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[Research article]

Method Development and Validation of RP-HPLC Method for Glimepiride, Pioglitazone Hcl and Metformin Hcl in Tablets

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ABSTRACT

A simple, accurate, precise and linear Isocratic RP-HPLC has been developed and subsequently simultaneous for the determination of glimepiride, pioglitazone Hcl and metformin Hcl in pharmaceutical dosage form. Kromosil C_{18} (150mm X4.6 mm) 5µ with flow rate of 1ml/min by using JASCO PU-1580 and UV/VIS JASCO UV-1570 at 217nm. The separation was carried out using a mobil phase consisting of the mixture of methanol and 25mM phosphate buffer (pH-2.0) in the ratio of 50 :50. The retention time for glimepiride, metformin Hcl and pioglitazine Hcl were found to be 2.24, 3.76 and 10.20 min. the correlation coefficient was found to be 0.999 for GLI, 0.9993 for PIO and 0.9997 for MET. The mean percentage recovery was found to be 98.58 (GLI), 98.30 (PIO) and 98.87 (MET). The percentage the accuracy of the drugs were found to be near to 100% representing the accuracy of the method. The proposed method was also validated and applied for the analysis of the drugs on tablet formulations.

Keywords : RP-HPLC, Glimepiride (GLI), Pioglitazone Hcl (PIO) and Metformin Hcl (MET).

.

INTRODUCTION

Glimepiride is chemically 1-P-2-3 ethyl methyl-2-oxo-3pyrroline-1-carboxamido ethyl phenyl-3-(trans-4-methyl cyclohexyl) urea, is antihyperglycemic agent. It is indicated for the treatment of the oral therapy of type -2 diabetes mellitus. It improves the insulin sensitivity of peripheral tissue and lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Pioglitazone Hcl is chemically ±-5-{[4(-(5-ethyl-2-pyridinyl) ethoxy) phenyl]methyl}-2,4 thiazodine dione mono hydrochloride, is an oral antihyperglycemic agent. It is used in the treatment of type-2 diabetes mellitus and also non-insulin dependent diabetes mellitus (NIDDM). It is selectively stimulates the nuclear receptor peroxisome proliferators activated receptor gamma (PPAR-γ). Metformin Hcl is chemically 1,1-dimethylbiguannide, is antilipolytic effect. It act by suppressing excessive hepatic glucose production and improving glucose clearance and decrease fasting plasma glucose levels and increase insulin-mediate glucose utilization in peripheral tissue and lowers serum free fatty acid concentrations[1-5]. From the survey of literature, this drugs using Spectrophotometric, LC/MS, IPLC, LC-ESI-MS/MS, RP-HPLC, HPTLC methods in single and combined with

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others drugs and in human plasma. But there is no work in RP-HPLC method for the simultaneous estimation of glimepiride, pioglitazone Hcl and metformin Hcl in their combination dosage form[6-32]. The proposed method presented here is simple, fast, accurate and precise and can be used for simultaneous determination in combination tablet dosage form. The method was validated as per ICH guidelines[33-37].

INSTRUMENT

Instrument used in present study was JASCO. HPLC. The pump used was JASCO PU-1580 pump. The samples were applied Kromosil c_{18} 5µ column with Rhedyne injector. The sample was performed using UV/VIS JASCO UV-1570 detector with flow rate 1ml/min and operated by JASCO LC –NeT II/ADC interface. The scaltech precision balance (0.001gm sensitivity) was used for weighing purpose.

MATERIALS USED

Glimepiride, Pioglitazone Hcl and Metformin Hcl raw materials were supplied by microlabs, Bangalore. Tripride-2 (Microlabs) was taken for study which contains 2mg of glimepiride, 15 mg of pioglitazone Hcl and 500mg of metformin Hcl. HPLC grade methanol (molychem, Mumbai), Potassium dihydrogen orthophosphate (RFCL-New Delhi), decane sulphonic acid sodium (S.D. Fine chem. Ltd), ortho phosphoric acid (Ranbaxy fine chemical. Ltd, Mumbai), HPLC grade water.

PREPARATION OF MOBILEPHASE

Methanol and 25mM phosphate buffer (pH-2.0) in the ratio 50:50 were mixed, sonicated for 10 minutes and filtered through the membrane filter of micron 0.45µ.

PREPARATION OF 25Mm PHOSPHATE BUFFER

3.4gm of potassium dihydrogen ortho phosphate and 0.244gm of decane sulphonic acid sodium were transferred into 1 litre volumetric flask, dissolved by adding 100ml of distilled water and made upto to volume with distilled water, pH adjusted to 2.0 using 1% o-phosphoric acid solution.

