DEVELOPMENT AND VALIDATION OF A HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND GLIMEPIRIDE IN BULK AND TABLET DOSAGE FORM

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Abstract

A simple high-performance liquid chromatographic (HPTLC) method has been developed and validated for simultaneous determination of pioglitazone and glimepiride in bulk and tablet dosage form. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of Benzene: Ethyl acetate: Diethyl ether 6:3:1 v/v. The detection of spot was carried out at 254 nm. The calibration curve of pioglitazone was found to be linear between response was determined of 600 ng/ml to 3600 ng/ml with regression coeficent 0.9984 and calibration curve of glimepiride was found to be linear between 200-1200 ng/ml for glimepiride with regression coefficient of 0.9991. The limit of detection was 57.22 ng/ml and 16.67 ng/ml and the quantification limit was 190.73 ng/ml and 55.58 ng/ml for pioglitazone and glimepiride respectively.The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 97.84 to 99.07 %. The proposed method is applicable to routine analysis of Pioglitazone in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

Keywords: Pioglitazone, Glimepiride, HPTLC, ICH guidelines

1. Introduction

Pioglitazone is a thiazolidinedione derivative and it is used for the treatment of type 2 diabetes mellitus, chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione. Pioglitazone is an oral antidiabetic agent and acts as an agonist at PPAR gamm receptors have acts primarily by reducing insulin resistance. Glimepiride is a second-generation sulfonylurea derivative

chemically it is 3-ethyl-4-methyl-N-[2-[4[(4methylcyclohexyl)carbamoylsulfa moyl]phenyl]ethyl]-2-oxo-5H-pyrrole-1-carboxamide. (Fig. 1) Glimepiride is used for the treatment of type 2 diabetes mellitus. Glimepiride is used with diet to lower blood glucose by increasing the secretion of insulin from pancreas on stimulating pancreatic β cells and increasing the sensitivity of peripheral tissue.

A literature survey reveals that various analytical methods like pioglitazone by HPLC and MECK2, HPLC and solid phase extraction method in human serum4,Human Plasma3,pioglitazone and glimepiride have been analysed separately by HPLC5,8. Simultaneous HPLC analysis of pioglitazone and glimepiride7, Simultaneous HPLC analysis of metformin and in pharmaceutical dosage form6.But these methods are sophisticated,expensive and time consuming when compared to simple method.

There is need for a interest to develop simple, accurate, specific, sensitive, precise and reproduciable simultaneous HPLC method for the estimation of pioglitazone and glimepiride in bulk and its formulation.

2. Experimental

2.1. Materials and Methods

Pure standard of Pioglitazone and glimepiride (Assigned purity 99.98%) was obtained as a gift sample from Micro labs Pvt. Ltd, Badi, India. The gift samples were used as standard without further purification. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Qualigens. Commercial pharmaceutical preparation (Pioryl) which was claimed to contain 15mg of pioglitazone and 2mg of glimepiride is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

2.2. Equipment

The instrument used in the present study was Camag Linnomat V-semiautomatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner 3, Cagmag Twin trough chamber of appropriate size (20X 20), Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH) were used throughout the experiment. Cagmag Wincats software was used for acquisition, evaluation and storage of chromatographic data.

2.3. Standard solution

Standard stock solutions 1 mg mL^{-1} of Pioglitazone and Glimepiride were prepared in methanol and further diluted in mobile phase. The working standard solutions were prepared in mobile phase to contain mixture of pioglitazone and glimepiride in over the linearity range from 600 –3600 ng/ml and 200 - 1200 µg/ml.

2.4. Assay in formulation

Twenty tablets each containing and their average weight was calculated. The tablet were crushed to furnish a homogenious powder and a quantity equivalent to one tablet were weighed in to a 100 ml volumetric flask, dissolve in methanol, sonicated for about 15 min and then made up to volume with mobile phase. The solution was stirred for 10 min using a magnetic stirrer and filtered into a 100 ml volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. Further add mobile phase to obtain a stock solution of 100µg/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up sufficient volume with the mobile phase to give an expected concentration of 1µg/ml.

