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CONTENTS

Development And Validation Of Reverse Phase Hplc Method For Simultaneous Estimation Of Pioglitazone Hydrochloride And Glimepiride

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Abstract:

The objective of the present work was to develop a reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of Pioglitazone hydrochlorideand Glimepiride. The optimized conditions of chromatographic system include column Agilent BDSHypersil C18 (250 x 4.6mm) 5 µm particle size, isocratic pump mode with UV detection at 225 nm, mobile phase consisting of buffer:methanol: acetonitrilemixture in the ratio of 37:42:21 as mobile phase and a flow rate of 1.5 mL/min. The systemappropriateness tests were done before starting the validation as per ICH guidelines and parameters were found to be within the acceptable criteria. The method exhibited retention time(RT) of 17 min and linearity between the concentration range of 72-168 µg/mL for Pioglitazone hydrochloride with a correlation coefficient of 0.997. Glimepirideexhibited a RT of 39 min and linearity between the concentration range of 8-24µg/mLalong with a correlation coefficient of 0.999.LOD and LOQ values for Pioglitazone hydrochloride were 1.816 and 5.504µg/mL respectively whereasforGlimepiride it was found to be 0.06375 and 0.1931µg/mL respectively.The calculated %RSD values for intra-day, inter-day, method precision and repeatability were found to be within the limits of <2%. The percentage recovery of Pioglitazone hydrochlorideand Glimepiride ranged between 98.73 to 99.13% and 95.50 to 98.38 %respectively.Robustness was indicated by insignificant variations in RT with deliberate variations in flow rate, mobile phase ratio and detection wavelength. The good percentage recovery of sample clearly indicates the reproducibility and accuracy of the developed method. All the results indicated that the proposed method was proved to be, simple, fast, sensitive, specific, precise, and robust. Hence, the developed method was successfully used for the estimation of above mentioned antidiabetic drugs in bulk drug and buccal film formulation.

Keywords: Reverse phase HPLC, Pioglitazone hydrochloride, Glimepiride, Methoddevelopment,Validation.

INTRODUCTION

Pioglitazone hydrochloride (Fig.1)is a drug used for the treatmentof type-II diabetes mellitus, it belongs tothe group of thiazolidinediones also called "glitazones", when used along with a proper diet and exercise program itcontrols high blood sugar. It is a white crystalline powder, soluble in dimethyl formamide and slightly soluble in ethanol. Pioglitazone hydrochloride

(Fig.1), 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione hydrochloride acts primarily by decreasing insulin resistance[1]. Glimepiride (GLP) (Fig.2),4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl)carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamideis an oral anti-diabetic drug.It belongs to the class of drugs known as sulfonylurea and is used to treat type II diabetes mellitus withanadvantage of being completely bioavailable, being effective at low doses in patients with non-insulindependent diabetes mellitus, showing linear pharmacokinetics, and having a prolonged effect. As with the other sulphonylureas,Glimepiride appears to lower blood glucose levels by stimulating insulin release from the pancreas [2].

Pioglitazone Hydrochloride:
Molecular weight: 356.44
Molecular Formula: $C_{19}H_{21}ClN_2O_3S$