PREPARATION OF STANDARD SOLUTIONS

10mg of glimepiride was dissolved in 1% formic acid in acetonitrile, 10mg of pioglitazone Hcl was dissolved in methanol and 32mg of metformin Hcl was dissolved in distilled water and made upto 10ml with same for each drugs. From this solution, 1ml of solution was made up to 10ml with diluent (methanol and water in the ratio 50:50) separately and 5ml of each solution made upto 50ml with mobile phase. The working standard solution concentration was prepared to the concentration 2µg/ml, 15µg/ml and 50µg/ml.

PREPARATION OF SAMPLE SOLUTION

A quantity of tablet powder equivalent to 0.04552 gm transfer to 10ml volumetric flask dissolved in few ml of diluent and made upto the volume by mobile phase. From this 1ml of solution was diluted to 20ml using the same diluent to get the concentration of 2µg of glimepiride, 15µg/ml of pioglitazone Hcl and 50µg/ml of metformin Hcl.

METHOD DEVELOPMENT

Selection of wavelength

Stock solution of 10mg/ml was prepared and further diluted to get the concentration 10µg/ml for glimepiride, pioglitazone Hcl and metformin Hcl with diluent. The wavelength was selected by scanning the above standard drugs between 200-400nm. The scan results showed that reasonably maximum absorbance was recorded at 217nm. Therefore 217nm was selected as the detection wavelength for the HPLC investigation [Figure-1].

METHOD

The samples were applied Kromosil C_{18} (150 x 4.6mm) 5µ with Rhedyne injector in reverse saturation mode using methanol and 25mM phosphate buffer (pH-2.0) in the ratio of 50:50 as mobile phase with the flow rate of 1.0ml/min. The samples were performed using UV/VIS JASCO UV-1570 detector with the flow rate 1ml/min. The instrument computes accurate results with minimal time. The retention time was obtained 2.24 for GLI, 3.76 for MET and 10.20 for PIO respectively. The results of assay were reported in the Table-1 and Figure-2.

VALIDATION OF THE METHOD

Accuracy

Accuracy was determined by tablets samples with different known concentrations of the drugs (50%,

100% and 150%). Each concentration was injected in six times and the assay was performed as per the developed method. From this % recovery and the amount present (or) recovery was calculated. Results of recovery study are reported in table-2.

Precision

Standard solution of GLI, PIO and MET were prepared in the same manner for the standard preparation. This solution containing 2µg/ml for glimepiride, 15 µg/ml for Pioglitazone Hcl and 50µg/ml for metformin Hcl. The repeatability was performed for six times. The result of precision was reported in table-3.

Linearity

Linearity was determination in the range of 50-150% (50, 75, 100, 125 and 150%) targeted concentration of assay procedure. 2-6ml of standard solution was made upto 10ml with mobile phase in separately flask. Five series of standard solution containing 0.21, 0.32, 0.412, 0.54 and 0.64 µg/ml of glimepiride, 1.65, 2.47, 3.30, 4.12 and 4.94 µg/ml of pioglitazone Hcl and 50.70, 76.05, 101.40, 126.75 and 152.10 µg/ml of metformin Hcl were injected. Linearity of each concentration and response of ratio of each concentration was found .Linearity of each concentration was reported in table-4.1-4.3 and graph 1-3.

Ruggedness

The above sample prepared solution and diluted to get the concentration of 2µg/ml of glimepiride, 15µg/ml of pioglitazone Hcl, and 50µg/ml of metfomin HCL of tablet sample. The 20µL was injected through column separately by two different analysis in the same HPLC system and same column. The result was reported in tablet-5.

Robustness

The above prepared was determined by the variation of flow rate and variation of wavelength. The result was reported in table-6.