2.5. Prewashing of TLC plates

HPTLC was performed on 20 cm × 10 cm precoated silica gel 60 F 254 TLC plates. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 min. at 110 C, with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

3. Results and Discussion

A methanolic solution of Pioglitazone (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 1µg/ml. Different concentrations of Pioglitazone in a concentration range of 600-3600ng/ml and Glimepiride in a concentration range of 200-1200 were applied on plates as 8 mm bands, 8 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic

sample applicator fitted with 100 µl Hamilton syringe. A methanol blank was applied to parallel track. The mobile phase, Benzene: Ethyl acetate: Diethyl ether 6:3:1 v/v was poured into the twin trough glass chamber and the glass chamber left to equilibrate for 10 min at 25 ± 2^0 C. After that the plate was placed in Camag twin trough glass chamber. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 254 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of Pioglitazone and glimepiride at various concentrations. This method was followed for all quantitative analysis. The Wincats software was used for data acquisition and processing of the plate. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

3.1. Validation of Analytical Method

Validation of an analytical method is process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Typical analytical parameters used in validation area:

1. Linearity
2. Accuracy
3. Precision
4. Specificity
5. Limit of detection
6. Limit of quantification
7. Range
8. Solution stability

3.2. Linearity

The response was determined to be linear over the range of 600ng/ml to 3600ng/ml (600, 1200,1800, 2400, 3000, 3600) for pioglitazone and 200-1200ng/ml (200, 400, 600, 800,1000, 1200) for glimepiride. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The purposed method was evaluated by its correlation coefficient and intercept value calculated by statistical study. They were represented by the linear regression equation. (Fig 2& 3 calibration curve & chromatogram no.1)

$Y_{Pioglitazone}$= 3.5107x + 1672.4
Coefficient of correlation (r^2) value = 0.998

$Y_{Glimepiride}$= 3.7791x -342.19
Coefficient of correlation (r^2) value =0.9991

3.3. Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.4 ml, 1ml and 1.6 ml of sample drug from pioglitazone solution of 10 µg/ml were pipetted into each of three volumetric flasks. To this 0.8 ml of standard drug solution of 10 µg/ml was added to each volumetric flask respectively. Then add 0.2ml, 0.4ml, 0.6ml of sample drug from glimepiride solution of 10µg/ml. To this 0.2 ml of standard drug solution of 10µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with mobile phase. The range of recovery studies were found between 98.34 to 99.40 %. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. (Table No. 1)

3.4. Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments. The repeatability study which was conducted on the solution having the concentration of about 1800 ng/ml for pioglitazone and 600 ng/ml for glimepiride (n =5) showed a RSD of 0.753% for pioglitazone and 1.42% for glimepiride. It was concluded that the analytical technique showed good repeatability.(TableNo.2)

3.5. Limit of Detection And Quantification

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

The limit of detection was 57.22 ng/ml and16.67 ng/ml and the quantification limit was 190.73 ng/ml and 55.58 ng/ml for pioglitazone and glimepiride respectively which represents that sensitivity of the method is high.

3.6. Specificity

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drugs was spiked and observe the chromatogram.

The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for pioglitazone and glimepiride.

3.7. Range

The specific range derived from the linearity studies. The range was calculated from the linearity graph. From the lower to higher concentration between which the response is linear, accurate and precise.

Acceptance criteria: RSD < 2.0

The range for Pioglitazone was found to be 600-3600 ng/ml.

The range for Pioglitazone was found to be 200-1200 ng/ml.

3.8. Solution Stability

The solution stability of the standard and sample prepared in methanol was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for period of more than 48 hours.

4. Conclusion

The proposed HPTLC method is found to be simple, accurate, precise, linear, and specific, and, for quantitative estimation of pioglitazone and glimepiride in bulk and its formulation.

Hence the present HPTLC method is suitable for routine assay of pioglitazone and glimepiride in raw materials and in pharmaceutical formulations in the quality control laboratories. The results show the methods could find practical application as a quality control tool for simultaneous analysis of drugs from their combined dosage forms in quality control laboratories.

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Bibliography:

1. www.drugbank.com
2. Radhakrishna T,Sreenivas Rao,D.,Om Reddy,G., *J Pharm Biomed Anal* .2002; 29 (4): 593-607
3. Sripalakit P,Neamhorn,P., *J Chrom B*. 2006; 843(2): 164-169.
4. Zhong W Z,WilliamsMG, *J Pharm Biomed Anal*. 1996; 14(4): 465-473.
5. Saber Lotfy AMR, *Pak J Anal Environ Chem*.2008;9(2):118-121.
6. Kolte B L,Raut BB,Deo AA,Bagool A, Shinde B, *J Chrom Science*, 2004; 42: 27-31.
7. Karthik A,Sumramanian G,Malikarjuna RC,Krishanamurthy B,Ranjitkumar A,Musmade P,Surulivelrajan M,Karthikeyan K, *Pak. J. Pharm. Sci*.2008; 21(4): 421-425
8. Khan I U, Aslam F,Ashfaq M,Asghar M N.J *Anal Chemistry*. 2009; 64(2):171–175.
9. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), November 2005.

