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A New RP-HPLC Method Development and Validation for Simultaneous estimation of Telmisartan and Pioglitazone in Pharmaceutical Dosage Form

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Abstract: Simple, rapid, fast and precise reversed-phase high performance liquid chromatographic method has been developed and validated for the simultaneous estimation of Telmisartan and Pioglitazone in tablet dosage form. The quantification was carried out using Phenomenex C_8 (250 × 4.6 mm, 5µ) column and mobile phase comprised of acetonitrile and ammonium di hydrogen phosphate (pH 4.5; 20mM) in proportion of 65:35 (v/v).
The flow rate was 1.0 ml/min and the effluent was monitored at 210 nm. The retention time of Telmisartan and Pioglitazone were found 2.38 min and 3.16 min respectively. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and limit of quantitation. Linearity of Telmisartan and Pioglitazone were in the range of 10 to 50µg/ml and 7.5 to 37.5µg/ml respectively. The percentage recoveries of both the drugs were 99.85% and 102.06% for telmisartan and pioglitazone respectively from the tablet formulation. This method was most suitable for simultaneous determination of Telmisartan and pioglitazone in pharmaceutical dosage form and bulk drug.
Keywords: Pioglitazone, Telmisartan, HPLC, method validation.

Introduction

Pioglitazone hydrochloride (PG-HCl), ((±)-5-{p- [2-(5-ethyl-2-pyridyl)ethoxy] benzyl}-2,4-thiazolidine-dione hydrochloride) is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus (also known as non-insulin-dependent diabetes mellitus [1] (NIDDM) or adult-onset diabetes). Pioglitazone decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Currently, it is marketed under the trad name Actos® [2].several HPLC methods have been reported for determining pioglitazone hydrochloride in tablets [3-6]. The quantitative determination of pioglitazone in human serum by direct-injection HPLC mass spectrometry and its application to a bioequivalence study has also been reported [7]. Yamashita determined pioglitazone and its metabolites in human serum and urine [8] and Zhang and Lakings reported an assay method for pioglitazone alone in dog plasma [9]. Potentiometric sensors [10] were fabricated for the

determination of pioglitazone in some pharmaceutical formulations. Telmisartan is a new angiotensin II receptor antagonist for the treatment of essential hypertension usually given in combination with ramipril. Telmisartan (TEL) is chemically 4'-((1, 4'-dimethyl-2'-propyl (2, 6'- bi-1H-benzimidazol)-1'-yl) methyl)-(1, 1'-biphenyl) - 2-carboxylic acid. Literature survey revealed that telmisartan is not yet official in any of the pharmacopoeia. There are numerous methods reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms[13-14] and or in biological fluids. However, no method has been reported so far for the estimation of these two drugs simultaneously in combined dosage forms. Hence, in this present study, a new reversed-phase high performance liquid chromatography method was developed a rapid, economical, precise and accurate method for the simultaneous estimation of telmisartan with pioglitazone in tablets.

Material and Methods

Instrumentation Chemicals and reagents

The HPLC system consisted of Shimadzu pump LC - 10AT VP and LC-20AD pumps connected with SPD-10A VP UV-Visible detector. The data acquisition was performed by Spincotech 1.7 software. Pioglitazone Hcl with Telmisartan Hcl was kindly supplied by Glenmark Pharmaceuticals Ltd., and Ajanta Pharmaceuticals Ltd., Mumbai, India, respectively as gift samples. Tablets containing Pioglitazone Hcl with Telmesarton Hcl were procured from local pharmacy. All the reagents were of analytical grade. Glass double distilled water was used throughout the experiment.

Chromatographic conditions

Analysis was carried out at 210 nm using an Inertsil ODS-3V, Reverse phase column of 250x 4.6 mm 5µm dimensions at ambient temperature. The mobile phase consisted of acetonitrile and ammonium di hydrogen phosphate (pH 4.5; 20mM) in proportion of 65:35 (v/v) filtered through 0.45µ Nylon that was set at a flow rate of 1.5ml/min.