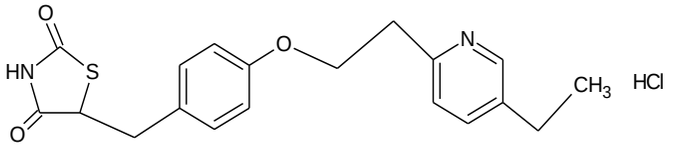

Fig.1: Structure of Pioglitazone hydrochloride

Glimepiride:
Molecular weight: 490.62
Molecular formula: $C_{24}H_{34}N_4O_5S$

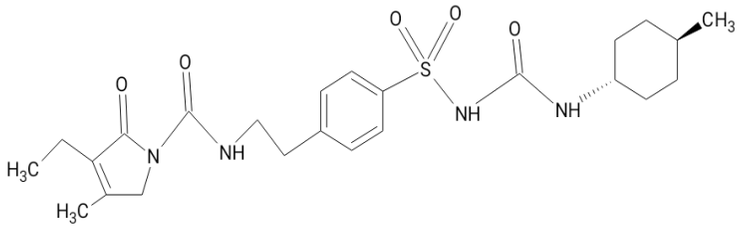

Fig.2:Structure of Glimiperide

Earlier several research papers have been described in the literature for the determination of Pioglitazone hydrochloride and Glimepiride. A single RP-HPLC method for the simultaneous determination of six active ingredients including Metformin hydrochloride, Pioglitazone, Glimepiride, Gliclazide, Glibenclamide and Glipizide in pharmaceutical products were reported earlier[3]. A rapidand precise reverse phase HPLC method was also reported for the simultaneousestimation of Pioglitazone hydrochlorideand Glimepiride in pharmaceutical dosage

forms[4]. In the present work, an attempt has been made to develop and validatea simple RP-HPLC method in terms of accuracy, precision, specificity, system suitability,linearity, and robustness along with limit of detection and limit of quantification for simultaneous determination of Pioglitazone hydrochloride and Glimepiride in bulk and buccal film dosage form**.**

Methodology

Reagents:

Pioglitazone hydrochloridewas obtained as gift sample from Aarathi drug limited, Mumbai, India. Glimepiride was obtained as gift sample from Hetero Drugs ltd, Medhak, Telangana, India. HPLC grade acetonitrile was purchased from Fischer scientific chemicals. HPLC grade water was prepared using Milli-Q water purification system. Orthophosphoric acid used was of AR grade and was purchased from Merck specialities Pvt Ltd. Class A glassware was used throughout the experiment.

Instrumentation:

The method development was carried out using Shimadzu LC (Grace Smart RPc18 model) which consisted of a column Agilent BDS Hypersil C18 (250 x 4.6mm) with 5 µm particle size, isocratic pump mode.

Preparation of buffer and mobile phase:

Thebuffer used was 0.05M disodium hydrogen phosphate, it was prepared by dissolving 3.40 g of potassium dihydrogen phosphate in 900mL of waterand was adjusted to pH 3.0using dilute ortho-phosphoric acid and diluted to 1000mL with water.To prepare the mobile phase, exactly 370 mL of buffer was taken into a 1000 mL beaker, to this 420 mL of methanol was added and to the above mixture 210 mL of the acetonitrile was added.The above prepared mixture was used as mobile phase and was filtered through 0.45µ nylon membrane filter and degassed by ultra-sonication for 30 min.

Preparation of standard stock solution:

Accurately 15 mg of Pioglitazone hydrochloride and 5 mg Glimepiride were weighedseparately and transferred into different a clean and dry 25 mL volumetric flasks and each was dissolved in 10 ml of methanol. The final volume was made up to the mark using mobile phase to get 600µg/mL and 200µg/mL respectively.

Sample Preparation:

Buccal films (no 3) units (each of 6 mm diameter) of each formulation equivalent to 15 mg of Pioglitazone hydrochloride and 2 mg of Glimepiridewere taken in separate 100 ml volumetric flasks, 100 ml of mobile phase was added and continuously stirred for 24 h. The solutions were filtered and degassed by sonication. Further 10µL of the sample solutions were injected into the chromatogram in triplicate and the peak area was recorded. [5-7]

Optimization of chromatographic condition:

Spectroscopic analysis of Pioglitazone hydrochloride and Glimepiride showed that it has a maximum UV absorbance at 225 nm. Therefore, the chromatographic detection was performed at 225 nm using a UV-Visible detector, the injection volume used was 10 µL. A mobile phase consisting of a mixture of buffer:methanol:acetonitrile was used with a flow rate of 1.5 µL/min (Table 1).

Specificity:

It was assessed by injectingplacebo sample containing all ingredients of the formula except the analyte which was set up as per test method. To recognize the interference by these excipients, a blend of the inert ingredients (placebo sample) was spiked with Pioglitazone hydrochloride and Glimepiride and chromatogram was recorded.