Fig.1-Overlain UV spectra of 3 standards

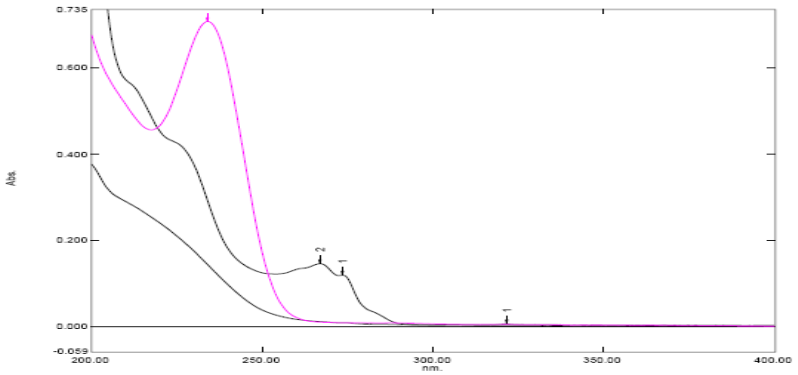
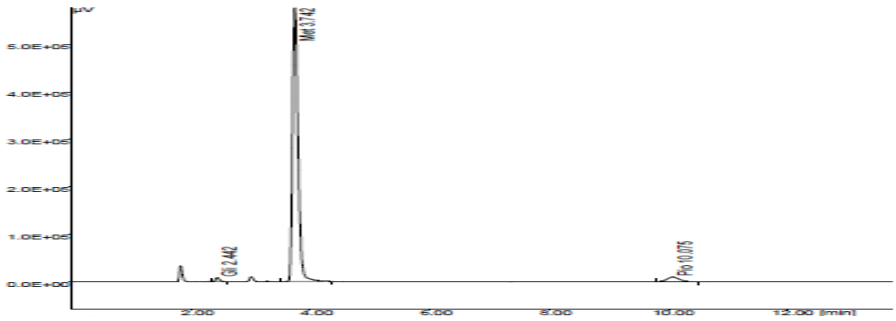
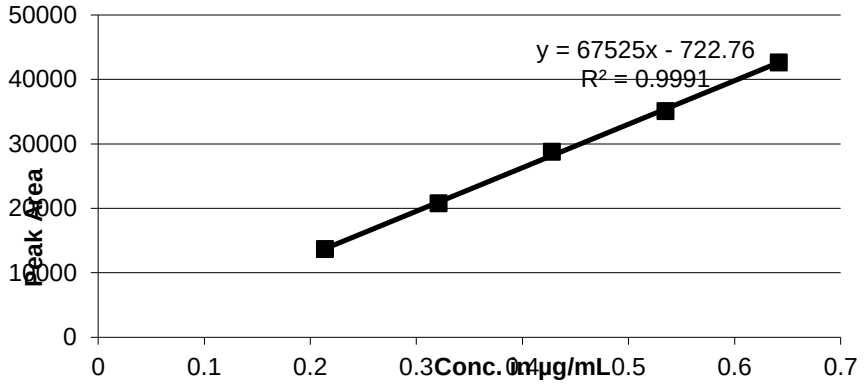


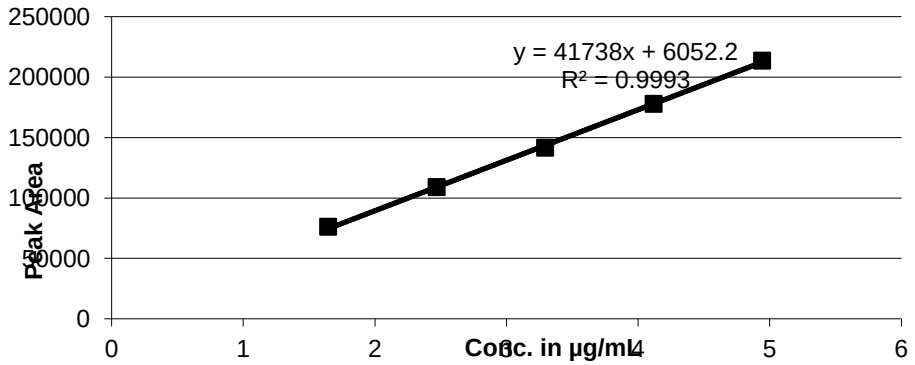
Fig-2- Assay chromatogram of GLI, MET and PIO



