

# SIMULTANEOUS DETERMINATION OF PIOGLITAZONE AND GLIMEPIRIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

KARTHIK A\*, SUBRAMANIAN G, MALLIKARJUNA RAO C, KRISHNAMURTHY BHAT, RANJITHKUMAR A,

MUSMADE P, SURULIVELRAJAN M, KARTHIKEYAN K AND UDUPA N

*Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal, India*

## ABSTRACT

A simple, fast, and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of pioglitazone and glimepiride in bulk drug and pharmaceutical dosage form. The quantification was carried out using Inertsil ODS (250 × 4.6 mm, 5 $\mu$ ) column and mobile phase comprised of acetonitrile and ammonium acetate (pH 4.5; 20mM) in proportion of 60:40 (v/v). The flow rate was 1.0 ml/min and the effluent was monitored at 230 nm. The retention time of pioglitazone and glimepiride were 7.0 $\pm$ 0.1 and 10.2 $\pm$ 0.1 min respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. Linearity of pioglitazone and glimepiride were in the range of 2.0 to 200.0 $\mu$ g/ml and 0.5-50 $\mu$ g/ml respectively. The percentage recoveries of both the drugs were 99.85% and 102.06% for pioglitazone and glimepiride respectively from the tablet formulation. The proposed method is suitable for simultaneous determination of pioglitazone and glimepiride in pharmaceutical dosage form and bulk drug.

**Keywords:** Pioglitazone, Glimepiride, HPLC, method validation.

## INTRODUCTION

Pioglitazone is a thiazolidine Dione derivative. It is one of the PPAR- $\alpha$  agonist, insulin sensitizer used to reduce the insulin resistance. Pioglitazone (fig. 1) is chemically [( $\pm$ )-5-[[4-[2-[5-ethyl -2- pyridinyl] ethoxy] phenyl]-methyl]-2,4-]thiazolidinedionemonohydro-chloride. Glimepiride is a sulfonyleurea urea derivative chemically-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-oxamide)ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea, widely used in patients with type 2 diabetes (non-insulin-dependent diabetes). The drugs are prescribed individually as well as multi component dosage forms available in the market. A number of methods have been published for the estimation of the above said analytes. Pioglitazone in human plasma (Venkatesh *et al.*, 2007; Xue *et al.*, 2006 and Sripalakit *et al.*, 2006) and HPLC method for antidiabetic drugs (Yao *et al.*, 2006; Jedlicka *et al.*, 2004 and Kolte *et al.*, 2004) were reported. Method for estimation of glimepiride in human plasma (Chakradhar *et al.*, 2007; Saleem *et al.*, 2004) was published. HPLC method for estimation of glimepiride and related substances (Deep *et al.*, 2005; Kovarikova *et al.*, 2004; Khan *et al.*, 2005) were also reported in the literature. Even though various methods were reported in the literature for estimation of glimepiride and pioglitazone individually or in combination with other drugs no method had been reported for simultaneous estimation of these two drugs using HPLC in bulk drug and pharmaceutical dosage forms. The present study was

aimed at the simultaneous estimation of pioglitazone and glimepiride by reverse phase HPLC method. The method was validated according to the ICH (Q2A 1995) guidelines.

## EXPERIMENTAL

### *Materials, reagents and chemicals*

Pioglitazone and Glimepiride were obtained as gift samples from Dr. Reddys Laboratories, Hyderabad. Ammonium acetate and glacial acetic acid were A.R grade from SD fine chemicals Mumbai. acetonitrile HPLC grade from Merck chemicals, Mumbai.

Chromatographic condition: Water alliance 2695-separation module with waters 2487 dual UV detector was used. Millennium software version 4.0 s used for Data acquisition. Inertsil ODS (25 cm × 4.6 mm, 5 $\mu$ ) column was used as a stationary phase. Mobile phase comprised of acetonitrile and 20mM ammonium acetate buffer (60; 40 v/v) with pH adjusted to 4.5 $\pm$ 0.2 was used. Injection volume was 10 $\mu$ l and run time was 12min and flow rate 1.0ml/min. The column was maintained at ambient temperature and the eluent was detected at 230 nm.

