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Original Article

RP- HPLC method for Simultaneous Estimation of Pioglitazone Hydrochloride Metformin Hydrochloride and Glibenclamide in Multicomponent Tablet Dosage Form

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ABSTRACT

A simple, sensitive, specific and accurate reversed-phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of Pioglitazone HCl, Metformin HCl and Glibenclamide in combined tablet dosage form is developed and validated. Chromatographic separation was carried out using Agilent TC-C_{18} column (250 mm × 4.6 mm i.d., 5 µm particle size) with mobile phase consisting of acetonitrile:methanol:water (70:10:20 v/v/v) at a flow rate of 1 ml/min. Detection was carried out at 227 nm. The elution technique was based on isocratic mode. The method was validated in accordance with ICH guidelines. The retention time of Pioglitazone HCl, Metformin HCl and Glibenclamide were found to be 6.82 min, 2.42 min and 9.40 min, respectively. The developed method illustrated excellent linearity (R^2>0.99) in the concentration range of 5-30 µg/ml, 50-300 µg/ml and 2-10 µg/ml for Pioglitazone HCl, Metformin HCl and Glibenclamide, respectively. No chromatographic interference from the tablet excipients was found. The mean recoveries were found in the range of 98-102 % which shows accuracy of the method. The developed method was found to be accurate, precise, reproducible and specific and can be successfully applied for the quantitative estimation of these drugs in pharmaceutical formulations and routine analysis in quality control laboratories.

Keywords: Pioglitazone HCl (PIO), Metformin HCl (MET), and Glibenclamide (GLB), RP-HPLC, Validation.

1. INTRODUCTION

Type-2 diabetes mellitus is a disorder characterized by disrupted insulin production leading to high blood glucose levels. To control this disease, combination therapy is often used. Hypoglycemic agents such as pioglitazone HCl, metformin HCl and glibenclamide in

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combination are widely prescribed to control blood sugar levels. These drugs combination provide the basis for the development of a quantitative multicomponent analytical method development.

Pioglitazone hydrochloride (PIO) is chemically, (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2, 4-thiazolidinedione hydrochloride (Fig 1a), is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus. PIO decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Literature survey reveals that chromatographic and spectroscopic methods are reported for its determination as an individual drug and in combination with other drugs in pharmaceutical formulations and in biological fluids [1-15].

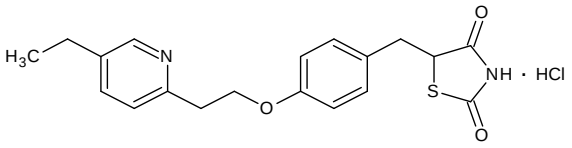

Fig. 1a

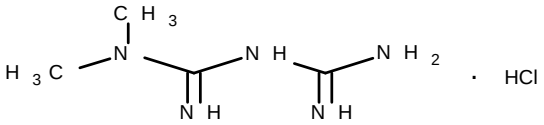

Fig. 1b

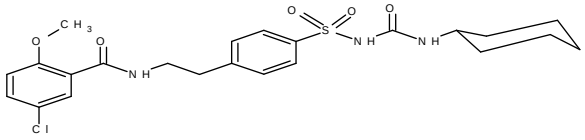

Fig. 1c

Fig 1: Chemical structure of analytes (a) Pioglitazone hydrochloride (b) Metformin hydrochloride (c) Glibenclamide

Metformin hydrochloride (MET) chemically, *N*,*N*-dimethyl-imidodicarbonimidic diamide hydrochloride (Fig 1b), is an antidiabetic agent from the biguanide class used in the management of type 2 diabetes. It does not cause insulin release from the pancreas and does not cause hypoglycemia, even in large dose. It decrease hepatic glucose production, decrease intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. It predominant effect is to decrease fasting plasma glucose. Some methods have been reported in the literature for the estimation of MET individually and in the presence of other drugs in formulations [16-27].

