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Analytical method development and method validation of metformin hydrochloride and pioglitazone hydrochloride in bulk and their pharmaceutical dosage forms by RP-HPLC

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ABSTRACT

A simple, sensitive, rapid, low cost, accurate and precise reverse phase high performance liquid chromatographic method was developed for the estimation of Metformin HCl (Mt) and Pioglitazone HCl (Pg) 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 (C_2H_3N,41.05 g mol^{-1}, 99.8 - 100%) and 0.01M Sodium dihydrogen phosphate solution (NaH_2PO_4, 119.98 g mol^{-1}) in the ratio of 60 : 40(v/v) respectively. The flow rate was 1 ml/min and effluents were monitored at 228 nm and eluted at 2.280 min (Mt) and 3.960 min (Pg). Calibration curve was plotted with a range from 20-120 µg/ml for Mt and 0.6 - 3.6 µg/ml for Pt. 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.

KEYWORDS

High-performance liquid chromatography (HPLC);
Reversed phase;
Mobile phase.

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,

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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 was used RP - HPLC method to analyze of Metformin HCl and Pioglitazone HCl in bulk and their pharmaceutical dosage forms. Metformin (I, N, N-dimethyldiguanide) (Figure 1) and Pioglitazone, (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione[6] (Figure 2) are used in the treatment of type 2 diabetes.

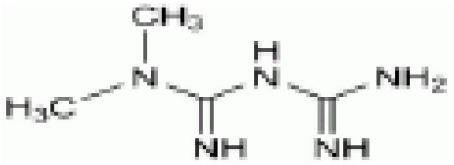

Figure 1 : Structure of Mt. HCl

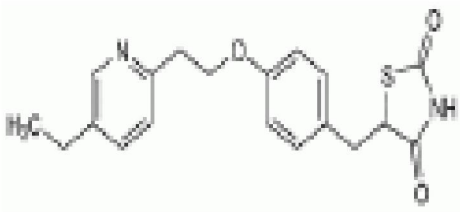

Figure 2 : Structure of Pg. HCl

The literature reveals that there are some of the methods have been reported for metformin UV[6, 7], HPLC[8] stability studies[9] and potentiometry, 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 Mt (batch No. WE 220 .), Pg (batch No. WVX 103) and all chemicals and reagents were obtained from Chandra Laboratories, Hyderabad, India.

Instrumentation

The HPLC system consisted of a pump (model Prominent, make Shimadzu Corporation, Japan) with manual injecting facility (Rheodyne Injector Model) programmed at 20 µl capacity per injection was used. The detector consisted of a UV–vis (thermo UV) model operated at a wavelength of 228 nm(was determined by uv – spectrophotometry). The column used was Hipersil BDS C18 (250mm×4.6 mm, 5.0 µm). Different mobile phases were tested in order to find the best conditions for separation of Mt and Pg compounds . The mobile phase contained Acetonitrile, 0.01 M Sodium dihydrogen phosphate solution (60:40) and the flow rate was maintained at 1 ml min-1 UV detection was carried out at 228 nm. The mobile phase and samples was filtered using 0.45 µm membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature.

Standard solutions and calibration graphs for chromatographic measurement

100 mg Mt and 3mg Pg were weighed accurately and transferred to 50 ml volumetric flasks. All the drugs were dissolved in HPLC-grade solvent (mobile phase) to prepare (2000 µg ml-1 of Mt and 60 µg ml-1 of Pg) 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-1 for Mt and 0.6, 1.2, 1.8, 2.4, 3, 3.6 µg ml-1 Pg and peak areas 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 500 mg of Mt and 15 mg of Pg were transferred to a 50 ml volumetric flask and dissolved in 50 ml of mobile phase, sonication was done for 10 min with swirling. After sonication, the solution was filtered through a Whatman filter paper . Before the assay of tablet formulations, 3 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 3 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 Mt and Pg respectively. The mixed standard stock solution injected in HPLC. Different solvents (four trials) were tried.

Method validation

The method was validated according to the ICH guidelines[19]. The following validation characteristics were addressed: linearity, accuracy, precision, and specificity, limits of detection and quantitation and system suitability.

Linearity and range

Linearity of the method was studied by injecting the mixed standard solutions in the concentration range of 20 -120 µg ml-1 for Mt and 0.6 -3.6 µg ml-1 Pg injected six times into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the proposed method was evaluated by carrying out five independent assays of test sample(with 100 µg ml-1 Mt and 3 µg ml-1 Pg . RSD (%) of five assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different times.