Graph 1- Linearity of standard glimepiride



Graph 2-Linearity of standard pioglitazone HCl



Graph 3- Linearity of standard metformin HCl

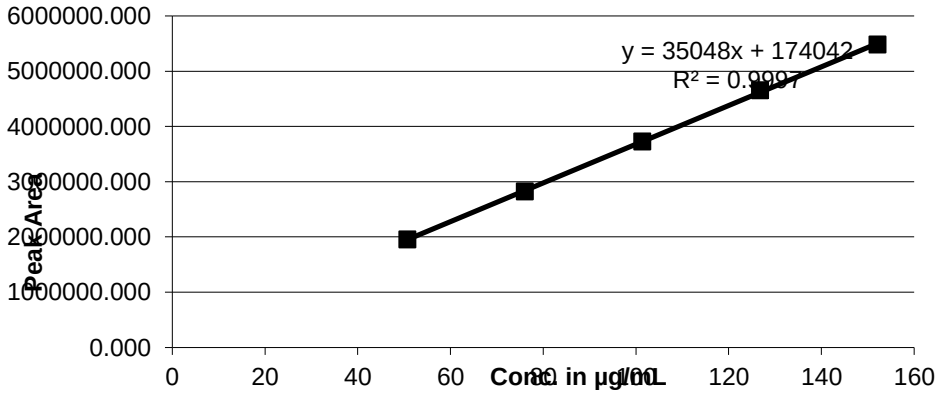


Table -1- Results of global assay

Parameters	Name of drug		
	GLI	MET	PIO
Mean of peak area	28462	3762411	157068
%RSD	0.3795	0.0132	1.0712
Amount in mg/tablet	1.966	494.336	14.746
Label claim	2	500	15
% Assay	98.45	98.863	98.20

Table -2- Results of global % recovery studies

Different levels	Name of drug	Amount taken in µg	Amount found in µg	%recovery	% of mean recovery
50	GLI	1	0.96	98.46	98.35
	MET	250	247.54	98.85	98.86
	PIO	7.5	7.23	98.62	98.63
100	GLI	2	1.96	98.58	98.58
	MET	500	494.42	98.97	98.93
	PIO	15	14.73	98.54	98.58
150	GLI	3	2.72	98.65	98.68
	MET	750	741.92	98.92	98.93
	PIO	22.5	21.87	98.65	98.59

Table -3- Results of precision studies of standard drugs

Parameters	Name of drug		
	GLI	MET	PIO
Mean of peak area	2853.52	376566.18	155874.26
Mean of retention time	2.448	3.714	9.95
SD	77.026	1836.42	3029.70
%RSD	0.2699	0.0487	1.64

Table 4.1- Results of linearity studies of standard glimepiride

Concentration in µg/mL	Standard peak area
0.21	15555.168
0.32	21467.320
0.43	28551.354
0.54	35238.494
0.64	43275.802

Table 4.2- Results of linearity studies of standard pioglitazone Hcl

Concentration in µg/mL	Standard peak area
1.65	71585.79
2.47	107404.81
3.30	156247.25
4.12	175537.78
4.94	212682.95

Table 4.3- Results of linearity studies of standard metformin Hcl

Concentration in µg/mL	Standard peak area
50.70	1951522.2
76.05	2818888.7
101.40	3765538.2
126.75	4650614.5
152.10	5442709.8

Table-5-Ruggedness data of analyst – I and II

Parameters	Analyst-I			Analyst-II		
	GLI	MET	PIO	GLI	MET	PIO
Mean of standard peak area	28405.15	3759668	155962.3	28599	3765489	157688
%RSD	0.710	0.13	0.127	0.354	0.401	1.353
Recovery Quantity	1.966	494.336	14.746	1.95	496.434	14.841
% Recovery	98.45	98.863	98.20	98.79	98.51	99.67

Table -6- Robustness study.

Variations	Retention Time in minutes			Standard peak area		
	GLI	MET	PIO	GLI	MET	PIO
Floe rate at 0.8ml\min	2.43	4.9	14.3	48299.26	4655856	1927303
Flow rate at 1.2ml\min	2.45	3.68	9.97	29650.1	3164563	134215.6
Wavelength at 215 nm	2.45	3.68	9.97	38552	3777252	152077
Wavelength at 219 nm	2.43	4.07	11.57	33245.67	3619664	154009

RESULTS AND DISCUSSION

The present study was aimed to developing an an accureate, precise and linear HPLC method for analysis of glimepiride, pioglitazone Hcl and metformin Hcl and in pharmaceutical dosage form as per ICH guidelines. It showed the linearity response over range 0.21-0.64µg/ml of glimepiride, 1.65-4.94µg/ml of pioglitazone Hcl and 50.70-152.10µg/ml of metformin Hcl. The correlation coefficient for three drugs was found to be 0.999, 0.9993 and 0.9997. The recovery studies of these three drugs were found to be 98.58, 98.30 and 98.87 respectively. The precision %RSD was found to be 0.0766 for glimepiride, 0.4102 for pioglitazone Hcl and 0.0612 for metformin Hcl. The ruggedness and robustness were studied with replicates standard solution of these drugs and the result was found to be acceptance criteria.

CONCLUSION

The proposed method gives good, resolution between glimepiride, pioglitazone Hcl and metformin Hcl within short time (11 minutes). The validation parameters are validated and the results

are complied with the ICH guidelines. The method is very simple, specific accurate, precise and linear for the determination of glimepiride, pioglitazone Hcl and metformin Hcl in combined dosage form. Therefore for the method can be used in routine quality control analysis of these drugs.

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