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Stability-Indicating RP-HPLC Method for Simultaneous Determination of Olmesartan Medoxamil and Pioglitazone in Fixed Dose Combination Tablet Dosage Form

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The aim of the present study is to develop and validate a novel, simple, selective and sensitive stability indicating reverse phase HPLC method for the simultaneous determination of olmesartan medoxamil (OLM) and pioglitazone (PIO) in tablet dosage form after being subjected to different stress conditions, such as hydrolysis (0.1 N HCl and 0.1 N NaOH), oxidation (30 % H_2O_2), heat (80 ºC for 48 h) and photolysis (UV, 254 and 366 nm). The validation studies were carried out as per the International Conference on Harmonisation (ICH) and United State Pharmacopoeia (USP) guidelines. An isocratic HPLC method was developed to separate olmesartan and pioglitazone from the degradation products, using a Hiber C_{18} column (250 mm × 4.60 mm, 5 µm). A mixture of 10 mM KH_2PO_4 buffer (pH 4.4) and acetonitrile (50:50, v/v) was used as mobile phase. The flow rate was 1.0 mL/min and the UV detection was carried out at 230 nm. Retention time for olmesartan and pioglitazone were 5.58 and 10.73 min, respectively. A linear response was observed in the range of 0.10-200.00 µg/mL ($r^2 > 0.99$) for both the drugs. Drugs were decomposed in acid, base and 30 % H_2O_2 but were found to be stable in heat and photolytic stresses. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and quantitation and robustness. The procedure was found to be specific, linear, precise (including intra and inter day precision), accurate and robust. Applicability of the method has been illustrated performing the assay of a fixed dose combination tablet.

Key Words: HPLC, Stability indicating method, Validation, Olmesartan, Pioglitazone.

INTRODUCTION

Patients with diabetes have a much higher rate of hypertension than would be expected in the general population. Regardless of the antihypertensive agent used, a reduction in blood pressure helps to prevent diabetic complications[1]. Hypertension is one of the important risk factors for cardiovascular morbidity and mortality in diabetic subjects. Control of blood pressure prevents or retards either microvascular and macrovascular complications, while control of only hyperglycaemia prevents or retards mainly microvascular complications, *e.g.*, nephropathy, retinopathy and

neuropathy. Nearly 70 % of deaths occur in diabetes due to macrovascular complications, *e.g*., myocardial infarction, stroke, neglected gangrene of the limbs due to peripheral vascular disease, *etc*. and all these risks can be prevented by control of blood pressure, along with optimal control of hyperglycaemia[2].

It appears reasonable that regular inclusion of an angiotensin-receptor antagonist is beneficial for its proven renoprotective action[1]. Angiotensin II receptor antagonists together with a glitazone are often prescribed to diabetic patients[3]. Angiotensin type 1 receptor (AT_1R) blockers are widely used in the treatment of hypertension and hypertension-related cardiovascular end-organ damage. Recent clinical trials have demonstrated that AT_1R antagonism substantially lowers the risk for type 2 diabetes[4]. In addition, AT_1R blockade improves insulin sensitivity in animal models of insulin resistance[5]. The nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) plays an important role in the regulation of insulin sensitivity[6]. The activation of PPAR-γ by Angiotensin receptor blockers (ARBs) provides a potential mechanism for their insulin-sensitizing/antidiabetic effects. The combination of PPAR-γ agonist and ARBs can be used to prevent atherosclerosis and coronary heart disease in hypertensive patients with type 2 diabetes[7]. Olmesartan medoxomil, [(5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-nethylethyl)-2-propyl-1-{4-[2-tetrazol-5-yl)-phenyl]phenyl}methyl- imidazol-5-carboxylate)] (Fig. 1) is one of the newest ARBs dose dependently reduces blood pressure and provides nephroprotective effect in hypertension and diabetes-induced nephropathy[8]. Glitazones are PPAR-γ agonists currently used in the treatment of type 2 diabetes[9]. These drugs are the most powerful insulin sensitizers available in clinical practice. Pioglitazone hydrochloride, [(±)-5-{*p*-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl}-2,4-thiazolidinedione hydrochloride] (Fig. 1) is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus[10].

