RP-HPLC method for simultaneous estimation of Metformin HCl and Pioglitazone in tablet dosage form

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Abstract

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Metformin HCl and Pioglitazone in tablet formulation. Chromatography was performed on a Zorbax Eclipse C18 (5 cm×2.1 mm I.D) column from agilent in isocratic mode with mobile phase containing acetonitrile: buffer pH 3.0 (20:80). The flow rate was 0.5 ml/min and the eluent was monitored at 218 nm. The selected chromatographic conditions were found to effectively separate Metformin HCl (RT- 0.928 min) and Pioglitazone (RT- 5.81 min). Linearity for Metformin HCl and Pioglitazone was found in the range of 800-1200 µg/ml and 24-36 µg/ml respectively. The proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in tablet formulation.

Keywords: Metformin HCl, Pioglitazone, Estimation, RP-HPLC Method, Method Validation.

Introduction

Metformin[1,2] is an oral antidiabetic drug of the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Its use in gestational diabetes has been limited by safety concerns. It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor. Metformin works by suppressing glucose production by the liver. Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It helps reduce LDL cholesterol and triglyceride levels and is not associated with weight gain.

As of 2010, metformin is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide). Pioglitazone[1,3] is used for the treatment of diabetes mellitus type 2. Pioglitazone selectively stimulates nuclear receptor peroxisone proliferator-activated receptor gamma (PPAR-gamma). It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the lipidic, muscular tissues and in the liver. Various methods like HPLC, HPTLC, UV spectroscopy and Mass spectroscopy were reported for the determination of Metformin HCl and Pioglitazone alone and/ or in combination[4-18]. But no simultaneous determination on fast LC method is available for this combination. Moreover, small amount of mobile phase is required thus reducing analysis time and cost.

Material and Methods

Analytical pure samples of Metformin HCl and Pioglitazone were obtained as gift samples from reputed pharma company for proposed study. The pharmaceutical dosage form used in this study was Cetapin®-P (Metformin HCl SR 500 mg and Pioglitazone 15 mg Tablet) procured from the local market and labeled to contain 15 mg of Pioglitazone and 500 mg of Metformin HCl per tablet. The solvents used in the study were of Merck HPLC grade and chemicals used in the study were of Merck AR grade.

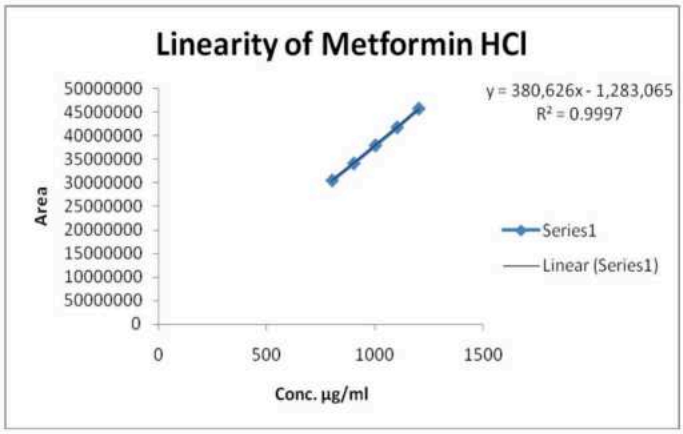
The chromatographic separation was carried out on Agilent technologies 1200 RRLC system consisting of pump, Column oven, Autosampler, detector and acquisition software EZ chrom elite version 3.1.2 (Merck KGaA, Darmstadt, Germany).

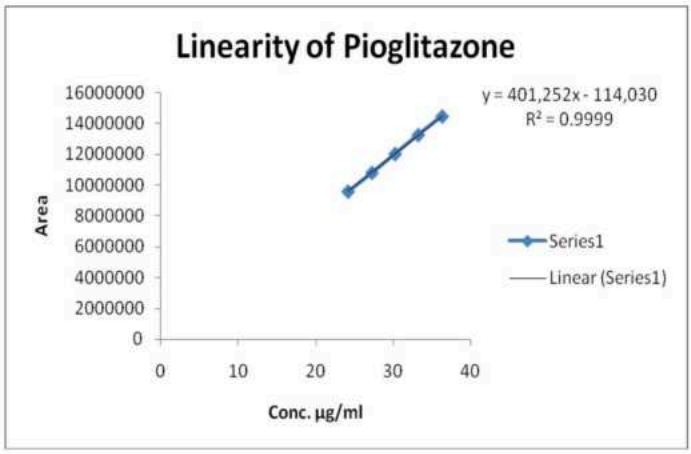
Preparation of Mobile Phase and Stock Solutions: 1ml of triethylamine was mixed in 1000 ml of water and pH of mixture was adjusted to 3.0 with *o*-phosphoric acid. Mobile phase consists of 80% of buffer pH 3.0 and 20% Acetonitrile was prepared. This mixture was sonicated for 10 min and filtered through 0.2 µm membrane filter and used as mobile phase. Stock solutions were prepared by weighing 200 mg of Metformin HCl and 30 mg of Pioglitazone. The weighed drugs were transferred to two volumetric flask 20 ml and 100 ml respectively. Volumes were made up to the mark with methanol to obtain a solution containing 10000 µg/ml of Metformin HCl and 300µg/ml of Pioglitazone.