Limit of detection and quantification (LOD,LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) for the procedure were performed on samples containing very low concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the minimum level at which the analyte can be reliably detected. LOQ was considered as the lowest concentration of analytes in standards that can be reproducibly measured with acceptable accuracy and precision.

System suitability

The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between Mt and Pg peaks were defined.

Specificity

Injections of the extracted placebo were performed to demonstrate the absence of interference with the elution of the Mt and Pg. For determining selectivity of the method, a 1 ml 0.1M NaOH (basic medium) added to 0.5 ml stock solution and make up to 10 ml with mobile phase and elution the drugs, return this procedure with 1 ml 0.1 M HCl (acidic medium) and third time by heating 0.5 ml stock solution and make up to 10 ml with mobile phase (thermal medium).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Mt and Pg combination tablets) to which known amounts of Mt and Pg standard powder corresponding to 80, 100 and 120 $\mu g\ ml^{-1}$ for Mt and 2.4, 3.0, 3.6 $\mu g\ ml^{-1}$ of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatograms in optimized mobile phase. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery was calculated.

Analysis of marketed formulation

The marketed formulation was assayed as described above. The peak areas were measured at 228 nm, and concentrations in the samples were determined using multilevel calibration developed on the same LC system under the same conditions using linear regression analyzed for Mt and Pg in the same way as described earlier.

RESULTS AND DISCUSSION

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 dihydrogen buffer (0.01 M), ammonium dihydrogen phosphate solution (0.01 M) and sodium dihydrogen phosphate solution (0.01 M) respectively in different ratios were tried. It was found that acetonitrile : sodium dihydrogen phosphate solution in ratio of 60: 40(v/v) gave acceptable

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retention time (RT 2.280 min for Mt and RT 3.960 min for Pg), plates, and good resolution for Mt and Pg at the flow rate of 1 ml/min)

Validation

Linearity

Linearity was evaluated by analysis of working standard solutions of Mt and Pg of six different concentrations. The range of linearity was from 20 -120 µg/ml for Mt and 0.6 -3.6 µg/ ml Pg. The regression data obtained are represented in TABLE 1. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration of each drug.

TABLE 1 : Linear regression data for the calibration curves, n=6; R2=coefficient of correlation (see Vogel's textbook of quantitative chemical analysis, 6th edition for formulation of R)

Compound	Y = bx + a		R^2
	a	b	
MT	112.49	35.384	0.9991
PG	17.126	216.25	0.9992

Precision

The results of the repeat of experiments in the same conditions to several times (5 times) are shown in TABLE 2. 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 Mt, and 0.09 and 0.28 . µg/ml for Pg.

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 3).

TABLE 3 : Specificity studies

Extra injection	Retention time,TR (min)		Resolution (Rs)		Asymmetry (As)	
	Mt	Pg	Mt	Pg	Mt	Pg
0.1 M HCl	2.313	3.663	-	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	-	7.948	1.762	1.538

TABLE 4 : Statistical analysis of parameters required for system suitability testing of the proposed HPLC method

Parameters	Mt	Pg
Theoretical plates	2948	5703
Resolution	-	8.256
LOD, (µg/ml)	2.38	0.09
LOQ, (µg/ml)	7.282	0.28
Peak asymmetry	1.714	1.538
% R.S.D (area)	0.765	0.986

TABLE 5 : Recovery studies

Compound	Label claim	Amount added	Total amount added	Amount recovered mcg	% Recovery
Mt	80	10	90	89.61447	99.57163
	100	10	110	109.7332	99.75743
	120	10	130	130.9213	100.7087
Pg	2.4	0.3	2.7	2.697388	99.90324
	3.0	0.3	3.3	3.330896	100.9362
	3.6	0.3	3.9	3.898629	99.96486

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 (4)

Recovery studies

Good recoveries of the Mt and Pg were obtained at various added concentrations for the tablets as shown in TABLE (5)

TABLE 2 : Precision studies of HPLC method (five times repeated)

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

TABLE 6 : Applicability of the HPLC method for the analysis of the pharmaceutical formulations

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

TABLE 7 : Summary of validation parameters

Parameter	Mt	Pg
Linearity range (µg/ml)	20 - 120	0.6 – 3.6
Correlation coefficient r2	0.9991	0.9992
Accuracy	100	99.89
Limit of detection (µg/ml)	2.38	0.09
Limit of quantitation (µg/ml)	7.282	0.28
Precision (% R.S.D.)	0.765	0.986

Analysis of a commercial formulation

Experimental results of the amount of Mt and Pg in tablets, expressed as a percentage of label claims 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. Fixed dose combination tablets were analyzed using the proposed procedures TABLE (6).

DEVELOPMENT

Validation; Metformin HCl; Pioglitazonee HCl

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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