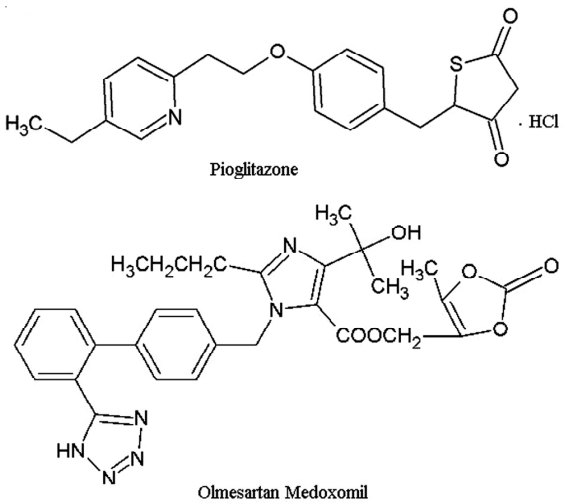

Fig. 1. Structural representation of olmesartan medoxamil and pioglitazone hydrochloride

According to current good manufacturing practices (cGMP), all drugs must be tested with a stability-indicating assay method before release. The objective of the study is to develop and validate a simple and sensitive stability-indicating reverse phase high performance liquid chromatographic (RP-HPLC) assay method for the determination of olmesartan medoxamil and pioglitazone in fixed dose combination tablet dosage form after forced degradation studies according to ICH and USP recommended test conditions.

EXPERIMENTAL

Olmesartan medoxamil (OLM) and pioglitazone (PIO) were obtained from Swiss Garnier Life Sciences (Himachal Pradesh, India). Acetonitrile and other organic solvents used were of HPLC grade and were obtained from Merck (Darmstadt, Germany). All aqueous solutions including the buffer for the mobile phase were prepared with water (resistivity of 18.2 MΩ cm) collected from a Milli-Q gradient system of Millipore (Elix 3, Milli-Q A10 Academic).

Instrumentation and chromatographic conditions: The liquid chromatography system used was of Knauer (Berlin, Germany). An isocratic HPLC method was developed to separate OLM and PIO from the degradation products, using a Hiber C_{18} column (250 mm × 4.60 mm, 5 μm). A mixture of 10 mM KH_2PO_4 buffer (pH 4.4) and acetonitrile (50:50, v/v) was used as mobile phase. The mobile phase was filtered through a 0.45 μm membrane filter and degassed using an ultrasonicator and the separations were achieved by isocratic elution with a flow rate of 1 mL/min injecting 50 μL of the sample. The UV detection was carried out at 230 nm.

Preparation of stock and standard solution: The stock solutions (1 mg/mL) were prepared by separately weighing and dissolving 10 mg of OLM and PIO into 10 mL of acetonitrile. Aliquots of the combined standard stock solution of OLM and PIO were prepared with mobile phase to get the required final concentrations.

Preparation of sample solution for assay: Twenty fixed dose combination tablets of OLM and PIO were powdered and powder equivalent to 20 mg of OLM and 30 mg of PIO was extracted into 100 mL of acetonitrile by vortex mixing followed by ultrasonication. It was then filtered through 0.45 μ filter and diluted with acetonitrile to 40 μg/mL of OLM and 60 μg/mL of PIO for analysis. The resulting solution was then injected into the column and chromatographed using the conditions mentioned above. The per cent dug content was determined from the area of the peak using the regression equation obtained in the calibration experiments.

Method development: For simultaneous analysis of OLM and PIO in bulk drug and in the formulation, a variety of mobile phases were tried in the development of an HPLC method. Sensitivity, suitability for stability studies, time required for the analysis and ease of preparation was considered for selecting the mobile phase.

Method validation: The method was validated according to ICH[11-14] and USP guidelines[15]. The validation parameters addressed were linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantitation (LOQ) and robustness[16,17].

Linearity: For linearity, the test solutions were prepared by diluting combined primary stock solution of OLM and PIO (1 mg/mL) at ten concentration levels from 0.10-200.00 µg/mL. The solutions were injected in triplicate and three separate linearity curves were constructed. The slope and intercept were calculated.