Selection of mobile phase

To develop a precise and robust HPLC method for simultaneous determination of Telmisartan and Pioglitazone HCl, their standard solutions were injected in the HPLC system. After literature survey and solubility data different composition of mobile phase of different flow rates were employed in order to determine the best condition for effective separation of both drugs.

Standard solutions

20 mg of Telmisartan and 15 mg of Pioglitazone standard powder was taken and dissolved in 10 ml of the mobile phase (stock-1). 1ml solution was taken and diluted to 10 ml with mobile phase (stock-2). From this 0.5, 1.0, 1.5, 2.0, 2.5 ml was taken and diluted to 10 ml with mobile phase. The working standard solutions were prepared and further diluted in mobile phase to contain a mixture of Telmisartan and PioglitazoneHCl in over the linearity range from 10-50µg/ml and 7.5-37.5 µg/ml respectively.

Assay Method

The standard solution of Telmisartan and Pioglitazone HCl contains 0.4 mg/each tablet from this solution, 20µl were injected and chromatographed. The mobile phase consisting of acetonitrile and ammonium di hydrogen phosphate in proportion of 65:35 (v/v) was pumped at a flow rate 1ml per min, the detection was monitored at 210 nm and the run time was 15 minutes.

Method Validation [15, 16, 17]

The method was validated for the parameters like system suitability, specificity, range and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, ruggedness and robustness,

System suitability

System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (symmetry factor), theoretical plates of the column, peak area and retention time.

Range and Linearity

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution by taking suitable volume and diluted up to 50 ml to get the desired concentrations (40, 80, 100, 120 and 140 µg/ml) for linearity in the range of 40-140 µg/ml. The prepared solutions were filtered through membrane filter and each of the dilutions was injected five times into the column. Absorbance at 210nm was measured and calibration curve for Telmisartan and Pioglitazone HCl was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Precision

The precision of method was investigated with respect to repeatability and ruggedness.

Method Precision (Repeatability):

Method precision for assay was established by determining the assay of six sample preparations under same conditions. Six replicates of Telmisartan and Pioglitazone HCl sample solution was prepared at

sample concentration by one analyst and analyzed on same day.

Intermediate Precision (Ruggedness):
Different analyst, using a different system, repeated the procedure followed for method precision on a different day using same lot of sample.

Accuracy
Accuracy was determined over the range 50% to 150% of the sample concentration. Calculated amount of Telmisartan and Pioglitazone HCl from standard stock solution was added in placebo to attain 50%, 100% and 150% of sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected and the chromatograms were recorded.

Specificity
Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Robustness
The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. flow rate of mobile phase to 1.2 ml/min. for -0.1 ml/min. change and 1.4 ml/min. for +0.1 ml/min. change to 25°C and 35°C to observe their effect on system suitability.

Standard and sample solution stability
Standard and sample preparation was prepared as per test procedure and assay of standard and sample was determined as per method. Standard and sample solution was stored for 24 hours at room temperature. Assay of standard and sample solution was determined after different hours till 24 hours using freshly prepared standard. The assay obtained was compared with the initial assay value and recorded.

Detection and Quantification limit
Limit of detection and limit of quantification was calculated by the proposed method which was based on the standard deviation (s) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD= 3.3 (s/S) and LOQ= 10 (s/S).

Results and Discussion

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Telmisartan and Pioglitazone HCL in combined tablet dosage form. The separation was carried out by using a mobile phase consisting of acetonitrile and 0.5%triethyl amine buffer of pH 4.5 in the ratio of 65:35 v/v. The column used was C8 phenomenex 5µ, 250cm x 4.6mm id with flow rate of 1.3ml/min using PDA detection at 210nm. For quantitative estimation 210nm was selected as suitable wavelength. The individual peaks of Telmisartan and Pioglitazone HCl was identified by knowing the retention time 2.38 and 3.16 minutes respectively. The chromatogram of Telmisartan and Pioglitazone HCL reference standard chromatogram were shown in figure-1.