Range and Linearity:

The linearity of the assay method was determined by multipoint calibration method consisting of sevendifferent concentrations of analyte and the data was statistically analysed by performing regression analysis. Aliquots of 3mL, 4mL, 5mL, 6mL and 7 mL were withdrawn from stock solution diluted to 25 mL using mobile phase to get a concentration between 72-168µg/mL for Pioglitazone hydrochloride. Similarly aliquots of 1mL, 1.5mL, 2mL, 2,5mL and 3mL were diluted to 25 mL with mobile phase to obtain a concentration ranging between 8 to 24µg/mL for Glimepiride.

About 10 µL of standard dilutions of Pioglitazone hydrochloride and Glimepiride were injected in to the chromatograph. Retention time and peak area obtained were recorded and standard curve was plotted.

Sensitivity:

LOD and LOQ of Pioglitazone hydrochloride and Glimepiridewere determined based on standard deviation of the response and slope. The LOD and LOQ were calculated as: LOD = 3.3 σ/S and LOQ = 10 σ/S; where σ is the standard deviation of the peak area of lowest standard concentration and S is the slope of the curve.

Precision:

Precision of the system is checked by injecting standard dilution of 120µg/mL and 16µg/mLsix times and then ensuring the reproducibility in the retention time and area.The repeatability, intra-day precision, inter-day precision of the analytical method was measured by analysing the dilutionsof same concentration (n=6). Peak area of all six replicates was determined and %RSD was calculated and reported (Table 2)

Accuracy:

The accuracy of the method was tested by determining the percentage recovery of the analyte in formulation by assaying the known amounts of analyte added. It was carried out at three different levels LQC, MQC and HQC by spiking the formulation with standard drug substances at 80%, 100%, and 120%

respectively. All the measurements were carried out (n = 3) and the recovery was expressed in terms of analyte found in spiked sample.

Robustness:

Robustness of the current method was studied by estimating the effect of deliberate changes such as variation in mobile phase composition, flow rate of 1.5±1mL/min and detection wavelength of225±15nm on analytes determination.

RESULTS AND DISCUSSION

Table 1: Chromatographic conditions

Column	BDS Hypersil C18 (250 x 4.6mm) with 5 µm particle size.
Mobile phase	Buffer: Methanol: Acetonitrile
Ratio	37:42:21
Flow rate	1.5 mL/min
Wavelength	225 nm
Injection volume	10 µL
Run time	20 min
Temperature	Ambient
Mode of operation	Isocratic elution

