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Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride in their Bulk and Formulation Dosage fForms by using RPHPLC Method

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ABSTRACT

A simple rapid, economically accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of Metformin HCl (MET) and Pioglitazone (PIO) in pure and in pharmaceutical dosage forms. A Hypersil BDS C18 column (250x4.6mm, 5µ) was used with a mobile phase containing a mixture of Acetonitrile and Sodium dihydrogen phosphate buffer in the ratio of 60: 40(v/v). The flow rate was 1 ml/min and effluents were monitored at 228 nm and eluted at 2.303 min (MET) and 3.850 min (PIO). Calibration curve was plotted with a range from 20-120 µg/ml for MET and 0.6 - 3.6 µg/ml for PIO. The assay was validated for the parameters like accuracy, precision and system suitability parameters. The proposed method can be useful in the routine analysis for the determination on metformin and pioglitazone in pharmaceutical dosage forms.

Key words: RPHPLC Validation, LOD, LOQ,
Accuracy and Precision Metformin HCl, Pioglitazone HCl.

INTRODUCTION

Analytical chemistry is basically concerned with the determination of the chemical composition of matter however, identification of substance, the elucidation of its structure and quantitative analysis of its composition are the aspects covered by modern analytical techniques[1]. The qualitative and quantitative analysis can be done by various analytical methods: various analytical techniques can be revised and some of them give accurate result, example, Chromatography by HPLC method[2].

Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system[3]. Reverse phase chromatography(RPC), the most widely used chromatographic mode[4] is used to separate neutral molecules in solution on the basis of their

hydrophobicity[5] which involves the use of a non-polar stationary phase and a polar mobile phase. RPC is usually a first choice for the separation of both neutral and ionic samples, using a column packing that contains a less polar bonded phase such as C8 or C18. The most common stationary phases in RPC are those in which a functional group is chemically attached to a silica support (bonded phases). The most popular bonded phases are the alkyl groups, such as -CH3,-C4H9,-C8H17, and -C18H37, phenyl (-C6H5) groups, cyano [(-CH2) 3CN] groups, and amino [(-CH2)3NH2] groups, with retention increasing exponentially with chain length. The researcher used RP - HPLC method to analyze of Metformin HCl and Pioglitazone HCl in bulk & their pharmaceutical dosage forms. Metformin (I, N, N-dimethyldiguanide) and Pioglitazone, (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione [6] (Illustration 2) are used in the treatment of type 2 diabetes.

The literature reveals that there are some of the methods have been reported for metformin UV [6,7] HPLC[8] stability studies[9] and potentiometer, spectrofluorimetry[10]. For pioglitazone HPLC method in pharmaceutical dosage forms[11] determination of its metabolites in human plasma[12,13] and simultaneous determination of metformin and pioglitazone[14-16] in pharmaceutical dosage forms. The present paper describes a simple, sensitive, validated and economic method, precise, and accurate for the determination of metformin and pioglitazone and can be used for routine analysis of tablets. It was validated as per ICH norm[17-19].

EXPERIMENTAL

Materials and Methods

Pharmaceutical grade working standards and all chemicals and reagents were obtained from Chandra Laboratories (a Govt.Aproved analytical testing laboratory), Hyderabad, and India.

Instrumentation

The LC system consisted of a pump (model prominence, make Shimadzu, Japan) with manual injecting facility (programmed at 20µl capacity per injection was used. The detector consisted of a UV–VIS (thermo UV 1575) model operated at a wavelength of 228 nm (determined by UV – spectrophotometry). The software used was Spin chrome system. The column used was Hypersil BDS C18 (250mm×4.6 mm, 5.0µm) THERMO Technologies Corporation. Different mobile phases were tested in order to find the best conditions for separation of MET and PIO. The mobile phase contained Acetonitrile, 0.01M Sodium dihydrogen phosphate (60:40) and the flow rate was maintained at 1 ml/min UV detection was carried out at 228 nm. The mobile phase and samples was filtered using 0.45µm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

RESULTS AND DISCUSSION

Standard solutions preparation

100 mg MET and 3mg PIO were weighed accurately and transferred to 50 ml volumetric flasks. All the drugs were dissolved in Mobile phase to prepare 2000 µg/ml of MET and 60 µg/ml of PIO standard stock solutions. Calibration standards at six levels were prepared from this standard stock solution (the concentrations were 20 . 40 . 60 . 80 . 100 . 120 µg/ ml for MET (the range was 20 – 120 µg /ml) and 0.6 , 1.2 , 1.8 , 2.4 , 3 , 3.6 µg /ml PIO (the range was 0.6 – 3.6 µg /ml) and peak areas