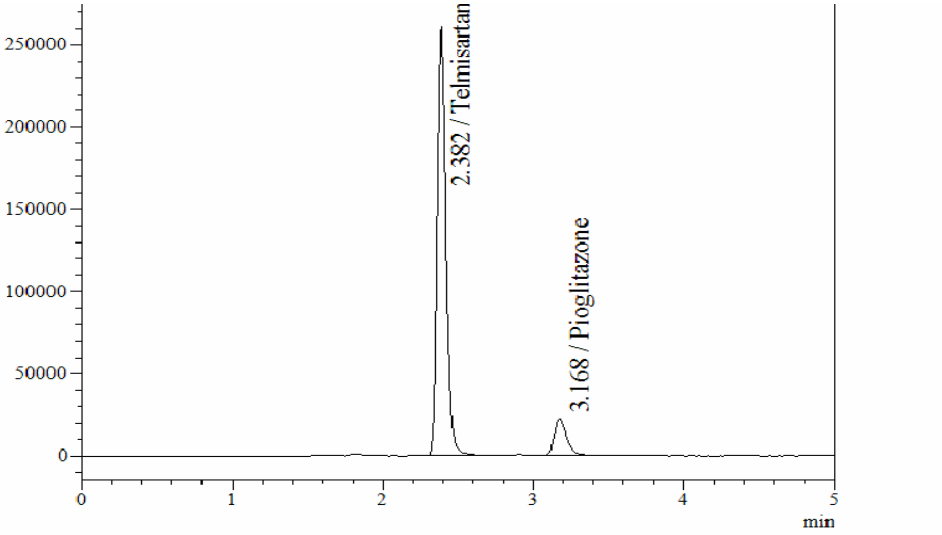

Figure: 1 Chromatogram for Telmisartan and Pioglitazone HCl

Linearity was evaluated by visual inspection of the plot of peak area as a function of analyte concentration for Telmisartan and Pioglitazone HCl**.** The linearity of the method was determined at concentration levels ranging from 10-50µg/ml for Telmisartan and 7.5-37.5µg/ml for Pioglitazone HCl it was presented in table-1 and table -2. The correlation co-efficient of Telmisartan was found to be 0.9997 and the correlation co-efficient of Pioglitazone HCl was found to be 0.9995, these are within limit was shown in fig.2 and fig.3 respectively. System suitability parameters such as resolution, tailing factor and number of theoretical plates are presented in table - 3.System precision was carried out, the RSD for peak area of Telmisartan and Pioglitazone HCl for five replicated injections was not more than 2.0% and the data was presented in table - 4.

Table: 1 Linearity Data for Telmisartan

Concentration (µg/ml)	Peak area
10.0	180692
20.0	333938
30.0	484976
40.0	652384
50.0	810050
Correlation Coefficient	0.9996

Table: 2 Linearity Data for Pioglitazone HCl

Concentration (µg/ml)	Peak area
7.5	17665
15.0	35330
22.5	53116
30.0	71628
37.5	89414
Correlation Coefficient	0.9999