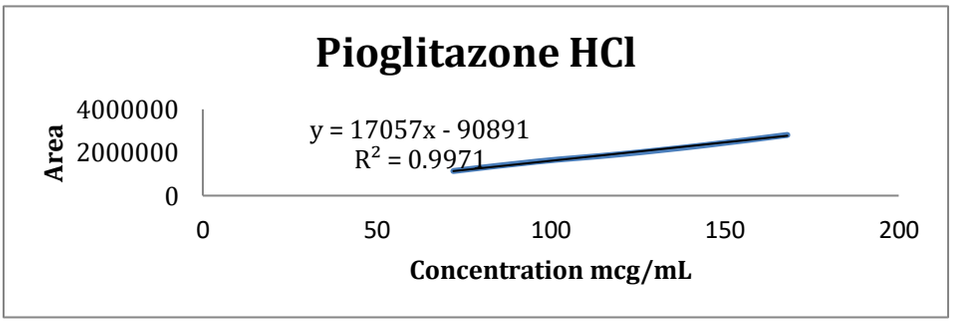

Fig.3: Calibration curve of Pioglitazone hydrochloride

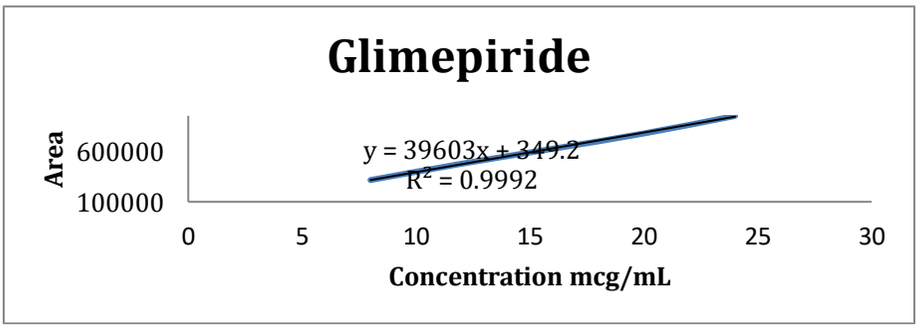

Fig.4: Calibration curve of Glimepiride

Table 2: System Suitability parameters

HPLC Parameters	Pioglitazone Hydrochloride	Glimepiride
Linearity range (µg/ml)	72-168	8-24
Correlation Coefficient (r^2)	0.997	0.999
Tailing factor	0.96	1.07
Number of theoretical Plates	3742	5255
RSD	0.5%	0.1%