Table 1: Linearity for MET

S. No	Conc.	% Peak Area
1	20	824.368
2	40	1519.58
3	60	2247.839
4	80	2967.824
5	100	3576.653
6	120	4399.867

Table 2: Linearity for PIO

S. No	Conc.	% Peak Area
1	0.6	147.746
2	1.2	267.519
3	1.8	410.263
4	2.4	546.377
5	3.0	665.715
6	3.6	789.841

Table 3: Precision studies, each values mentioned above are mean of five replicates

Compound	Conc.	AVG		ST. DV		%RSD	
	(µg/ml)	RT	AREA	RT	AREA	RT	AREA
MET	100	2.3046	3333.304	0.003912	25.5117	0.169727	0.765358
PIO	3	3.7788	608.8152	0.032599	6.003634	0.862683	0.986118

Table 4: Precision studies, each value mentioned above are mean of five replicates

Extract	Retention time, TR (min)		Resolution (Rs)		Asymmetry (As)	
Injections	MET	PIO	MET	PIO	MET	PIO
0.1 M HCl	2.313	3.663	0	7.009	1.625	1.577
0.1 M NaOH	2.347	3.247	1	3.783	1.750	1.600
Heat	2.317	3.780	0	7.948	1.762	1.538

Table 5: Statistical analysis of parameters required for system suitability

Parameters	MET	PIO
Theoretical plates	2948	5703
Resolution	0	8.256
LOD, (ìg/ml)	2.38	0.09
LOQ , (ìg/ml)	7.282	0.28
Peak asymmetry	1.714	1.538
% R.S.D	0.765	0.986

were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample Preparation

For the analysis of a tablet dosage form, 10 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder amount equivalent to 100 mg of Mt and 3mg of Pg were transferred to a 50 ml volumetric flask and dissolved in 50 ml of mobile phase, sonication was

Table 6: Recovery Studies

sample	Standard area	Sample area	Standard Wt. mg	Sample Wt. mg	Label claim mg	Average Wt. mg	Standard purity%	Assay %
MET	3359.15	3421.652	100.3	210.5	500	1024.5	99.8	99.24937
PIO	615.1717	619.737	3.05	210.5	15	1024.5	99.8	99.49702

Table 7: Recovery studies for 3 replicates

Compound	Label claim	Amount added	Total amount added	Amount recovered µg	% recovery
MET	80	10	90	89.614	99.57
	100	10	110	109.7332	99.757
	120	10	130	130.92	100.70
PIO	2.4	0.3	2.7	2.697	99.90
	3.0	0.3	3.3	3.02	99.819
	3.6	0.3	3.9	3.898	99.96

Table 8: Summary of validation parameters

Parameter	MET	PIO
Linearity range (µg/ml)	20-120	0.6-3.6
Correlation coefficient r2	0.9991	0.9992
Accuracy / RECOVERY	100	99.89
Limit of detection (µg/ml)	2.38	0.O9
Limit of quantization (µg/ml)	7.282	0.28
Precision (% R.S.D.)	0.765	0.986

done for 10 min with swirling. After sonication, the solution was filtered through a Watt man filter paper. Before the assay of tablet formulations, 6 replicate aliquots (Each 20 ml in volume) of the appropriately diluted tablet stock solution were sonicated for 8 min, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times. The possibility of excipient interference with the analysis was examined.

Optimization of HPLC Method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for MET and PIO respectively. The mixed standard stock solution injected in HPLC. Different solvents (four trials) were tried.

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis of Mt and Pg in fixed dose combined dosage form. Initially Acetonitrile and water in different ratios were tried, but unacceptable retention times and no asymmetry in beaks ., so water was replaced by potassium dehydrogenate buffer (0.01 M), ammonium dihydrogen phosphate (0.01 M) and by sodium dihydrogen phosphate (0.01 M) in different ratios were tried (illustration 2-5) . It was found that Acetonitrile : sodium dihydrogen phosphate buffer in ratio of 60: 40(v/v) gave acceptable retention time , (RT 2.280 min for MET and RT 3.850 min for PIO), plates, and good

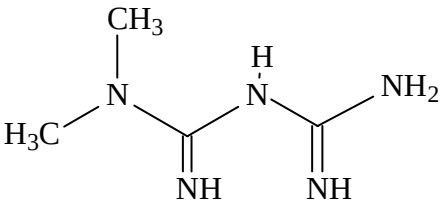

Fig. 1: Structure of Metformin. HCl (MET)

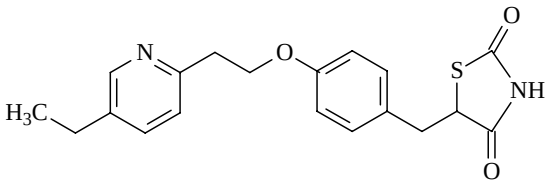

Fig. 2: Structure of Pioglitazone.Hcl (PIO)