### *Solutions*

#### **Standard solutions**

Standard stock solution (1000  $\mu$ g/ml) of pioglitazone, glimepiride were prepared separately in methanol. The

\*Corresponding author: Fax: +91-820-2571998, e-mail: millinkarthik@yahoo.co.in

**Table 1:** Recovery studies

Name of the Drug	Spiking level (%)	Amount added ( $\mu\text{g/ml}$ )	Amount recovered ( $\mu\text{g/ml}$ ) n=3	Percentage recovery	Average percentage recovery
Pioglitazone	80	8	7.89	98.63	99.85
	100	10	10.21	102.1	
	120	12	11.86	98.83	
Glimepiride	80	0.8	0.82	102.5	102.06
	100	1	1.02	102	
	120	1.2	1.22	101.67	

working standard solutions were prepared and further diluted in mobile phase to contain a mixture of pioglitazone and glimepiride in over the linearity range from 2-200  $\mu\text{g/ml}$  and 0.5-50  $\mu\text{g/ml}$  respectively.

#### Assay in formulations

Twenty tablets, Pioryl, (Panacea Biotech), each containing 15mg of pioglitazone and 2mg of glimepiride were weighed and finely powdered. A quantity of powder equivalent to 15mg of pioglitazone and 2mg of glimepiride was weighed and transferred to a Standard flask. The drug was diluted using methanol to get a concentration of 10 $\mu\text{g/ml}$  of pioglitazone, 1 $\mu\text{g/ml}$  of glimepiride. The contents were mixed thoroughly and filtered through a 0.45  $\mu$  filter. 10 $\mu$  of the sample was injected in to HPLC system.

## RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatograms of pioglitazone and glimepiride were shown in (fig. 4). There was clear resolution between pioglitazone and glimepiride with retention time of 7.0 and 10.2 minutes respectively.

#### Validation of the method

##### Linearity

The response for the detector was determined to be linear over the range of 2 to 200 $\mu\text{g/ml}$  (2, 5, 10, 25, 50, 100, 200) for pioglitazone and 0.5-50 $\mu\text{g/ml}$  (0.5, 1, 2, 5, 10, 25, 50) for glimepiride. Each of the concentration was injected in duplicate to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. They were represented by the linear regression equation (figs. 2, 3).

$$Y_{\text{Pioglitazone}} = 27494X + 32335, 'r' \text{ value} = 0.9995$$

$$Y_{\text{Glimepiride}} = 37719X - 3261, 'r' \text{ value} = 0.9987$$

Slopes and intercepts were obtained by using regression equation ( $y=mx+c$ ) and least square treatment of the results used to confirm linearity of the method developed.

#### Precision and accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and % relative standard deviation was calculated. From the data obtained, recoveries of standard drugs were found to be accurate (table 1).

The %CV of interday and intraday precision obtained was less than 1% for both the drugs. The intraday and interday precision of pioglitazone was 0.47 and 0.86 and glimepiride was 0.76 and 0.94 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

#### Specificity of the method

The PDA chromatograms of the pioglitazone and glimepiride in standard and sample were recorded. In the chromatograms of the formulations, some additional peaks were observed which may be due to excipients present in the formulations. These peaks however did not interfere with the standard peaks, which demonstrate that the assay method is specific. Furthermore, the purity of the peaks was studied by peak purity studies. The results revealed that the peak is free from interferences, which shows that the HPLC method is specific.

#### Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (1:3) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (1:10). The LOD of pioglitazone and glimepiride found to be 0.2 $\mu\text{g/ml}$  and 0.1 $\mu\text{g/ml}$  respectively. The LOQ of pioglitazone and glimepiride found to be 2 $\mu\text{g/ml}$  and 0.5 $\mu\text{g/ml}$  respectively

