International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 50-53, Jan-Mar 2010

METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS DETERMINATION OF PIOGLITAZONE AND GLIMEPIRIDE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

B.Praveenkumar reddy, D.Boopathy*, Bibin Mathew, M.Prakash, and P.Perumal

Department of Pharmaceutical Analysis,

J.K.K.Nataraja College of pharmacy, Natarajapuram,

Komarapalayam (P.O) - 638183, Namakkal (D.T), Tamil Nadu, India.

***E-mail:drpmrmp@gmail.com**

Abstract: A simple, selective, rapid, and precise reverse phase HPLC method has been developed for the simultaneous estimation of pioglitazone and glimepiride in pharmaceutical dosage form. A phenomenex Luna c_{18} column (4.6x150mm) was used for the separation. The mobile phase was acetonitrile: KH2PO4 buffer (60:40%v/v) (Ph6) at a flow rate of 1.5ml/min with detection at 230nm.The retention time of pioglitazone and glimepiride was 4.4 and 2.7 minutes respectively. The developed method was validated in term of accuracy, precision, specificity, system suitability, linearity, and robustness, limit of detection and limit of quantification. Linearity of pioglitazone and glimepiride were in the range of 240 to 360µg/ml and 32 to 48µg/ml respectively. The proposed method is suitable for simultaneous determination of pioglitazone and glimepiride in pharmaceutical dosage form.
Keywords: pioglitazone and glimepiride.

Introduction

Pioglitazone is a thiazolidine dione derivative and it is used in patient with NIDDM type of anti-diabetic, chemically it is 5-(4-[2-(5-ethypyridin-2-yl] benzyl) thiazolidine-2, 4-dione. The molecular formula is $C_{19}H_{20}N_2O_3S$. Glimepiride is a sulfonylurea derivative chemically 3-ethyl-2,5-dihydro-4-methyl-N-[2-[4-s(trans-4-methyl cyclohexyl) amino] carbonyl]amino] sulfonyl] phenyl]ethyl]-2-oxo-1H-pyrrole-1-carboxamide[1], widely used in patient with type 2 diabetic mellitus. Its molecular formula is $C_{24}H_{34}N_4O_5S$. Many methods have been described in the literature for the determination of pioglitazone and glimepiride individually and in combination with other drug[2-11]. Our present plan is to develop new, simple, precise, & accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated. The method was validated according to the ICH (Q_2A 1995) guidelines[12].

Materials and methods

Pioglitazone and glimepiride were obtained as gift samples from Dr. Reddys laboratories, Hyderabad. Acetonitrile & methanol were HPLC grade and potassium dihydrogen phosphate was A R grade from Merk chemicals, Mumbai.

Analysis of formulation:

Twenty tablets from a batch were randomly selected and powdered, Weigh accurately 1346.7mg of ground tablet powder (equivalent to 150mg of pioglitazone and 20.3 mg of glimepiride) transfer it in 100 ml of volumetric flask, and add 100ml of mobile phase, shake the flask on a rotator shaker for 30 min and sonicate for 15 min with inmmediate shaking. Keep the solution on a rotatory shaker for 30 min at

200 rpm. Centrifuge the portion of above solution at 4000 rpm for 5 min. pipette out 5 ml of above clear solution and transfer it to 50 ml volumetric flask and make up the volume with mobie phase. The sample solution was suitably diluted and used for the analysis. After setting chromatographic condition and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20µl fixed-sample loop of the injection port. The solution was injected and chromatogram was recorded. A representative chromatogram has been given in the figure-1.The result of analysis reported in (table-1).The stability of the sample in mobile was analyzed after 24 hrs, it was found no change in analytical parameter.

Recovery studies:

To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Result of recovery studies were found to be satisfactory and are reported in [Table- 1]

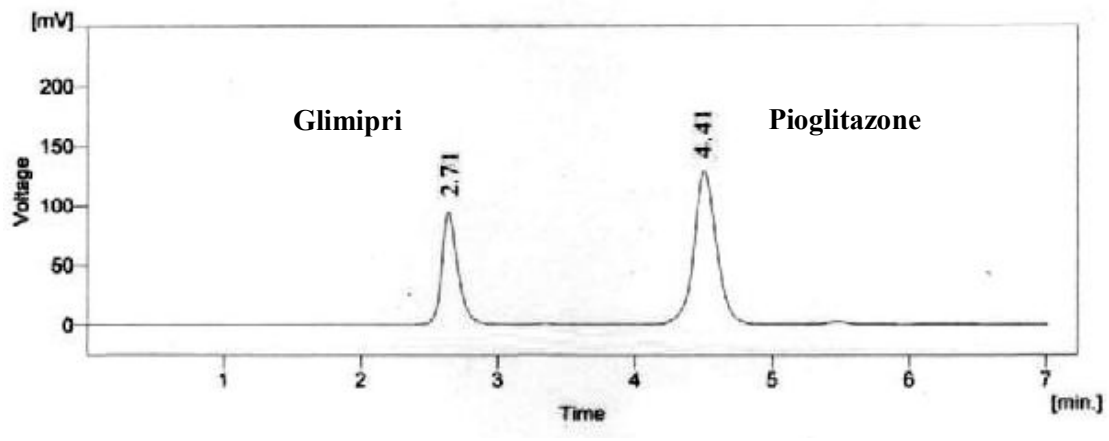
Table1: Analysis of formulation.

