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Research Article

SIMULTANEOUS DETERMINATION AND VALIDATION OF PIOGLITAZONE AND GLIMEPIRIDE IN TABLET DOSAGE FORM BY HPTLC METHOD

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ABSTRACT

The objective of this work is to develop a novel high performance thin layer chromatographic method for the determination of Pioglitazone and Glimepiride in tablet dosage form. The quantification was carried out using 16x10cm Aluminum backed HPTLC silica gel 60 F_{254} plates. Scanning was done by Camag TLC scanner-3 equipped with winCATS software, using a Deuterium light source with the slit dimension of 6.00x0.45mm. The elution was achieved with a mobile phase mixture of toluene, ethyl acetate, methanol and glacial acetic acid at a ratio of 70:15:10:5 v/v/v/v. The wavelength 235nm was selected for detection, the R_f value was found to be 0.42 and 0.27 for pioglitazone and glimepiride respectively as observed in a Twin Through Chamber at room temperature. The procedure was validated as per ICH rules for Accuracy, Precision, Detection limit, Linearity, Reproducibility and Quantification limit which are within the limit. The pioglitazone and glimepiride results are linear over the range of 3-15 µg/mL (r^2=0.999) and 0.4-2 µg/mL (r^2=0.999), respectively. The method can be used to analyze commercial solid dosage containing Pioglitazone and Glimepiride with good recoveries for routine analysis.

Keywords: Pioglitazone, Glimepiride, HPTLC, Validation.

INTRODUTION

Pioglitazone [1] is one of the PPAR-alpha agonist, insulin sensitizer used to reduce the insulin resistance. It is a thiazolidine dione derivative and chemically (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione. Glimepiride is a sulfonylurea urea derivative chemically [[p- [2-(3-ethyl-4-methyl-2-oxo-3-pyyroline-1-oxamide) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea, widely used for type 2 diabetes (non-insulindependent diabetes). The prescription of this drug may be individual or multi component dosage forms [2]. It is official in BP [3], USP [4]. A number of methods have been published for the estimation of the above said analytes by UV, HPLC etc [5-9]. Even though various methods were reported in the literature for estimation of Glimepiride and Pioglitazone individually or in combination with other drugs no method was reported for simultaneous estimation of these two drugs using HPTLC in bulk drug and pharmaceutical dosage forms. Paper aims to develop an HPTLC method for the estimation of Pioglitazone and Glimepiride in tablet dosage forms and validate it according to ICH guide lines [10, 11].

Reagents and Materials

Pure pioglitazone and glimepiride used as working standards were gift samples from Dr. Reddy's Laboratories, Hyderabad. Tablets containing pioglitazone 15mg and glimepiride 2mg (Dibiglim-P) were obtained from local market and used within their shelf life period. All other chemicals including methanol, toluene and ethyl Acetate employed of analytical grade are purchased from Merck, India.

Instrumentation

The chromatographic system comprised of Camag Linomat 5 sample applicator equipped with a 100µL syringe, Densitometer Camag TLC scanner-3, using a deuterium light source, the slit dimension is 6.00 x 0.45mm. Twin though chamber and 16x10cm aluminum backed HPTLC silica gel 60 F_{254} plates. Data integration was carried out using Win-CATS software. A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Preparation of Standard Solutions

The standard stock solutions were prepared separately by transferring 15mg of pioglitazone and 2mg of glimepiride into 100 mL standard volumetric flask. To that about 50 mL of methanol was added, the solution was sonicated to dissolve and the volume is made up to the mark. Further secondary dilution was done with the mobile phase to get the final concentrations of pioglitazone (3-15µg/mL) and glimepiride (0.4-2µg/mL).