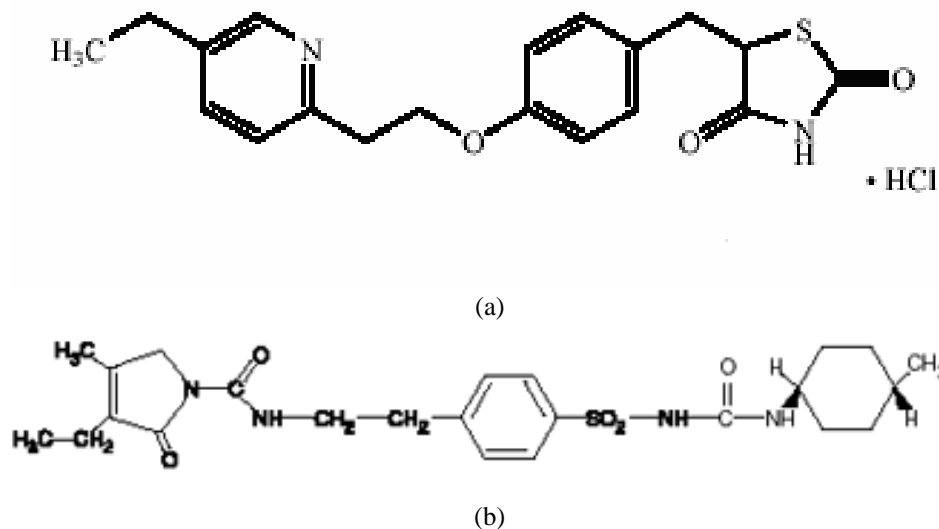


Fig. 1: Chemical structures of (a) Pioglitazone (b) Glimepiride

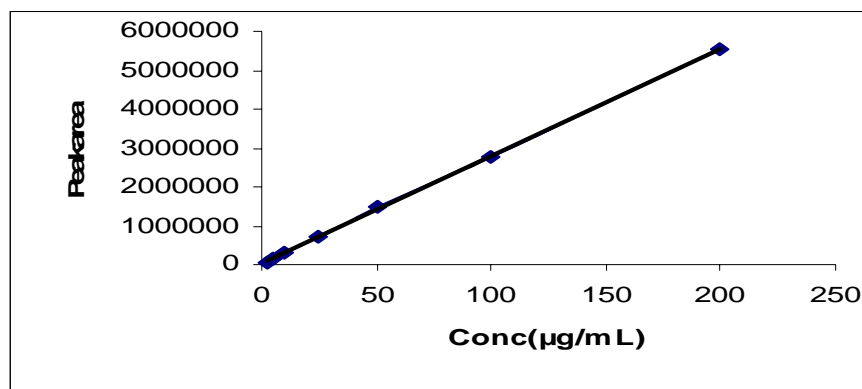


Fig. 2: Calibration curve of pioglitazone

### Robustness

The robustness of the method was studied by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic content, changes in the wavelength. It was observed that there was no marked changes in the chromatograms demonstrate that the HPLC methods have developed are robust.

### Solution Stability

In this study, the mobile phase, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions.

### System suitability

The resolution, capacity factor, theoretical plates/meter,  $R_f$  values and peak symmetry were calculated for the

standard solutions. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations. System suitability parameters might be fall within  $\pm 3\%$  standard deviation range during routine performance of the method. The summary of the method validation results were showed in the (table 2).

Table 2: Summary of analytical method validation

Validation parameters	Pioglitazone	Glimepiride
Recovery (%)	99.85	102.06
Intraday precision (%CV)	0.47	0.76
Interday precision (%CV)	0.86	0.94
Linearity ( $r^2$ )	0.9995	0.9987
Robustness (%CV)	0.66	0.82
LOD (µg/ml)	0.2	0.1
LOQ (µg/ml)	2	0.5
Specificity	Passed	Passed

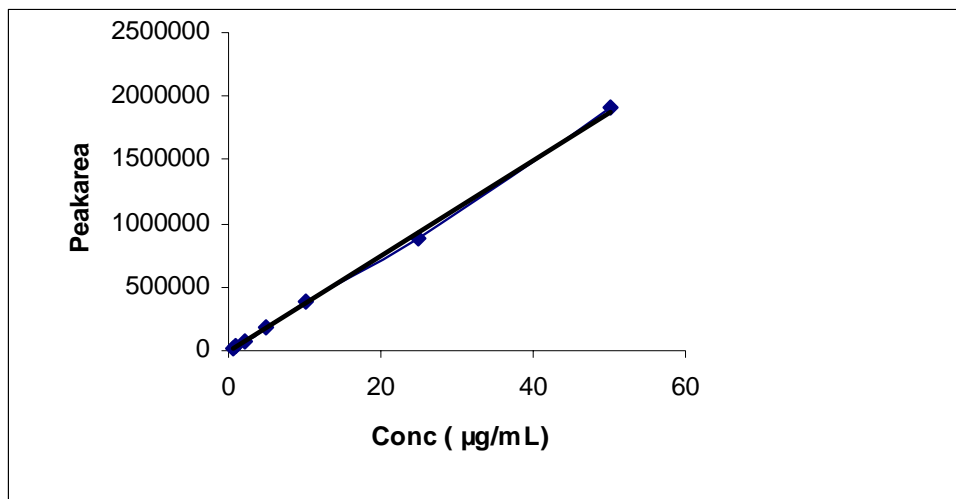


Fig. 3: Calibration curve of glimepiride.