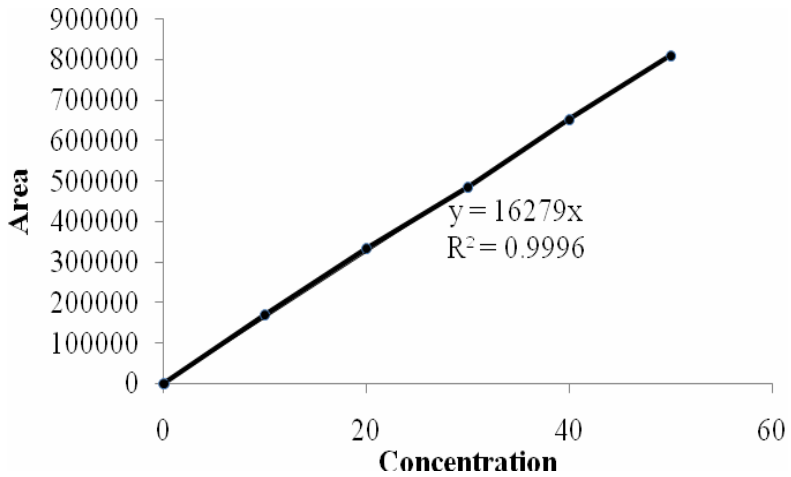

Figure: 2 Linearity graph of Telmisartan

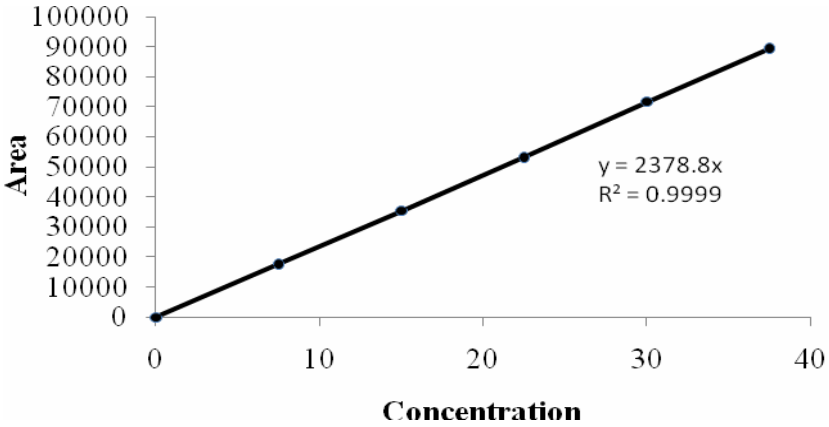

Figure: 3 Linearity graph of Pioglitazone HCl

Table: 3 Data derived from System suitability experiment for Telmisartan and Pioglitazone HCl

Parameters	Obtained values	
	Telmisartan	Pioglitazone HCl
Retention Time	2.38min	3.16min
Peak Area	333636.2	35236
Standard Deviation	1024.3462	87.7639
RSD	0.3070	0.2491

Table: 4 Data derived from Method Precision experiment of Telmisartan and Pioglitazone HCl.

Parameters	Area for Telmisartan	Area for Pioglitazone HCl	Telmisartan assay		Pioglitazone assay	
			Amount Present (mg)	% Purity	Amount Present (mg)	% Purity
Average	333636.2	35236	19.9819	99.9096	14.9601	99.7339
SD	1024.3462	87.7639	0.0613	0.3067	0.0373	0.2484
%RSD	0.3070	0.2491	0.3070	0.3070	0.2491	0.2491