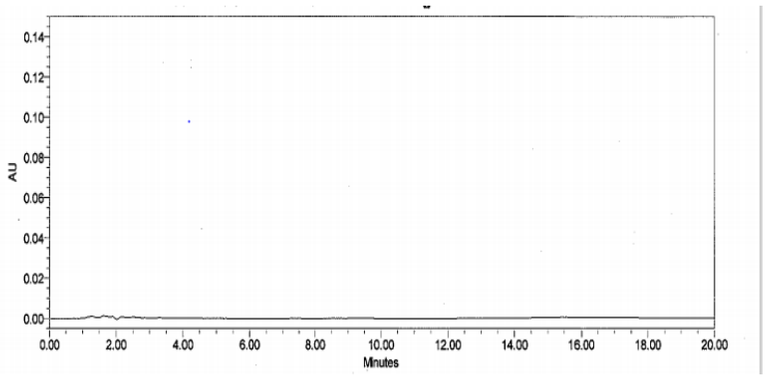

Fig.5: Blank Chromatogram

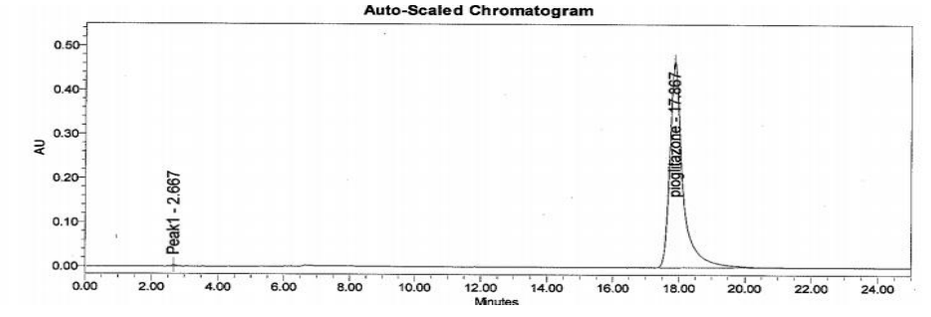

Fig.6: Chromatogram of Pioglitazone hydrochloride

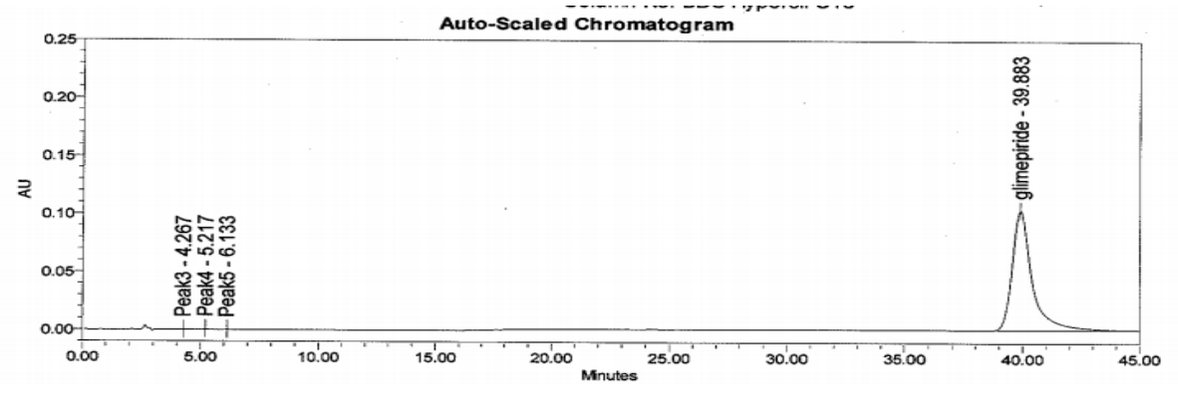

Fig.7: Chromatogram of Glimepiride

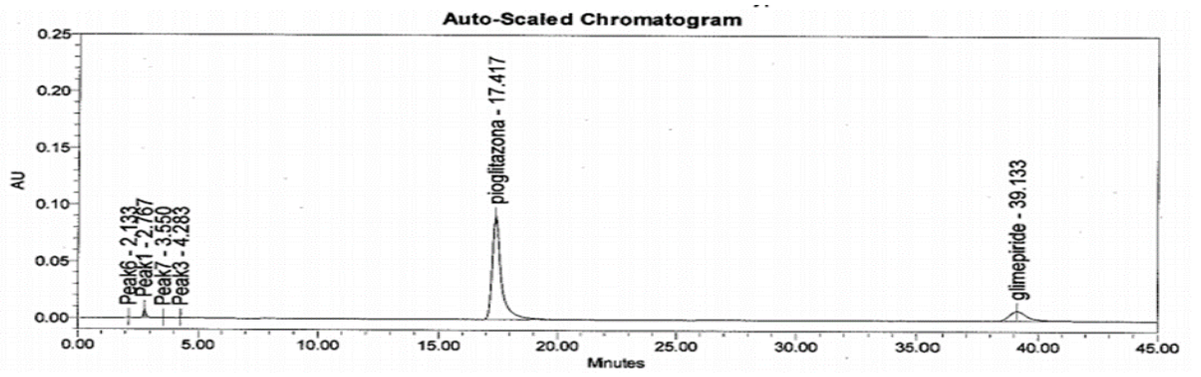

Fig.8: Chromatogram of Pioglitazone hydrochloride and Glimepiride

Table 3: Repeatability (n=6)

Parameters	Pioglitazone Hydrochloride Concentration (120 µg/mL)	Glimepiride Concentration (16µg/mL)
Mean(peak area)	2082837.3	912199.2
Standard deviation (SD)	9389.2	765.1
Relative standard deviation (%)	0.5%	0.1%

Table4:Statistical data of calibration curve

Parameters	Pioglitazone Hydrochloride	Glimepiride
Linearity (µg/mL)	72-168	8-24
Regression equation	y = 17057 x -90891	y = 39603 x + 349.2
Correlation coefficient (r^2)	0.9971	0.9992
Limit of detection (LOD) µg/mL	1.816	0.06375
Limit of quantification (LOQ) µg/mL	5.504	0.1931

Table5: Intraday precision

Time	Area of Pioglitazone Hydrochloride (120µg/mL)	RT (min)	Area of Glimepiride (16µg/mL)	RT (min)
1	2085604.0	7.45	911563.0	15.65
2	2097657.0	7.47	911720.0	15.67
3	2082702.0	7.44	911308.0	15.64
4	2085230.0	7.45	913085.0	15.65
5	2075248.0	7.46	912963.0	15.64
6	2070583.0	7.44	912560.0	15.63
Average	2082837.3	7.45	912199.8	15.65
SD	9389.3		765.1	
%RSD	0.5		0.1	

Table6: Inter day-precision

Day	Area of Pioglitazone Hydrochloride (120 µg/mL)	RT (min)	Area of glimepiride (16µg/mL)	RT (min)
1	2085504.0	7.47	911199.8	15.65
2	2086704.0	7.43	912890.7	15.54
3	2074807.0	7.52	912560.0	15.49
Average	2082338	7.47	912216.8	15.595
SD	6549	0.0451	896.1631	0.0778
RSD	0.3145	0.6034	0.09824	0.4988

Table7: Results of Robustness

Flow rate	Assay of Pioglitazone Hydrochloride %	Assay of Glimepiride%
1.4 mL/min	98.7	97.4
1.6 mL/min	96.5	95.8
Wave length		
210nm	96.7	95.8
240nm	94.7	93.6
Mobile phase ratio		
32:45:23	93.8	94.6
42:39:19	97.0	96.5