Preparation of Sample Solution

Twenty tablets each containing 15mg of pioglitazone and 2mg of glimepiride were weighed. Finely powder the tablets in a mortar, a quantity of the powder equivalent to one tablet content was accurately weighed and transferred into 100 mL of standard volumetric flask containing 50 mL of methanol, sonicated for 20 min and made up the volume with mobile phase. Further secondary dilutions were made with the mobile phase to get final concentration of pioglitazone 15µg/mL and glimepiride 2µg/mL. Quantification was achieved by peak area-ratio method with reference to the standards.

RESULTS AND DISCUSSION

In order to achieve simultaneous elution of the two components, initial trials were performed with the objective to select adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wavelength, optimum pH and concentration of the standard solutions were carefully studied. Several solvents were tested by using different proportions, such as methanol-toluene-hexane (60:20:20 v/v), methanol-acetonitrile-ethyl acetate (70:20:10 v/v/v) and methanol-acetonitrile-toluene-acetone (50:20:20:10 v/v/v/v). Finally, toluene, ethyl acetate, methanol and glacial acetic acid at a ratio of 70:15:10:5 v/v/v/v was selected as the optimum mobile phase. The wavelength 235nm was selected for detection of pioglitazone and glimepiride because it resulted in better detection sensitivity. The R_f value was found to be 0.42 and 0.27 for pioglitazone and glimepiride respectively as observed. ΔR_f obtained from the standard freshly prepared solutions was 0.1 for both drugs. HPTLC plate with drugs spot and chromatogram were shown in Figures 1 and 2, respectively.

Method Validation

To prove that the above developed method can be useful for routine quality control of these drugs, the method is validated according to ICH guidelines as follows.

The calibration plot was constructed by plotting peak area versus concentration (µg/mL) of pioglitazone and glimepiride which were found to be linear in the range of 3-15 µg/mL

(r^2=0.999) and 0.4-2 µg/mL (r^2=0.999), respectively (Figure 3). Limit of detection (LOD) values of pioglitazone and glimepiride were experimentally verified to be 0.05 µg/mL and 0.02µg/mL respectively. Limit of quantification (LOQ) values of pioglitazone and glimepiride were found to be 0.15µg/mL and 0.06µg/mL respectively, which indicated that the method can be used for analysis of pioglitazone and glimepiride over a very wide range of concentrations.