Precision: Intra-day precision was performed after injecting six replicate of the combined drug solution in three different concentrations (0.30, 100.00 and 160.00 µg/mL). The same study is repeated on three different days to determine inter-day precision. The concentrations were calculated from the areas obtained and the results were expressed as percentage relative standard deviation (RSD %).

Accuracy: Accuracy was evaluated by fortifying a mixture of decomposed reaction solutions with three different concentrations (0.30, 100.00 and 160.00 µg/mL) of the drugs. The recovery of the added drug was determined.

Specificity: The specificity of the method was established by injecting sample solutions of the drugs in presence of their degradation products and determining the peak purity.

Limit of detection and limit of quantitation: The LOD and LOQ of the drugs was determined by using a signal to noise ratio of 3 and 10, respectively. The LOQ was verified by injecting 6 replicates at its concentration.

Robustness: The robustness of the developed method was established in different deliberately varied chromatographic conditions (flow rate, temperature, column from different manufacturers, solvents of different lots).

System suitability: The system suitability test was performed to check whether the complete testing system was suitable for the required application. A combined standard solution of 100 µg/mL was injected for six times. Peak area, retention time, theoretical plates and tailing factor were measured.

Stress studies: Tablet samples were prepared after grinding the fixed dose combination tablets and weighing of the powder equivalent to weight of the drugs. Powder equivalent to 20 mg of OLM and 30 mg of PIO was weighed for each stress study separately and exposed to different experimental stress conditions described below.

Acidic hydrolytic stress: The powder was dissolved in 10 mL of 0.1 N HCl and kept at 60 ºC for 3 h in water bath. After attaining the ambient temperature, the solution was neutralized by 0.1 N NaOH and volume was made upto 100 mL with water. 2 mL of the resulting solution was then diluted upto 10 mL with acetonitrile to make a final solution containing 40 µg/mL of OLM and 60 µg/mL of PIO which was then centrifuged at 5000 rpm for 5 min and injected to the HPLC system.

Alkaline hydrolytic stress: The powder was dissolved in 10 mL of 0.1 N NaOH and kept at room temperature for 1 h. Then the solution was neutralized by 0.1 N HCl and volume was made upto 100 mL with water. The solution was then diluted and centrifuged as above and injected to HPLC system.

Oxidative stress: The powder was dissolved in 10 mL of 30 % H_2O_2 and kept at 60 ºC for 3 h in a water bath. After attaining ambient temperature, volume was made upto 100 mL with water and treated as same discussed above before injecting into the HPLC system.

Thermal stress: Powder was kept at 80 ºC for 48 h and the solution was prepared and diluted accordingly to achieve a final solution containing 40 µg/mL of OLM and 60 µg/mL of PIO which was then centrifuged and injected to the HPLC system.

UV Photolytic stress: The same amount of powder was exposed to UV short (254 nm) and UV long (366 nm) light for 48 h. Then the solution was prepared, diluted and centrifuged as above before injecting to HPLC system.

RESULTS AND DISCUSSION

Method development: We have tried a mobile phase composition starting from 30:70 v/v of 10 mM KH_2PO_4 buffer (pH 4.4) and acetonitrile. In such condition, though the chromatogram showed well resolved peak for OLM and PIO, but it was difficult to separate the degradation products. Again, due to increase in acetonitrile composition in the mobile phase above 50 % v/v, the peak of PIO was with a long tail giving difficulties in data integration. After performing the chromatographic run with several solvent mixtures, the mobile phase consisting of a mixture of 10 mM KH_2PO_4 buffer (pH 4.4) and acetonitrile (50:50, v/v) was found to furnish sharp, well-defined peaks with good symmetry. It was observed that the developed chromatographic conditions provides better separation of OLM (5.58 min) and PIO (10.73 min) as well as their degradation products in the chromatogram of forced degradation analysis of tablet samples. The typical representative chromatograms are shown in Fig. 2.

Method validation

Linearity: A linear response was observed in the range of 0.10-200.00 µg/mL for both the drugs. The mean correlation coefficient (± RSD) for OLM and PIO were 0.9978 (0.002) and 0.9975 (0.003), respectively (Table-1) (Fig. 3).

Precision: Data obtained for precision experiments are given in Table-1. The % RSD values for intra- and inter-day precision study were < 1.5 and < 2.0 %, respectively, which confirms that the method was sufficiently precise.

