylurea

anti-diabetic drug. It is marketed as Amaryl by Sanofi-Aventis. Glimepiride is the first third-generation sulfonylurea, and is very potent. It is sometimes classified as third-generation, and sometimes classified as second-generation. The primary mechanism of action of Glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. In addition, extrapancreatic effects may also play a role in the activity of sulfonylureas such as Glimepiride. This is supported by both preclinical and clinical studies demonstrating that Glimepiride administration can lead to increased sensitivity of peripheral tissues to insulin. These findings are consistent with the results of a long-term, randomized, and placebo-controlled trial in which Glimepiride therapy improved postprandial insulin/C-peptide responses and overall glycemic control without producing clinically meaningful increases in fasting insulin/C-peptide levels. However, as with other sulfonylureas, the mechanism by which Glimepiride lowers blood glucose during

long-term administration has not been clearly established. Glimepiride is effective as initial drug therapy. In patients where monotherapy with Glimepiride or Metformin has not produced adequate glycemic control, the combination of Glimepiride and Metformin may have a synergistic effect,

since both agents act to improve glucose tolerance by different primary mechanisms of action. This complementary effect has been observed with Metformin and other sulfonylureas, in multiple studies.

EXPERIMENTAL WORK:

Reagents and Chemicals:

Milli Pore & Milli Q water, Sodium Lauryl Sulphate, Disodium Hydrogen Phosphate, Methanol 0.2µ micron glass filtered HPLC grade, Acetonitrile 0.2µ micron glass

filtered HPLC grade, Ortho Phosphoric acid Working reference standard Pioglitazone HCl & Glimepiride, Tablet brand used Pionorm – G.

Instruments required:

Single pan balance, Vacuum pump with filtration kit, HPLC- VARIAN PRO STAR. UV 310 VARIAN – UV detector, Chromatographic data

software- STAR WORKSTATION Version:5.0, Stationary phase C₁₈ (250x 4.6 mm) 5µm particle size (VARIAN), Sonicator, pH meter

Chromatographic conditions:

The following optimized parameters were used as a final method for the

simultaneous estimation of Pioglitazone HCl and Glimepiride.

Instrument	VARIAN PRO STAR
Column	C ₁₈ (250x4.6mm) with 5µm particle size.
Mobile phase	Buffer: methanol: Acetonitrile (45:30:25) 0.05M Disodium hydrogen Phosphate adjusted to pH 3.0
Flow rate	1 ml/minute

Wavelength	225nm
Injection volume	10µl
Run time	10 minutes
Temperature	Ambient
Mode of operation	Isocratic elution

Preparation of Disodium Hydrogen Phosphate buffer pH 3.0:

1gm of Sodium Lauryl Sulphate and 0.71gm of Disodium Hydrogen phosphate was taken in a 500ml graduated glass container and to that

500ml of milli pore water is added to makeup the final volume. To adjust the pH 3 ± 0.2 , ortho phosphoric acid is added.

Preparation of mobile phase:

From the prepared buffer 45ml is added with 30ml of Methanol and 25ml of

Acetonitrile to prepare mobile phase and the solution is sonicated for 15 minutes.

Standard preparation:

Weigh accurately 25mg Pioglitazone HCl and it is transferred to 25ml standard flask. To that 10ml methanol is added to dissolve. Then it is added with mobile phase to makeup 25ml mobile phase. This is filtered with 0.45micron filter and it is degassed with sonicator. This is stock solution A. Similarly 25mg of Glimpride was weighed accurately and it was transferred to 25ml standard

flask. To that 10ml methanol is added to dissolve it. Then it is added with mobile phase to makeup 25ml. This is filtered with 0.45micron filter and it is degassed with sonicator. This is stock solution B. For Standard mixture preparation, 0.15ml of stock A and 0.02ml (20µl) of stock B are pipetted out and then it is transferred to 1ml Vial and the volume is made up with mobile phase

Sample preparation:

Weigh 0.2570gm of powdered sample is weighed and transferred to 25ml standard flask. To that 10ml methanol is added to dissolve and then the volume is makeup with mobile phase. Then it is filtered with 0.45micron filter and it is