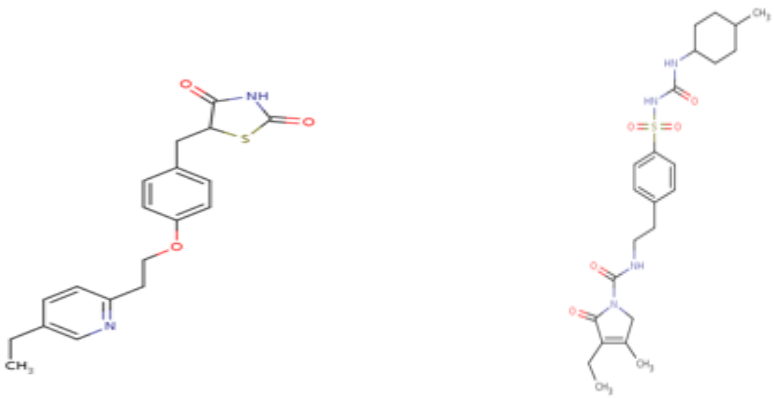


Fig. 1: Chemical structure of Pioglitazone and Glimepiride

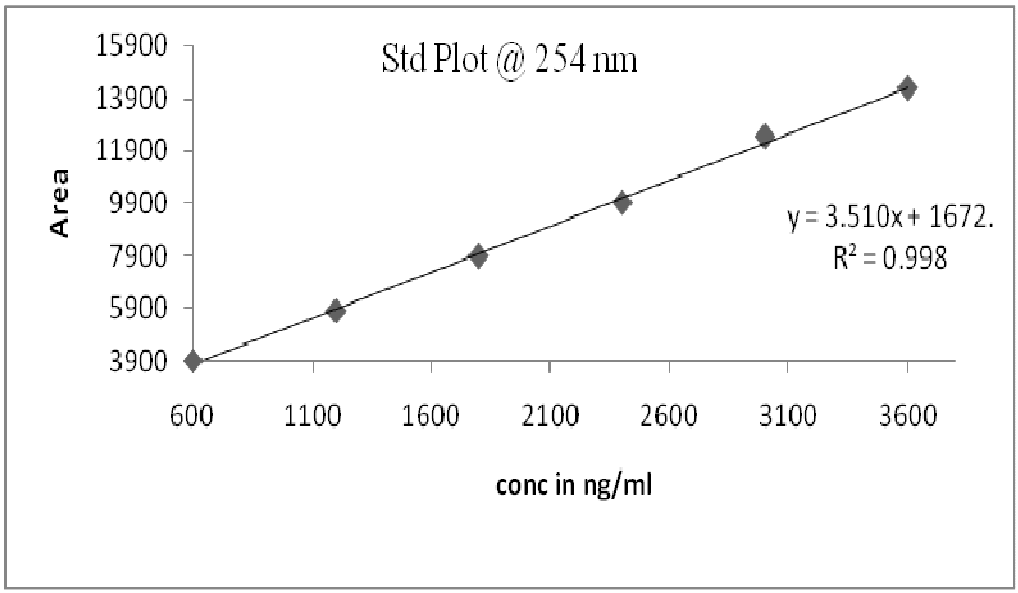


Figure 2: Calibration curve for Pioglitazone

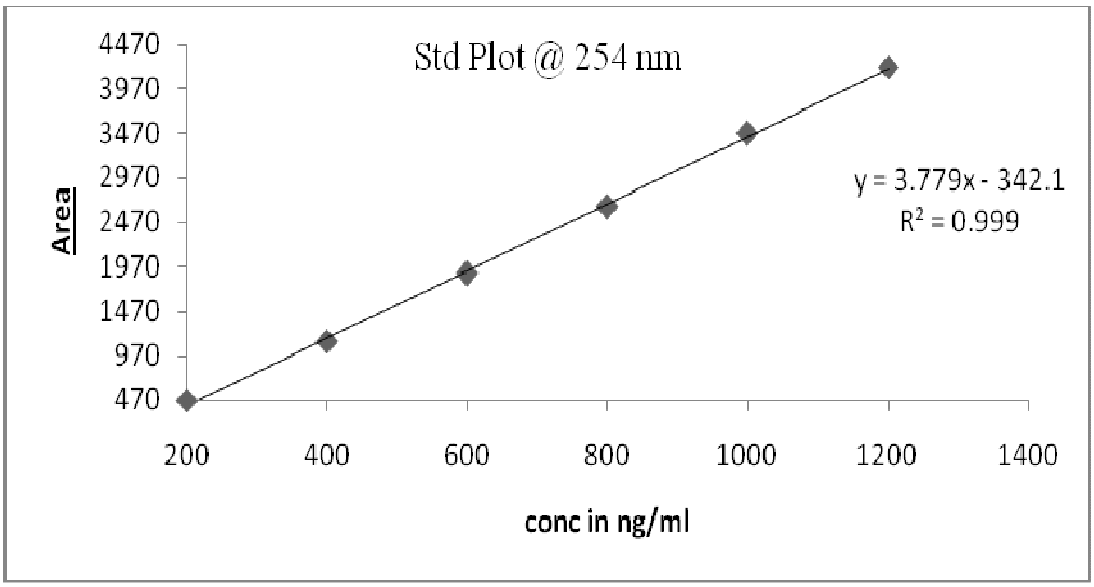
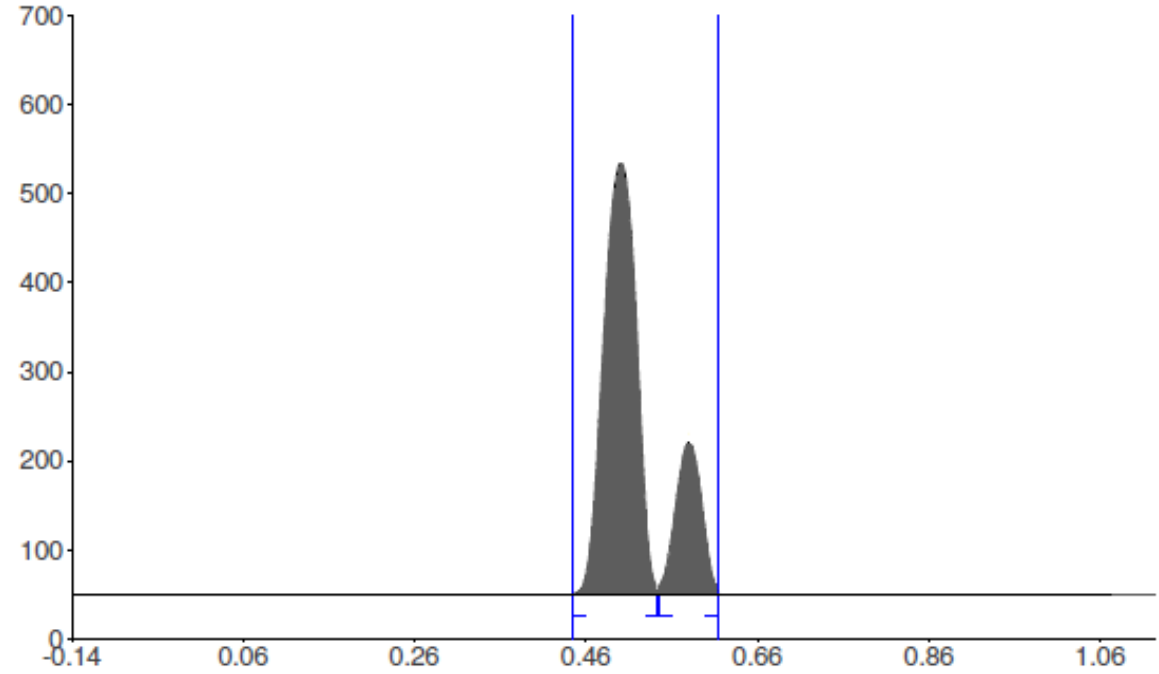


Figure 3: Calibration curve for Glimepiride



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.44	0.3	0.50	484.5	73.96	0.54	8.3	14927.3	77.99
2	0.54	9.0	0.58	170.6	26.04	0.61	2.1	4211.9	22.01

Chromatogram No 1: Peak Area for Pioglitazone and Glimepiride

Table No.1: Result of recovery studies

S.No.		Conc. taken in (µg/ml)	Std addition in (µg/ml)	Total Conc. in (µg/ml)*	% recover ±SD
1	PIO	800	400	1200	98.77±0.620
	GLP	200	200	400	98.44±0.878
2	PIO	800	1000	1800	98.88±0.951
	GLP	200	400	600	97.84±1.33
3	PIO	800	1600	2400	99.07±0.820
	GLP	200	600	800	98.16±0.875
Mean ± SD		PIO	98.90±0.151		
		GLP	98.14±0.300		

Table No. 2: (Precision of Pioglitazone and Glimepiride)

S.NO	Conc. (µg/ml)	Peak Area (µV*sec)	Mean ± SD	% RSD
1 PIO	(PIO 1800+GLP 600) ng/ml	7895.3	PIO	PIO
1 GLP		1867.4	7808.64±58.83	0.753
2 PIO		7832.2		
2 GLP		1887.3		
3 PIO		7796.3		
3 GLP		1832.3		
4 PIO		7780.2	GLP	GLP
4 GLP		1890.1	1875.26±26.66	1.42
5 PIO		7739.2		
5 GLP		1899.1		