Drug	**Pioglitazone**		**Glimepride**	
Amount of drug(mg/tab)	Labeled amount	Estimated amount	Labeled amount	Estimated amount
	15mg	14.45mg	2mg	2.004mg
% Label claim	99.16%		100.2%	
%RSD	0.21%		1.24%	

Table2: Validation and system suitability parameters

S.No	**Parameters**	**Pioglitazone**	**Glimepride**
1	System suitability (%RSD of tailing factor)	1.122	1.273
2.	Specificity	specific	Specific.
3	Precision: A)System Precision B).Method precision	0.21 0.72	1.25 0.61
4	Linearity	0.997	0.990
5	Accuracy	99.9	100.3
6	Robustness	Robustted	Robustted
7	LOD	7.5	0.96
8	LOQ	22.74	2.93

Figure-1 Chromatogram for formulation



Result and Discussion

A simple, precision and accuracy HPLC method was developed the simultaneous estimation of pioglitazone and glimepiride analysis of uncoated formulations, consisting of an Acetonitrile: phosphate buffer system (60: 40 % v/v). The chromatographic condition was set at a low rate of 1.5 ml/min with the UV detector at 230 nm. The above method was optimized with a view to develop an assay method for pioglitazone and glimepiride.

Several mobile phase compositions were tried to resolve the peaks of Pioglitazone and Glimepiride.

The optimum mobile phase containing Acetonitrile: KH_2PO_4 buffer (60: 40 % v/v) was selected because it was found ideal to resolve the analyte peaks of both the drugs. Quantification was achieved with UV detections at 230 nm based on peak area and absorbance. As per USP requirements system suitability studies were carried out and freshly prepared standard solutions are Pioglitazone and Glimepiride. Various parameters obtained with 20 µl of injection volume are summarized in the table given below.

References

1. The Merck index, 13th edition pp- 4453, 7537.
2. Chandana shveta; Kasture A. V. ; Yeole P.G.; Simultaneous spectrophotometric determination of Pioglitazone hydrochloride Glimepiride in tablets, Indian journal of pharmaceutical sciences, 2005 vol.67 157 627-629
3. Yannis Dotsikas, Constantinos kousoulos, Georgia tsatsou, yannis L.loukas, development of rapid method for the determination of glimipride in human plasma using liquid; liquid extraction based on 96-well format micro-tubes and liquid chromatography/tandem mass spectrometry, Journal of Rapid communication in mass spectrometry, 2005; 19(14); 2055-2061.
4. Hohyunkim, Kyu Young chang, Chang Hun park, Moon Sun Jung-Ae Lee, Hee Joo Lee, Kyung Ryul Lee, Determination of Glimepride in human plasma by LC-MS-MS and comparison of sample preparation methods for Glimepiride, 2004, *Chromatographia,* 60, 93-98.
5. Kolte B.L., Raut B.B., Deo A.A., Bagool M.A., Shinde D.B. ; Simultaneous high performance liquid Chromatographic determination of Pioglitazone and Metformin in pharmaceutical dosage form, 2004,vol.42,pp27-31 Journal of chromatographic sciences.
6. Madhira B Shankar, vaibhav D.Modi Estimation of Pioglitazone Hydrochloride and metformin Hydrochloride in tablets By Derivative Spectrophotometry and Liquid Chromatographic Methods. Journal of AOAC International 2005, July-August.
7. Constantinos pistos, maria koutsopoulou ,irne panderi, Improved liquid chromatography tandem mass spectrometric determination and pharmacokinetic study of glimepride in human plasma, Journal of Biomedical Chromatography, Vole 19(15), 2005 Jan

11, 294-40.1

8. Basniwal,pawan; Srivastava, Prabhat Spectrophotometric Estimation of Pioglitazone Hydrochloride in Tablet Dosage Form. Asian Journal of pharmaceutics, October 1, 2008; pg 15-20
9. Xeu,X-j(yj); Turner Quantitative Determination of Pioglitazone in Human serum by Direct-injection high performance liquid chromatography mass spectrometry and its application to a bioequivalence study. Journal of Chromatography B,October 2003,Vol-795,Pg no:215-226.
10. Khan, Mubeen Ahmad (MA); Sinha, Sukumar (S); LC determination of glimepiride and its related impurities. Journal of pharmaceutical and biomedical analysis. 2005-Oct; vole 39 (issue 5) : pp 928-43.
11. Yao. Jing(j) shi Ya-Qin (YQ): Development of a RP-HPLC method for screening potentially counterfeit anti-diabetic drugs. Journal of Chromatography B,October 2007,Vol-853,Pg no:254-259.
12. International conference on harmonization "Validation of analytical procedures Methodology", 14, Federal Register Nov.1996, 1-8.

*****