The solutions were further diluted with the same solvent to obtain final concentrations of 1000 µg/ml of Metformin HCl and 30µg/ml of Pioglitazone drug. The HPLC analysis was performed on reversed-phase fast liquid chromatographic system with isocratic elution mode using a mobile phase of Buffer pH 3.0: Acetonitrile (80:20, v/v) on Zorbax Eclipse XDB-C18 column (50×2.1 mm, 1.8 µm particle size) with 0.5 ml/min flow rate at 218 nm using UV detector.

Calibration curves for Metformin HCl and Pioglitazone: Marketed formulation contains Metformin HCl and Pioglitazone in a ratio of 33:1. Appropriate aliquots of Metformin HCl and Pioglitazone stock solutions

were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 800-1200 µg/ml and 24-36 µg/ml of Metformin HCl and Pioglitazone respectively. The solutions were injected using a 5 µl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak areas versus concentrations and regression equations were computed for both the drugs (Table 1).





Analysis of Marketed Formulations: About 5 intact tablets were weighed and crushed to fine powder. 1050 mg of powder containing 500 mg of Metformin HCl and 15 mg of Pioglitazone was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 minutes, cooled the flask to room temperature and diluted up to the mark with same solvent. Further dilute 5ml of this solution to 25ml with same solvent to get final concentrations of 1000 µg/ml and 30µg/ml of Metformin HCl and Pioglitazone respectively. The above solution was filtered using 0.22µ syringe filter. A 5 µl volume of above sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions.

Method Validation: The method of analysis was validated as per the recommendations of ICH and USP for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness[19].

The accuracy of the method was determined by calculating percentage recovery of Metformin HCl and Pioglitazone. For both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of Metformin HCl and Pioglitazone corresponding to 80, 100 and 120% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared.

Intraday and interday precision study of Metformin HCl and Pioglitazone was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of 1000 µg/ml and 30 µg/ml of Metformin HCL and Pioglitazone respectively.

System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 1000 µg/ml and 30 µg/ml for Metformin HCl and Pioglitazone respectively to check the reproducibility of the system and the results are shown in table 2.

For robustness evaluation of HPLC method a few parameters like flow rate, percentage of acetonitrile in the mobile phase and pH of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 1000 µg/ml and 30 µg/ml for Metformin HCl and Pioglitazone respectively.

Forced degradation studies: Forced degradation studies[20] of both the drugs were carried out under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis. Metformin HCl (200 mg) and Pioglitazone (30 mg) were weighed separately and transferred into 20 ml and 100 ml volumetric flasks respectively and diluted up to the mark with methanol to give 1000 µg/ml concentration of Metformin HCl and 300 µg/ ml of Pioglitazone drug. These stock solutions were used for forced degradation studies.

Forced degradation in basic media was performed by taking 5 ml of stock solution of Metformin HCl and Pioglitazone each in separate round bottom flasks. One combined solution was prepared. Then 10 ml of 1 N NaOH was added and these mixtures were heated for up to 3 h at 60ºC in order to exclude the possible derivative effect of light. Forced degradation in acidic media was performed by keeping the drug in contact with 1N HCl for upto 3 h at 60ºC in dark. Degradation with hydrogen peroxide was performed by taking 10 ml of stock solution of Metformin HCl and Pioglitazone in two different flasks and one flask with combination and adding 10ml of 30% (w/v) hydrogen

peroxide in each of the flasks. These mixtures were kept for upto 3 hr in the dark.

To study neutral degradation, 10 ml of stock solution of Metformin HCl and Pioglitazone was taken in two different flasks, then 10 ml of HPLC grade water was added in each flask, these mixtures were heated for 3 h at 60ºC in the dark. For dry heat degradation, solid drugs were kept in Petri dish in oven at 100ºC for 12 h. Thereafter, by weighing suitably working level of Metformin HCl and Pioglitazone were weighed and transferred to two separate 10 ml volumetric flasks and diluted up to the mark with methanol. The photostability was also studied by exposing above stock solutions of both the drugs to direct sunlight in summer days for 5 h on a wooden plank. For UV degradation study, the stock solutions of both drugs (1000 µg/ml) were exposed to UV radiation of a wavelength of 254 nm for 12 h in UV chamber.

For HPLC analysis, all the degraded sample solutions were diluted with methanol to obtain final concentration of 1000µg/ml of Metformin HCl and 30µg/ml of Pioglitazone. Similarly mixture of both drugs in a concentration of 1000 µg/ml of Metformin HCl and 30µg/ ml of Pioglitazone each was prepared prior to analysis by HPLC. Besides, solutions containing 1000 µg/ml of Metformin HCl and 30 µg/ml of drug separately were also prepared without performing the degradation of both the drugs. Then 5 µl solution of above solutions was injected into HPLC system and analyzed under the chromatographic condition described earlier.