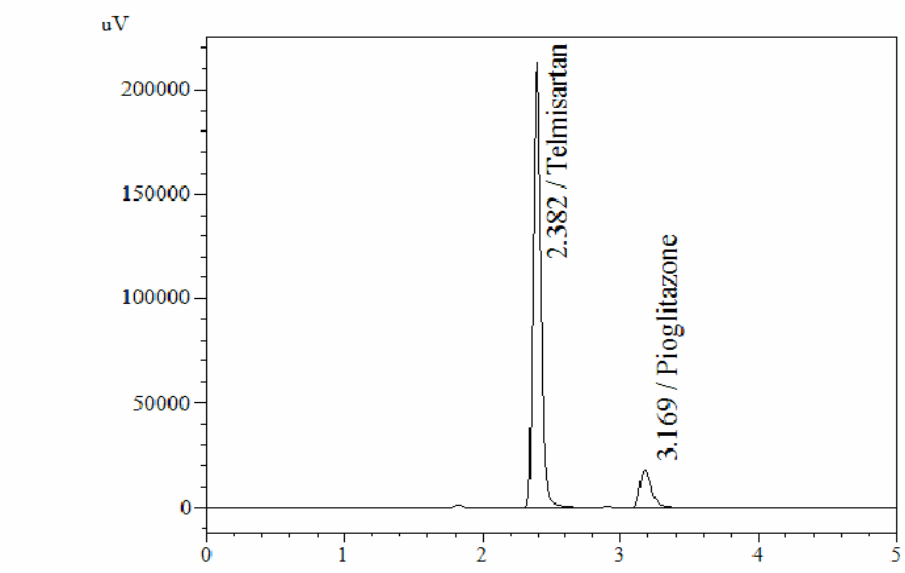

Figure: 4 Chromatogram of STD solution of Telmisartan and Pioglitazone HCl

Estimation of Telmisartan and Pioglitazone HCl in tablet dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. The peak area ratio of standard and sample solutions was calculated. The result of an analysis shows in figure - 4 the quantitative estimation was carried out on tablet by taking the same concentration as for standard solution. The table -5 shows percentage purity values ranging from 99.37-100.10 for Telmisartan and 99.52-100.01 for Pioglitazone HCl respectively. The acceptance criteria of method precision were found to be RSD NMT 2.0% and the method shows precision of 0.3070 for Telmisartan and 0.2491 for Pioglitazone HCl.

The method was validated as per ICH guidelines. The accuracy of the method was determined by recovery experiments. The recovery study was carried out and the percentage recovery range found to be within the limit, 99.21 - 99.70 percentage for Telmisartan and 98.16-98.87 percentages for Pioglitazone HCl. The robustness of the method was performed by flow rate variation and change pH of mobile phase, and the result was found to be within the limit. The method shows system suitability and precision within limits under given set of condition. 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 5.92µg/ml for Telmisartan and 0.82µg/ml for Pioglitazone HCl respectively, the data was presented in table - 6.

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 10µg/ml for Telmisartan and 7.50µg/ml for Pioglitazone HCl respectively. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown**.** Since this developed method can be used for routine analysis of two components in formulation.

The proposed high-performance liquid chromatographic method has been evaluated as per ICH guidelines, Parameters such as linearity, precision, accuracy, LOD, LOQ, specificity and robustness are proved to be convenient for the quality control of Telmisartan and Pioglitazone HCl in tablet dosage form. The proposed RP-HPLC method enables the determination of Telmisartan and Pioglitazone HCl because of good separation of chromatographic peaks. This method can be used successfully for the analysis of Telmisartan and Pioglitazone HCl in tablet dosage form. The developed chromatographic method for Telmisartan and Pioglitazone HCl is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis in research institution, quality control department in industries, approved testing laboratories, and clinical pharmacokinetic studies.

Table: 5 % Purity of the Telmisartan and Pioglitazone HCl

Sample no.	Area for Telmisartan	Area for Pioglitazone HCl	Telmisartan assay		Pioglitazone assay	
			Amount Present (mg)	% Purity	Amount Present (mg)	% Purity
1	333936	35332	20.00	100.00	15.00	100.01
2	333984	35331	20.00	100.01	15.00	100.00
3	334171	35188	20.01	100.07	14.94	99.60
4	331820	35169	19.87	99.37	14.93	99.54
5	334270	35160	20.02	100.10	14.93	99.52
Average	333636.2	35236	19.9819	99.9096	14.9601	99.7339
Std. Deviation	1024.3462	87.7639	0.0613	0.3067	0.0373	0.2484
%RSD	0.3070	0.2491	0.3070	0.3070	0.2491	0.2491

Table: 6 Validation Parameters of the Telmisartan and Pioglitazone HCl

Validation parameters	Telmisartan	Pioglitazone HCl
Linearity range (µg/ml)	10-50	7.5-37.5
Correlation co-efficient	0.9997	0.9995
LOQ (µg/ml)	10	7.5
Accuracy	99.42	98.57
System precision (% RSD)	0.39	0.51
Robustness pH (% RSD)	0.46	0.48
Robustness flow rate (% RSD)	0.38	0.64
No. of theoretical plates	5828.43	5466.42
Tailing factor	1.217	1.237

Acceptance criteria

The % RSD (Relative Standard Deviation) of assay result should be not more than 2.0%.

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