Table8: Results of accuracy for Pioglitazone hydrochloride(Recovery Studies) (N=3)

S. NO	Percentage level of standard	Peak area	SD	%RSD	Sample concentration µg/mL	Spiked	Found	Recovery (%)
1	80	1908504.0	6618	0.3212	120	96	94.79	98.73
2	100	2375203.0	6743	0.2133	120	120	118.95	99.13
3	120	2723191.0	6932	0.4013	120	144	140.68	97.70

Table9: Results of accuracy for Glimepiride (Recovery Studies) (N=3)

Sl No.	Percentage level ofstandard	Peak area	SD	%RSD	Sample concn µg/mL	Spiked	Found	Recovery (%)
1	80	702216.8	874.23	0.112	20	16	15.41	96.31
2	100	791710.8	901.24	0.2133	20	20	19.1	95.50
3	120	845616.0	929.14	0.4013	20	24	23.61	98.38

Table 10: Assay

Drug	Peak area	Drug content (mg)	% Drug content
Pioglitazone hydrochloride	1893589	14.89	99.30
Glimepiride	590093	1.97	98.32

DISCUSSION

The method was developed by varying the chromatographic conditions such as flow rate, mobile phase ratio and detection wavelength(Table 3). The method development was carried out by optimizing many chromatographic conditions in terms of solubility of Pioglitazone hydrochloride and Glimepiride in different solvent systems, suitability of the method, cost, ease of preparation and applicability of the method to different purposes. Specifically, the mobile phase composition was optimized by trial and error basis using different solvent mixture systems with varying proportions, among which a mixture of buffer, methanol and acetonitrile in the ratio of 37:42:21 v/v was selected as suitable system. Further, suitability of the method was tested by analysing system suitability parameters (Table 2)viz theoretical plates, and tailing factor; all these parameters found within the limits.

Specificity is described as the ability of a method to discriminate the analyte from all interfering substances. From the Fig.5,6,7 and 8 it is clear that the excipients used in the formulation did not interfere with the Pioglitazone Hydrochloride and Glimepiride peak indicating specificity of the method. The linearity of a given test procedure is its ability (within given range) to produce results that are directly proportional to concentration of the analyte in the sample. Range is the interval between upper and the lower levels of analyte that have been determined with precision, accuracy and linearity using the method. ICH guidelines specifies a minimum of five concentration levels, along with certain minimum specified ranges. Linearity is an important parameter for the confirmation of method's sensitivity for the analysis of analyte's concentration with a defined range. The concentration range was within 72 – 168 µg/mL and r^2 value of 0.9971 for Pioglitazone hydrochloride and 8 – 24 µg/mL and r^2 value of 0.9992 for Glimepiride (Fig.3 and 4), which indicated good linear relationship between analyte concentration and the response, peak area (Table4). The developed RP-HPLC method was highly sensitive to detect and determine the drugs content as the LOD and LOQ values for Pioglitazone hydrochloride and Glimepiride were found to be 1.816, 0.0637µg/mL and 5.504,0.1931µg/mLrespectively (Table 4). The precision measurements were expressed in % RSD. The % RSD of repeatability (Table 3) and intermediate precision (inter-day, intra- day) (Table 5 and 6) were found to be <2% which indicated the precision of the method as it complies with acceptable limits. The percentage recovery of Pioglitazone hydrochloride and Glimepiride was determined by standard addition method and ranged between 98.73 to 99.13% and 95.50 to 98.38 % (Table 8 and 9) which showed that the method is accurate. Robustness was indicated by negligible variations in drug content with deliberate variations in flow rate, mobile phase ratio and detection wavelength (Table 7). The estimated amount of Pioglitazone hydrochloride and

Glimepiride in the formulation was found to be 99.30 %and 98.32 % respectively.

CONCLUSION

The RP-HPLC method for analysis of Pioglitazone Hydrochloride and glimepiride in the prepared buccal film was successfully developed and validated in terms of validation parameters as per ICH guidelines. Hence, it can be concluded that the proposed method can be used for routine analysis of Pioglitazone Hydrochloride and glimepiride

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