Method development and Optimisation:**Selection of wavelength for detection of components:**

Solutions of Pioglitazone HCl and Glimepiride were scanned in the UV region and spectrum was recorded. The solvent used was 0.05M Disodium Hydrogen Phosphate buffer (pH 3.0),

Selection of chromatographic method:

Proper selection of the method depends on the nature of the sample (ionic or ionisable or neutral molecules), its molecular weight, pka value and stability. The drugs selected in the present study are polar and so reversed phase or ion exchange chromatography can be used. The reversed phase HPLC was selected for the initial separation because of its

Initial separation conditions:

The following chromatographic conditions were fixed initially to improve the separation

sonicated. This is stock solution C. From the stock C 0.25ml is pipetted out and transferred to the vial and volume is makeup to 1ml to prepare 150µgm/ml and 20µgm/ml final concentration of Pioglitazone HCl and Glimepiride.

Methanol and Acetonitrile in the ratio of 45:30:25. It was seen that at 225nm these compounds have very good absorbance which can be used for the estimation of compounds by RP- HPLC

simplicity and suitability. From the literature survey and with the knowledge of properties of the selected drugs, C₁₈ 5µ column was chosen as stationary phase and mobile phase with different compositions such as solvent and buffer was used. From all the data observed, obtained, available the initial separation conditions were set to work around.

Effect of ratio of mobile phase:

Under the chromatographic conditions mentioned above, the different ratios of mobile phase were tried. The chromatograms were observed for each

Effect of pH of mobile phase:

Several trials were made using different Buffer solutions of different pH range. The best separation was achieved with

Method validation:

The developed method was validated for simultaneous assay determination of

Linearity:

Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Pioglitazone HCl and

Precision:

To demonstrate agreement among results, a series of measurements are done with Pioglitazone HCl and Glimepiride six replicate injections of the specific standard at various time

RESULT AND DISCUSSION:

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both Pioglitazone HCl and

of the trails, out of which 45:30:25 i.e. 45 Buffer: 30 Methanol and 25 Acetonitrile were selected.

0.05M Disodium Hydrogen Phosphate at the pH 3.0.

Pioglitazone HCl and Glimepiride using following parameters.

Glimepiride which were found to be linear in the range of 30-150 µg/ml and 4-20 µg/ml respectively. Coefficient of correlation was 0.9982 and 0.9978 Figure-1 and 2.

intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.16 and 0.48 for Pioglitazone HCl and glimepiride Table 1.

Glimepiride. The mobile phase combination of 0.05M Disodium Hydrogen Phosphate buffer (pH 3.0), Methanol and Acetonitrile in the ratio of

45:30:25 found to be satisfactory and gave two symmetric and well resolved peaks for Pioglitazone HCl and Glimepiride. The retention time for Pioglitazone HCl and glimepiride were 3.18 and 5.02, respectively Figure 3. The calibration curve for Pioglitazone HCl was obtained by plotting the peak area of Pioglitazone HCl versus the concentrations of Pioglitazone HCl over the range of 30-150 $\mu\text{g/ml}$, and it was found to be linear with $r^2 = 0.9982$. Similarly, the calibration curve for Glimepiride was obtained over the range of 4-20 $\mu\text{g/ml}$ and was found to be linear with $r^2 = 0.9978$. The recoveries of Pioglitazone HCl and Glimepiride were found to be in the range of 99.78% and 100.06% within precision RSD of 0.16 and 0.48 for Pioglitazone HCl and Glimepiride. The system suitability parameters such as theoretical plates and tailing factor were found to be 2132, 1.05 and 729, 1.45 respectively for Pioglitazone HCl and Glimepiride. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the

smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The detection limit (LOD) was found to be 0.28 $\mu\text{g/ml}$ for Pioglitazone HCl and 2 $\mu\text{g/ml}$ for Glimepiride respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The quantitation limit (LOQ) was found to be 0.16 $\mu\text{g/ml}$ for Pioglitazone HCl and 0.6 $\mu\text{g/ml}$ for Glimepiride respectively. Proposed study describes a new RP-HPLC method for estimation of Pioglitazone HCl and glimepiride combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise Table 2. Percentage recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Pioglitazone HCl and Glimepiride their combined dosage form.