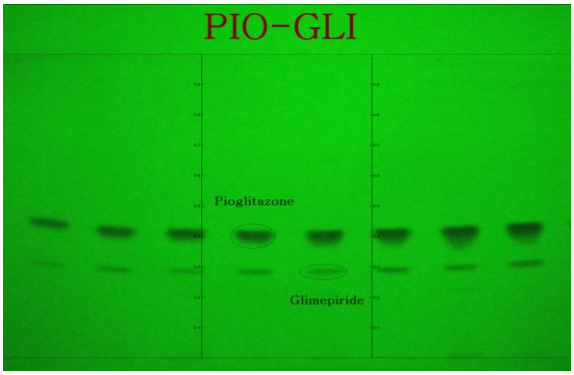

Fig. 1: HPTLC plate with eight tracks of mixture compound

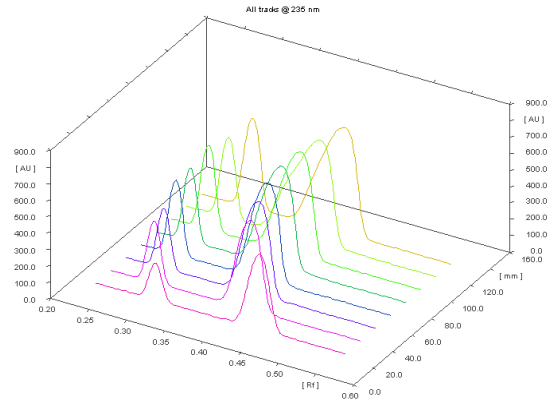

Fig. 2: Fingerprints of eight tracks

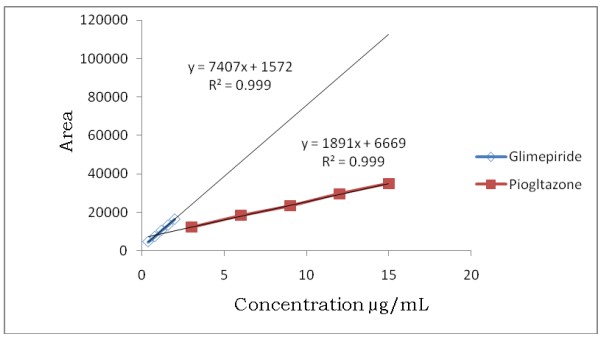

Fig. 3: Linearity curves of pioglitazone and glimepiride

The percentage recoveries of pioglitazone and glimepiride were found to be in the range of 100.01-100.09% and 99.97-100.07%, respectively. The results were shown in Table 1, which indicates that there is no interference with excipients, so the developed method is accurate.

Table 1: Recovery studies report for pioglitazone and glimepiride

Pioglitazone			
Initial Amount (ng)	***Amount found (ng)**	**Recovery (%)±SD**	**%RSD**
750	750.12	100.01±0.82	0.81
1500	1500.32	100.02±0.92	0.91
2250	2250.20	100.09±0.78	0.77
Glimepiride			
Initial Amount (ng)	***Amount found (ng)**	**Recovery (%)±SD**	**%RSD**
100	99.97	99.97±0.84	0.84
200	200.15	100.07±0.42	0.41
300	299.97	99.99±0.56	0.56

* mean of six sampling

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homologous sample. Results from determination of repeatability and intermediate precision, expressed as %RSD, were given in Table 2 for pioglitazone and Table 3 for glimepiride. There were no significant differences between %RSD values for intra-day and inter-day precision, which indicated that the method was precise.

Table 2: Precision data for pioglitazone

Param-eters	Reproducibility precision				Intermediate precision			
	3µg	6µg	9µg	12µg	3µg	6µg	9µg	12µg
Area under curve	11234	19087	24390	29623	11345	19126	24450	29703
	11456	19236	24582	29782	11468	19289	24620	29845
	11658	19657	24673	29879	11785	19689	24780	29748
Mean	11449	19326	24548	29761	11532	19368	24616	29765
SD	212.1	295.6	144.4	129.2	227.0	289.6	165.02	72.56
%RSD	1.85	1.52	0.58	0.43	1.90	1.49	0.67	0.24

Table 3: Precision data for glimepiride

Param-eters	Reproducibility precision				Intermediate precision			
	0.4µg	0.8µg	1.2µg	1.6µg	0.4µg	0.8µg	1.2µg	1.6µg
Area under curve	4542	7235	10032	13242	4538	7389	10265	13265
	4623	7364	10235	13356	4689	7564	10365	13459
	4752	7585	10465	13672	4892	7627	10532	13782
Mean	4639	7394	10244	13423	4706	7526	10387	13502
SD	55.91	77.0	26.6	222.76	77.6	23.3	34.89	61.16
%RSD	1.20	1.04	0.25	1.65	1.63	0.30	0.33	0.45

Robustness was done by small deliberate changes in the chromatographic conditions. There were no significant changes in the area under curve of pioglitazone and glimepiride when the variation in development time and chamber saturation time were done. The results (Table 4 and 5) indicated that the proposed method was robust.

Table 4: Difference in development time data for pioglitazone and glimepiride

Drugs	Development time (min)	Average area of six injections	SD	%RSD
Pioglitazone	14	52324	542.21	1.03
	15	52658	604.32	1.14
	16	52572	643.58	1.22
Glimepiride	14	19762	140.23	0.71
	15	19536	151.67	0.77
	16	19642	148.48	0.75

Table 5: Difference in chamber saturation time data for pioglitazone and glimepiride

Drugs	Chamber saturation time(min)	Average area of six injections	SD	%RSD
Pioglitazone	19	52683	568.36	1.07
	20	52656	524.59	0.99
	21	52782	536.74	1.01
Glimepiride	19	19462	146.60	0.75
	20	19768	149.83	0.75
	21	19352	137.95	0.71

Specificity of the method was determined by spots of pioglitazone and glimepiride from the samples, were identified by comparing its R_f value and its absorbance / reflectance spectrum with those of standard. The peak purity of pioglitazone and glimepiride were tested by comparison of spectra acquired at the peak start(S), peak – apex (A) and peak-end (E) positions of the spot were found to be specific. Figures 4 and 5 show the typical HPTLC chromatograms of pioglitazone and glimepiride, respectively.