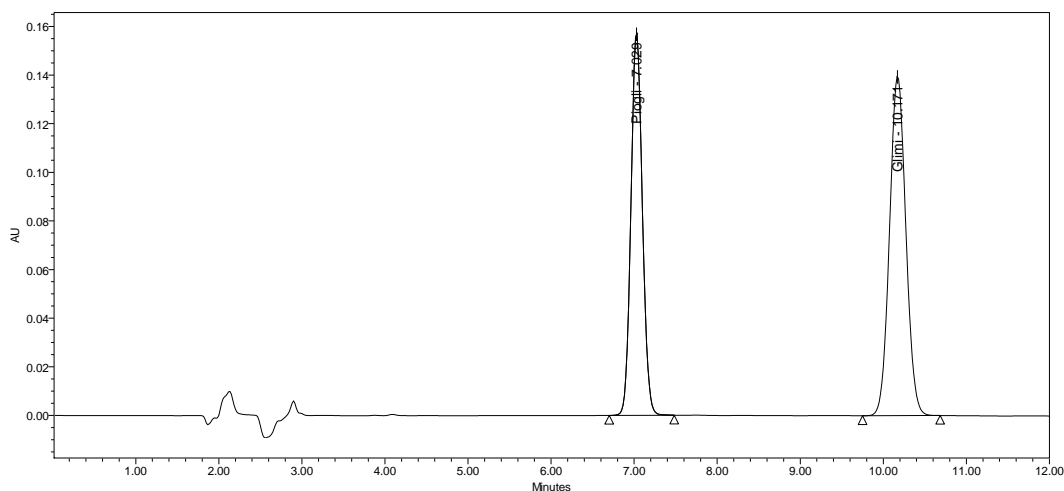


Fig. 4: Typical chromatogram of pioglitazone and glimepiride

## CONCLUSION

This method is simple, specific and easy to perform and requires short time to analyze the samples. Low limit of quantification and limit of detection makes this method suitable for use in quality control. This method enables simultaneous determination of Pioglitazone and Glimepiride because of good separation and resolution of the chromatographic peaks. The method was found to be accurate, precise, linear, robust and rugged.

## REFERENCES

Chakradhar L, Kallem R, Karthik..A, Sundari BT, Ramesh S, Mullangi R and Srinivas NR (2007). A rapid and highly sensitive method for the determination of Glimepiride in human plasma by liquid chromatography-electrospray ionization tandem mass

spectrometry-application to preclinical Pharmacokinetic study. *J. Biomed. Chromatogr.*, **22** : 58-63.

Eldeep S, Schepers U and Wätzig H (2006). Fast HPLC method for the determination of glimepiride, glibenclamide, and related substances using monolithic column and flow program. *J. Sep. Sci.*, **29**: 1571-1577.

ICH Draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register. Vol. 60, IFPMA, Switzerland, 1995, p.11260.

Jedlicka A, Klimes J and Grafnetterova T (2004). Reversed-phase HPLC methods for purity test and assay of pioglitazone hydrochloride in tablets. *Pharmazie*. **59**: 178-82.

Khan MA, Sinha S, Vartak S, Bhartiya A and Kumar S (2005). LC determination of Glimepiride and its related impurities. *J. Pharm. Biomed. Anal.*, **39**: 928-943.

Kolte BL, Raut BB, Deo AA, Bagoool MA and Shinde DB (2004). Simultaneous high-performance liquid chromatographic determination of pioglitazone and

- metformin in pharmaceutical-dosage form. *J. Chromatogr. Sci.*, **42**: 27-31.
- Kovariková P, Klimes J, Dohnal J and Tisovská L (2004). HPLC study of glimepiride under hydrolytic stress conditions. *J. Pharm. Biomed. Anal.*, **36**: 205-209.
- Reynolds, JEF (Eds.). *In: Martindale, the Extra Pharmacopoeia* (1989). 29<sup>th</sup> Edn., The Pharmaceutical Press, London, p.1240.
- Salem II, Idrees J and Al Tamimi JI (2004). Determination of glimepiride in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **799**: 103-109.
- Sripalakit P, Neamhom P and Saraphanchotiwitthaya A (2006). High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **843**: 164-169.
- United States Pharmacopoeia (1996). 23<sup>rd</sup> Edn, The USP convention, Inc, Rockville, MD.
- Venkatesh P, Harisudhan T, Choudhury H, Mullangi R and Srinivas NR (2006). Simultaneous estimation of six anti-diabetic drugs--glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay. *J. Biomed. Chromatogr.*, **20**: 1043-1048.
- Xue YJ, Turner KC, Meeker JB, Pursley J, Arnold M and Unger S (2003). Quantitative determination of pioglitazone in human serum by direct-injection high-performance liquid chromatography mass spectrometry and its application to a bioequivalence study. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **795**: 215-226.
- Yao J, Shi YQ, Li ZR and Jin SH (2007). Development of a RP-HPLC method for screening potentially counterfeit anti-diabetic drugs. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **853**: 254-259.