Accuracy: Difference between the peak areas obtained for fortified and unfortified solutions were used to calculate percentage recovery of the drugs. The recovery data indicates that excellent recoveries observed despite the presence of the degradation product of the drugs (Table-1).

Specificity: The specificity of the method can be justified from Fig. 2 where complete separation of the drugs from their degradation product was noticed. The average retention time for six replicates was 5.58 ± 0.04 and 10.73 ± 0.02 for OLM and PIO, respectively.

TABLE-1
SUMMERY OF VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameter	Olmesartan	Pioglitazone
Linearity range (µg/mL)	0.10-200.00	0.10-200.00
Correlation coefficient (r^2) ± RSD	0.9978 ± 0.002	0.9975 ± 0.003
LOD (µg/mL)	0.03	0.04
LOQ (µg/mL)	0.10	0.10
Accuracy (%)	98.46	99.08
Intra-day (n = 6) precision (RSD %)	< 1.42	< 1.25
Inter-day (n = 18) precision (RSD %)	< 1.28	< 1.13
Robustness	Robust	Robust
% RSD of peak area	1.03	1.19
Resolution	12.584	9.975
Theoretical plates	3846	3325
Tailing factor (asymmetry factor)	1.03	1.49

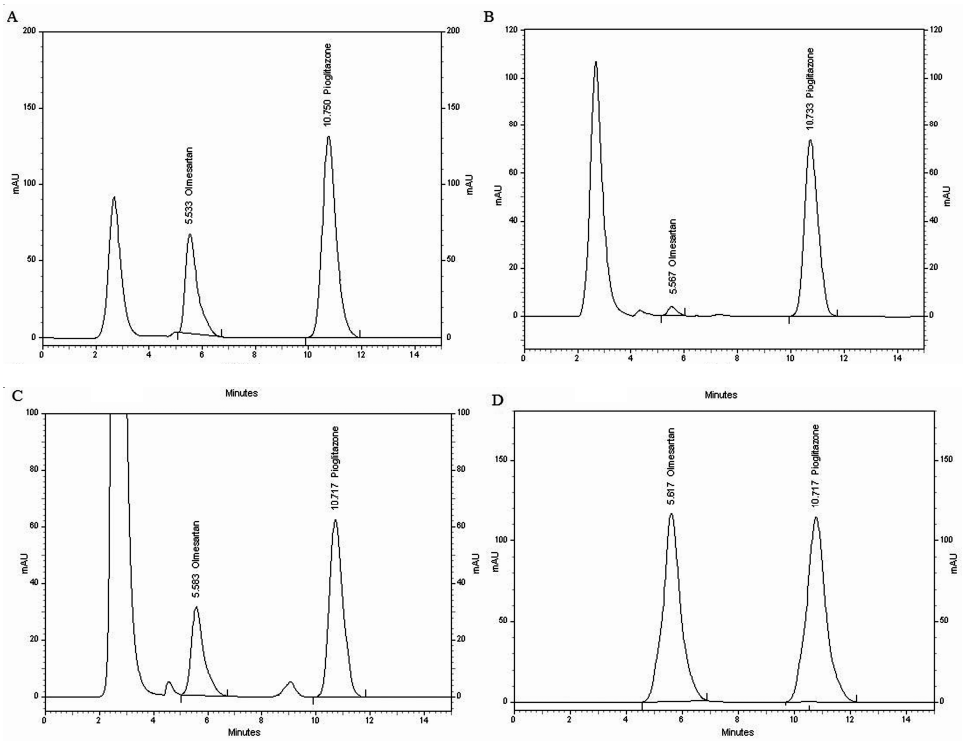

Fig. 2. Chromatograms of acid (A), base (B), oxidative (C) degradation study and assay in tablets (D)

Limit of detection and limit of quantitation: The LOD for OLM and PIO were 0.03 and 0.04 µg/mL, respectively. The LOQ for both the drugs were 0.10 µg/mL. Precision at LOQ was checked by analyzing six replicates and calculating the % RSD of the area which was < 0.85 % for intra-day and inter-day analysis.