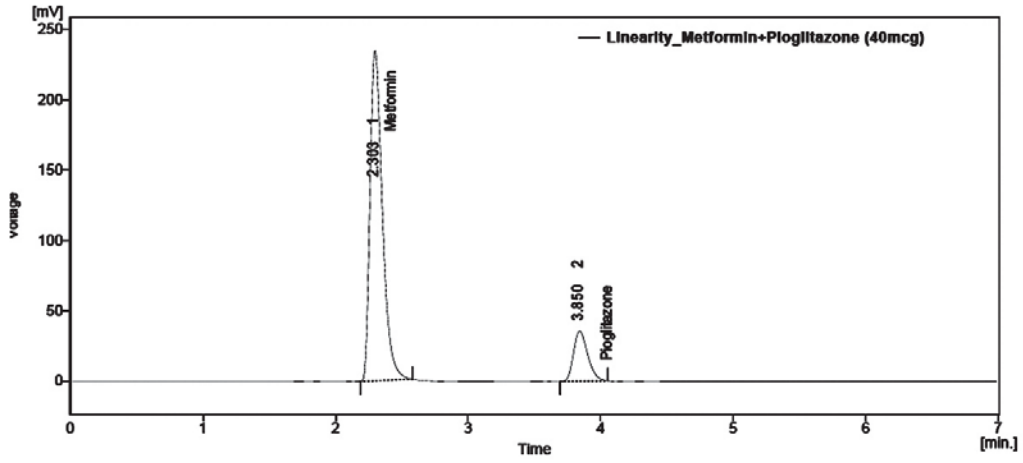

Fig. 3: HPLC chromatogram for MET and PIO

resolution for MET and PIO at the flow rate of 1 ml/ min).

Linearity

Linearity was evaluated by analysis of working standard solutions of MET and PIO of six different concentrations. The range of linearity was from 20 -120 µg/ml for MET and 0.6 -3.6 µg/ ml for PIO.

The regression data obtained are represented in table -1. The results show that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration of each drug as shown as in Fig. 1.

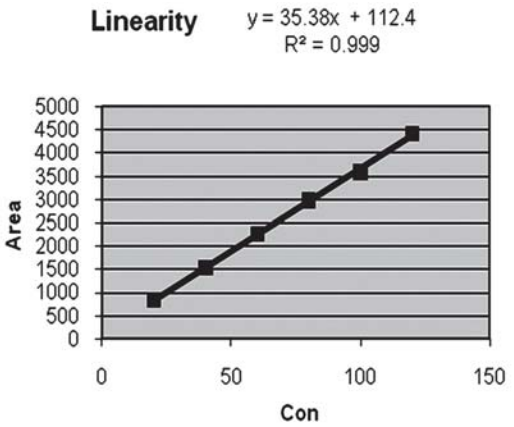

Fig. 4: Linearity graph for MET

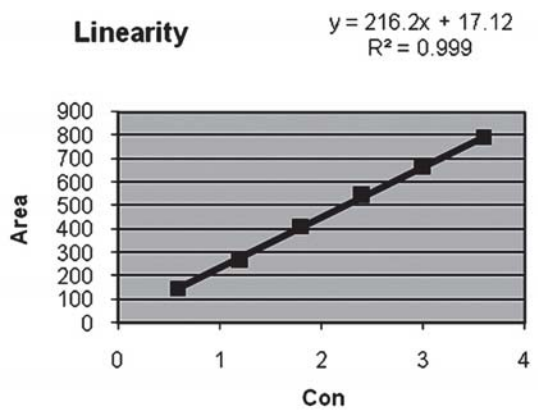

Fig. 5: Linearity graph for PIO

Specificity

Injections of the extracted commonly used excipients were performed to demonstrate the absence of interference with the elution of the drugs. These results demonstrate that there was no interference from other materials in the tablet formulation; therefore, confirm the specificity of the method (Table-4).

System suitability

System suitability parameters such as the number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table-5

Recovery studies

Good recoveries of the MET and PIO were obtained at various added concentrations for the tablets as shown in table-6 and 7

Precision

The results of the repeat of experiments in the same conditions to several times (5 times) are shown in Table-3. The developed method was found to be precise, with RSD values for repeatability and intermediate precision <2%, as recommended by ICH guidelines. Separation of the drugs was found to be similar when analysis was performed on different times LOD and LOQ

The LOD and LOQ values were found to be 2.38 and 7.282 µg/ml for MET, and 0.09 and 0.28. µg/ml for PIO.

CONCLUSION

The new HPLC method described in this paper provides a simple, convenient and reproducible approach for the simultaneous identification and quantification that can be used to determine metformin hydrochloride, pioglitazone hydrochloride in routine quality control.

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