DRUG	Method Precision		Assy Amount found
	Mean %	RSD (%)	
Pioglitazone HCl	99.77	0.16	15.25
Glimepiride	100.06	0.48	2.02

Table.1. Assay of Pioglitazone HCl and Glimepiride

Parameters	Pioglitazone HCl	Glimepiride
Linearity range $\mu\text{g/ml}$	30-150	4-20
Correlation Coefficient (r^2) S.D	0.9982	0.9978
Retention time (min) \pm S.D	3.18	5.02
Resolution	10.52	5.05
Tailing factor	1.05	1.45
Theoretical Plate	2132	729
Limit of detection ($\mu\text{g/ml}$)	0.28	2
Limit of Quantification ($\mu\text{g/ml}$)	0.16	0.6
Precision (RSD %) intraday (n=6)	0.21	0.39

Table.2. Validation and system suitability parameters

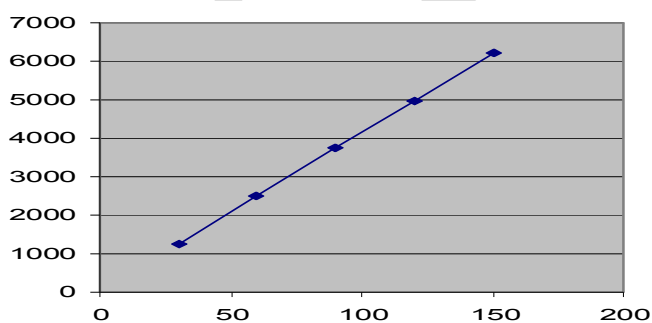


Figure 1. Linearity curve of Pioglitazone HCl

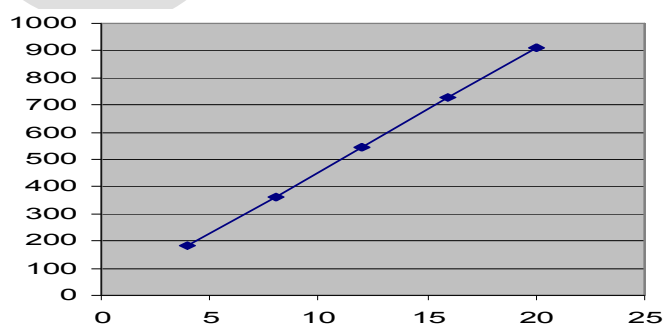


Figure 2. Linearity curve of Glimepiride

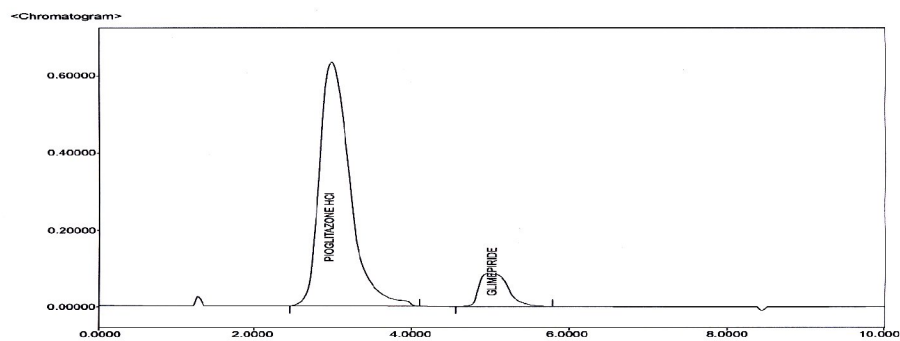


Figure 3. Typical Chromatogram of Pioglitazone Hcl and Glimepiride

CONCLUSION:

In the current study a new RP-HPLC method for the estimation of Pioglitazone HCl and Glimepiride combination in mixture using simple mobile phase was developed, optimized

and validated. The developed method is simple, sensitive, accurate and precise. The developed method can be used for routine analysis of Pioglitazone HCl and Glimepiride in a combined dosage form.

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