Results and Discussion

The mobile phase consisting of buffer pH 3.0: acetonitrile (80:20, v/v) at 0.5ml/min flow rate was optimized which gave two sharp, well-resolved peaks with minimum tailing factor for Metformin HCl and Pioglitazone. The retention times for Metformin HCl and Pioglitazone were 0.983 min and 5.671 min respectively. UV overlain spectra of both Metformin HCl and Pioglitazone showed that both drugs absorbed appreciably at 218 nm so this wavelength was selected as the detection wavelength. The calibration curve for Metformin HCl and Pioglitazone was found to be linear over the range of 800-1200 µg/ml and 24-36 µg/ml respectively. The data of regression analysis of the calibration curves is shown in table 1.

The proposed method was successfully applied to the determination of Metformin HCl and Pioglitazone in their combined tablet dosage form. The results for the combination were comparable with the corresponding labeled amounts. The developed method was also found to be specific since it was able to separate other excipients present in tablet from the two drugs. The LOD for Metformin HCl and PIOGLITAZONE were found to be 0.20 µg/ml and 0.25 µg/ml respectively while LOQ were 0.50 µg/ml and 0.45 µg/ml respectively. The results for validation and system suitability test parameters are summarized in table 2.

Table 1
Regression Analysis

Parameter	Metformin HCl	Pioglitazone
Correlation Coefficient	0.9998	0.9999
Intercept	-1283065	-114030
Slope	380626	401252
Range	800µg/ml-1200µg/ml	24µg/ml-36µg/ml

Table 2
Validation Result summary

Parameter	Metformin HCl	Pioglitazone
Correlation Coefficient	0.9998	0.9999
% RSD at Precision	0.3%	0.7%
Accuracy at 80%	100.5	99.0
Accuracy at 100%	99.6	100.3
Accuracy at 120%	98.7	99.7

Results for robustness evaluation for both the drugs are presented in table 3. Insignificant differences in peak areas and less variability in retention times were observed.

The degradation study indicated that Metformin HCl was stable to acid, base, H_2O_2, neutral conditions, direct sunlight, UV radiation and dry heat under experimental conditions. Pioglitazone was found to be susceptible to base with maximum degradation under basic condition; however it shows stability towards acidic, neutral hydrolysis peroxide, direct sun light, UV Radiation as well as dry heat degradation. Pioglitazone gets degraded into one or two degradation products in the stress conditions of alkaline condition as well as photolytic exposure. Summary of degradation studies of both the drugs is given in table 4.

Table 3
Robustness

Robustness Parameters	Conditions Changed	Metformin HCl	Pioglitazone
Wavelength (± 3nm)	215nm	100.4	99.3
	221nm	99.9	99.6
Column Temp (± 5ºC)	20 ºC	99.6	99.5
	30ºC	99.8	99.8
Mobile Phase Composition (± 10%)	MP A 78%	100.1	98.9
	MP A 82%	100.4	99.3
Flow Rate (± 0.1)	0.4 ml/ min	99.6	100.4
	0.6 ml/ min	99.6	100.0

Table 4
Degradation studies of Metformin and Pigolitazone

Degradation Condition	% Assay of Metformin HCl	%Assay of Pioglitazone
Control	99.8	99.1
Neutral Condition (3Hrs 60ºC)	100.9	99.4
Acid Hydrolysis (3hrs 60ºC 1N HCl, 10ml)	100.0	98.6
Base Hydrolysis (3hrs 60ºC 1N NaOH, 10ml)	99.6	83.6
Oxidation (3hrs dark 30% H_2O_2, 10ml)	99.1	98.7
Photolytic degradation (UV 254nm 12hrs)	100.2	99.5
Direct sunlight Sunny day 5hrs	100.5	99.1
Thermal degradation (12 hrs 100ºC)	99.3	100.6

Conclusion

In the proposed study, stability-indicating HPLC method was developed for the simultaneous determination of Metformin HCl and Pioglitazone and validated as per ICH guidelines. Statistical analysis proved that method was accurate, precise and repeatable. The developed method was found to be simple, sensitive and selective for analysis of Metformin HCl and Pioglitazone in combination without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. Assay results for combined dosage form using proposed method showed 99.06±1.40 % of Metformin HCl and 98.95±0.66 % of Pioglitazone. The results indicated the suitability of the method to study stability of Metformin HCl and Pioglitazone under various forced degradation conditions viz. acid, base, dry heat, neutral, photolytic and UV degradation. It can be concluded that the method separates the drugs from their degradation products; it may be employed for analysis of stability samples of Metformin HCL and Pioglitazone. However characterization of degradation products was not carried out.

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(Received, accepted)