Glibenclamide (GLB), 5-chloro-*N*-[2-[4[[[(cyclohexylamino)carbonyl]-amino]sulphonyl]phenyl]ethyl]-2-methoxy benzamide (Fig 1c), is a potent, second generation oral sulfonylurea antidiabetic agent widely used to lower blood glucose levels in patients with type 2 diabetes mellitus. It acts mainly by inhibiting ATP-sensitive potassium channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, which cause voltage dependent calcium channels to open, which causes an increase in intracellular calcium in the beta cell, which stimulates insulin release. The literature survey reveals that few methods are reported for estimation of GLB [29-33].

For many patients with Type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control. The fixed dose combination of PIO, MET and GLB showed significant efficacy in improving the glycemic control in type 2 diabetics.

In the present investigation, an attempt has been made to develop simple, sensitive, specific and accurate RP-HPLC method for the simultaneous determination of PIO, MET and GLB in multicomponent tablet dosage form. The developed method was validated as per ICH and USP guidelines [34, 35].

2. EXPERIMENTAL WORK

2.1 Materials and Methods

PIO, MET and GLB, reference standards were obtained as a generous gift sample from USV Lab. Pvt. Ltd., Mumbai, India. Triglycomet tablets labeled to

contain PIO (15mg), MET (500mg) and GLB (5mg), manufactured by Tristar Formulations Pvt. Ltd., Mettupalayam, Puducherry, India, were purchased from local market. All the chemicals used were of HPLC grade, obtained from Merck Co, Mumbai, India. All HPLC solvents and solutions were filtered through Nylon membrane filter of 0.45µ and 0.2µ pore size.

2.2 Instrumentation and optimization of chromatographic conditions

The HPLC analysis was carried out on Agilent 1120 Compact LC system composed of binary pump, manual injector, UV detector and Ezchrome EliteCompact software. Chromatographic separation was performed on Agilent TC-C18 (250 mm×4.6 mm i.d., 5µm partical size) and the mobile phase consisted of acetonitrile: methanol: water (70:10:20 v/v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 227 nm. The injection volume was 20 µl; analysis was performed at ambient temperature.

2.3 Preparation of standard solution

An accurately weighed quantity 10 mg each of PIO, MET and GLB were transferred separately to 100 ml volumetric flask, dissolved in methanol and diluted up to the mark with same solvent to obtained standard stock solution 100 µg/ml of each drug.

2.4 Preparation of Calibration Curve

The series of standard solutions were prepared by dilution of aliquots of the standard stock solution with methanol to get concentration in the range of 5-30 µg/ml for PIO and 50-300 µg/ml for MET and 2-10 µg/ml for GLB, respectively. Twenty microlitre of the each standard solution was injected to HPLC system. The peak areas were plotted against the corresponding concentrations to obtain the calibration graph.

2.5 Study of system suitability parameters

The system suitability is used to verify whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions. A 20 µl standard drug solution was injected separately and system suitability parameters were recorded.

2.6 Analysis of tablet formulation

Twenty tablets were weighed and their mean weight was determined. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 100 mg of MET was transferred to 100 ml volumetric flask and dissolved in sufficient quantity of methanol. The mixture was sonicated for 15 min. The volume was made up to the mark with same solvent. The solution was filtered and aliquots of the filtrate was diluted with methanol to get final concentration 7.5 µg/mL, 250 µg/mL and 2.5 µg/mLof PIO, MET and GLB, respectively. Twenty micro liters of the test and standard solutions were injected separately after the equilibration of mobile phase with stationary phase. The chromatograms were recorded up to 10 min and area of each peak was noted.

2.7 Method Validation

The optimized RP-HPLC method was completely validated according to the procedure described in ICH guidelines and United State Pharmacopoeia for validation of analytical methods. The performance parameters evaluated for the method were linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and ruggedness.

2.7.1 Linearity: Linearity was studied by diluting standard stock solution at six different concentrations (n=3) covering the range of 5-30 µg/ml, 10-300 µg/ml and 1-10 µg/ml, for PIO, MET and GLB, respectively. A graph was plotted for the concentration of the corresponding drug versus peak area. The correlation coefficient (r^2) for each drug was calculated.