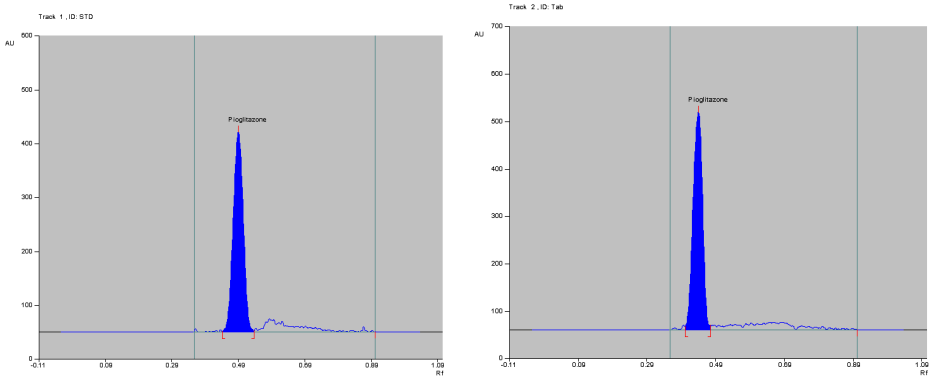

Fig. 4: Spectra for pioglitazone standard and sample

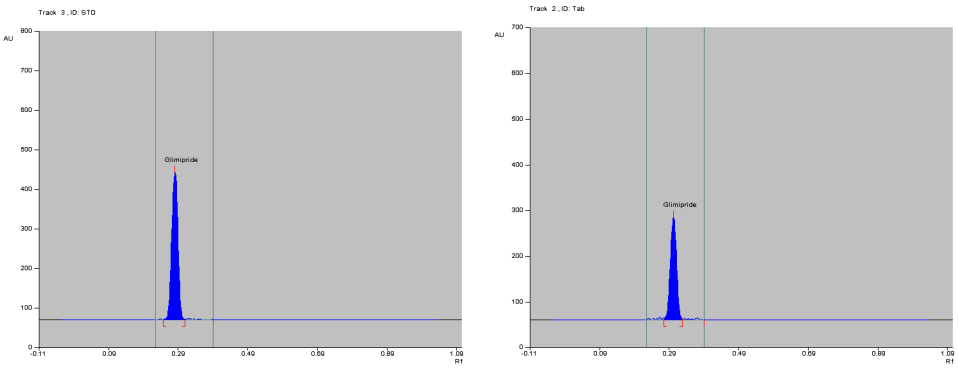

Fig. 5: Spectra for glimepiride standard and sample

The assay results show that the proposed method was selective for the simultaneous determination of pioglitazone and glimepiride without interference from the excipients used in the tablet dosage form. The values are shown in Table 6. The assay results and low %RSD values indicated that the developed method can be used for routine analysis of pioglitazone and glimepiride in pharmaceutical dosage forms.

Table 6: Estimation of amount of drug present in tablet dosage form

Tablet Formulation	Label claim (mg/tablet)	*Amount present (mg/tablet) ±SD	%RSD	*Percentage Label claim (%w/w)
Pioglitazone	15	15.12±0.24	1.58	100.8
Glimepiride	2	1.99±0.02	1.00	99.5

* mean of six sampling

CONCLUSION

The present method is precise, specific and accurate. The advantages of proposed method are its short analysis time, more resolution and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of mixtures of Pioglitazone and Glimepiride in pharmaceuticals.

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