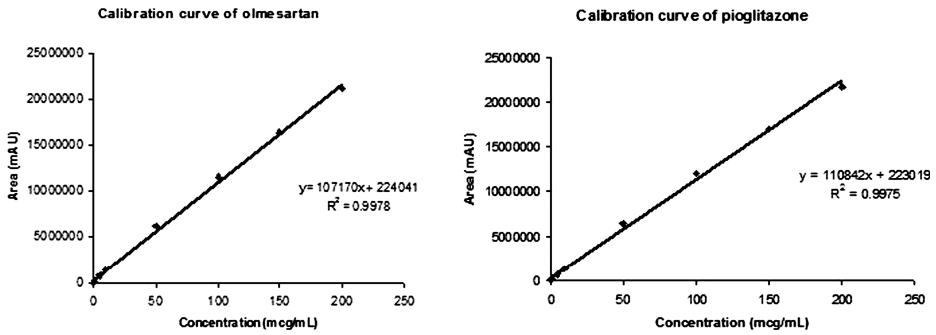

Fig. 3. Calibration curve of olmesartan and pioglitazone

Robustness: Good separation of the drugs and the degradation products achieved after changing the flow rate from 0.8-1.2 mL/min, temperature from 25-35 ºC, column from different manufacturers and solvents of different lots.

System suitability: The experimental result shows that the parameters tested were within the acceptable limit for % RSD of peak area (< 2 %), resolution (> 2 %), theoretical plates (> 3000) and tailing factor (< 2) (Table-1).

Stress studies: No additional peaks were found in the chromatogram of the sample undergone thermal (80 ºC for 48 h) and photolytic stresses (UV, 254 and 366 nm for 48 h). But additional peaks were observed in the chromatogram of the sample undergone hydrolytic (0.1 N HCl, 60 ºC for 3 h and 0.1 N NaOH, room temperature for 1 h) and oxidative (30 % H_2O_2, 60 ºC for 3 h) stresses (Fig. 2). This indicates that both the drugs were stable in heat and under UV light but susceptible to degradation in hydrolysis and oxidation. The percentage degradation and percentage recovery data for stress degradation studies are summarized in Table-2. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability-indicating.

TABLE-2
SUMMERY OF DEGRADATION STUDIES FOR OLMESARTAN AND PIOGLITAZONE

Stress condition	Time (h)	Degradation (%)		Recovery (%)	
		OLM	PIO	OLM	PIO
Acid, 0.1 N HCl, 60 ºC	3	59.68	16.03	40.32	83.97
Base, 0.1 N NaOH, RT*	1	96.07	53.05	3.93	46.95
30 % H_2O_2, 60 ºC	3	80.50	60.34	19.50	39.66
80 ºC	48	–	–	100	100
UV, 254 and 366 nm	48	–	–	100	100

*RT: Room temperature.

Assay: Experimental results of the amount of OLM and PIO in tablets expressed as percentage of label claim and were in good agreement suggesting no interference

from the excipients of the tablet. The drug content was found to be 98.34 and 103.88 % for OLM and PIO, respectively.

Conclusion

Literature survey reveals that there was no stability-indicating assay method for simultaneous determination of OLM and PIO in either bulk drug or in any pharmaceutical dosage form and hence the method developed in present investigation is a novel one. Different chromatographic methods have been described for the quantitative determination of either olmesartan medoxamil[18,19] or pioglitazone[20] separately. However, those reported methods are not applicable to perform the stability indicating assay for determination of OLM and PIO simultaneously in either bulk drugs or in a fixed dose combination tablet. The chromatographic method developed is adequate for quantitation of OLM and PIO in pharmaceutical dosage forms at different concentration levels. It is very simple, accurate and effective and provided no interference peaks for pharmaceutical excipients. Acceptable values of precision and accuracy have been obtained at all levels by this method regarding the guidelines for assay validation. The method uses simple mobile phase and is very beneficial for column life. The retention time of the two drugs is such that it distinguishes well from the degradant peaks. Applicability of the method has been illustrated performing the assay of a fixed dose combination tablet. So the developed analytical method will be of immense help to the pharmaceutical industries for stability testing as well as routine quality control analysis of OLM and PIO simultaneously in bulk drug and pharmaceutical formulations.

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