2.7.2 Precision: Repeatability study was carried out by analyzing sample solution six times, at 100% of test concentration within the same day using proposed

method. Similarly, the intra and inter day precision was evaluated by analyzing tablet sample on the same day and on different days at different time interval, respectively. The contents of drugs and the % relative standard deviation (% R.S.D.) value were calculated.

2.7.3 Accuracy: To check the accuracy of the developed method and to study interference of formulation additives, analytical recovery studies was carried out by the standard addition method. Pure drug standard solution was added to tablet samples at three different concentrations level. At each level, samples were prepared in triplicate and the mean percentage recovery and R.S.D. value were determined.

2.7.4 Detection and quantitation limits: Series of diluted standard solutions were prepared and analyzed by both methods. The limit of detection (LOD) and limit of quantitaton (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$$LOD = \frac{3.3\,\delta}{S} \quad \text{----- (1)} \qquad LOQ = \frac{10\,\delta}{S} \quad \text{--- (2)}$$

Where, δ: standard of y-intercept and S: slope of calibration curve.

2.7.5 Specificity: A sample solution of tablet was prepared in the test concentration range and injected into the chromatograph, to evaluate possible interfering peaks. This parameter was performed to know the retention time of each drug in a mixture and in the sample to understand if any drug-drug interaction or drug-excipient interaction is present.

2.7.6 Ruggedness: To test the ruggedness of the method, the analysis was done on different time intervals, days and different analysts to check for any changes in the chromatogram. The % R.S.D. was determined.

3. RESULTS & DISCUSSION

3.1 Method development and optimization

Preliminary tests were performed to select adequate optimum conditions. The parameters such as detection wavelength, ideal mobile phase and their proportions, flow rate and concentration of the standard solutions were studied. After several permutation and combination, it was found that mixture of methanol: acetonitrile: water gave sharp, well resolved peaks with symmetry within the limits and significant reproducibility as compared to other mobile phases.

The chromatographic separation was carried out using C_{18} column and a mobile phase composed of methanol: acetonitrile: water (70:10:20 v/v/v) at a flow rate of 1.0 ml/min. The eluent was monitor at 227 nm. An adequate peak symmetry and short run time was achieved as demonstrated in the chromatogram Figure 2. The retention time of PIO, MET and GLB were found to be 6.82 min, 2.42 min and 9.40 min, respectively. The system suitability parameters are shown in Table 1.

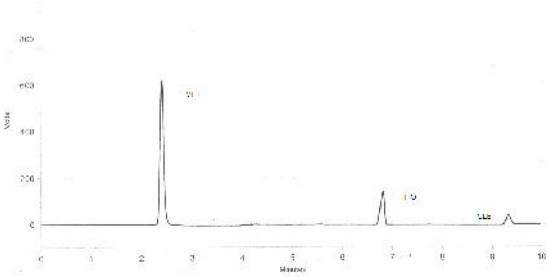

Fig 2: Chromatogram of PIO, MET and GLB

Table 1: System suitability parameters

Parameters*	PIO	MET	GLB
Retention time (min)	6.82	2.42	9.40
Asymmetric factor	0.92	0.89	1.26
No. of theoretical plate	5532	7149	4203
Capacity factor	2.01	1.69	1.32
Resolution	6.03	3.94	-

***Average of five determinations.**

3.2 Method validation

A linear relationship was found between the concentration and peak area (Fig 3, 4 and 5). The

correlation coefficient values (r^2) obtained was higher than 0.99 which attest the linearity of the method. The results are shown in Table 2. The precision data obtained for the evaluated method are demonstrated in Table 3. Mean contents of PIO, MET and GLB in precision analysis (n=6) were closed to labeled claim of respective drugs.

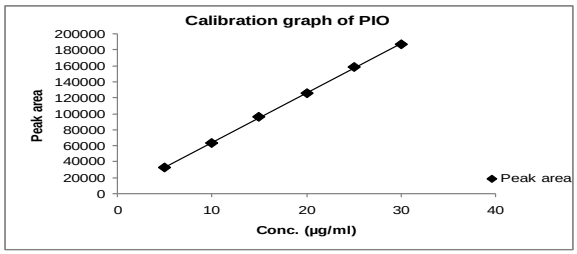

Fig 3: Linearity graph of PIO

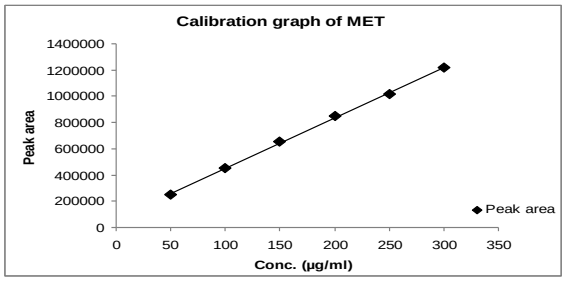

Fig 4: Linearity graph of MET

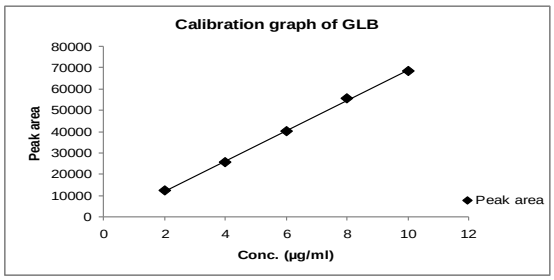

Fig 5: Linearity graph of GLB

Table 2: Regression analysis data

Regression parameters	PIO	MET	GLB
Concentration range (µg/ml)	5-30	50-300	2-10
Correlation coefficient (r^2)	0.9989	0.9990	0.9994

Table 3: Results of analysis of tablet formulation

Drug	Labeled claim mg/tablet	Mean (%) (n=6)	±S.D.	%R.S.D
PIO	15	99.62	0.6738	0.6763
MET	500	99.41	0.4341	0.4367
GLB	5	99.35	0.4952	0.4984

Accuracy was investigated by means of recovery studies using the proposed method. The percent recoveries after spiking with additional standard drug afford recovery in the range of 98-102% and the results are listed in Table 4. The LOD and LOQ were found to be 0.09 µg/ml and 0.16 µg/mL for PIO, 0.46 µg/ml and 1.2 µg/ml for MET and 0.32 µg/ml and 0.91 µg/ml for GLB, respectively. The % R.S.D. value for each parameter reported was found to be less than 2% which shows ruggedness of the RP-HPLC method. The results of ruggedness studies are presented in Table 5. The chromatogram obtained with the tablet sample solution with excipients shows no interfering peaks in the retention time of drugs.

Table 4: Results of recovery studies

Drug	Level (%)	% Recovery*	±SD
PIO	80	98.76	0.3356
	100	99.46	0.7325
	120	99.31	0.5632
MET	80	100.12	0.4421
	100	99.53	0.6228
	120	99.24	0.3692
GLB	80	98.88	0.8537
	100	99.09	0.5539
	120	99.32	0.3911

*Mean of three determinations

Table 5: Results of ruggedness parameters

Drug	Parameters		Intraday	Interday	Different analysts
PIO	(%) (n=6)	Mean	99.45	99.22	99.36
	%R.S.D		0.5562	0.6378	0.7640
MET	(%) (n=6)	Mean	99.63	99.45	100.23
	%R.S.D		0.7268	0.3978	0.5035
GLB	(%) (n=6)	Mean	99.11	98.87	98.66
	%R.S.D		0.3671	0.4585	0.3673

3.3 Analysis of tablet formulation

The proposed validated method was successfully applied for determination of PIO, MET and GLB in their combined dosage form. The results of analysis of pharmaceutical dosage form by the proposed method

(Table 1), expressed as percentage of labeled claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets.

4. CONCLUSION

The results of the analysis of pharmaceutical dosage forms by the proposed RP-HPLC method are highly reproducible, reliable and are in good agreement with the labeled claim of the drugs. The mobile phase is easy to prepare and the drugs are eluted within short run time. The results of recovery studies show that the method is free from interference of the excipients used in the formulation. The proposed RP-HPLC method is found to be simple, sensitive, accurate, precise, specific and economical and can be used for the routine simultaneous estimation of PIO, MET and GLB in pharmaceutical formulations.

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