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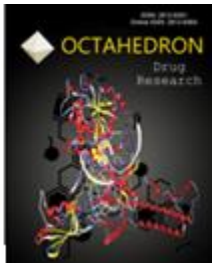

Determination of alogliptin benzoate and pioglitazone hydrochloride in their dosage forms, validation and stability-indicating studies using RP-HPLC method

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ABSTRACT

A recent, sensitive, stability-indicating RP-HPLC method was A recent, sensitive, stability-indicating RP-HPLC method was used to determine alogliptin benzoate and pioglitazone hydrochloride in their dosage form simultaneously. Stability studies were done via application of stress conditions on the analyte's solution. Resolution of the drugs was processed on BDS C18 Column (250x 4.6 mm) and the mobile phase consisted of sodium phosphate pH 3.6, and acetonitrile (60:40; v/v). The flow rate was 1 ml/min and the detector was set at 271 nm. The analytical method was tested for Linearity, accuracy, specificity, precision, ruggedness and robustness. The described HPLC method is valid for quantitation of alogliptin benzoate and Pioglitazone HCl in Tablets and valid as stability indicating method.

1. Introduction

Alogliptin, an orally hypoglycemic drug belongs to the DPP-4 inhibitor group. It is inhibitor of dipeptidyl peptidase 4 enzyme (DPP-4), which breaks the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1

(GLP-1). The active plasma incretins level which helps with glycemic control increases because of DPP-4 inhibition. It is not an official drug in any Pharmacopoeia. Few studies were reported for the estimation of Alogliptin individually[1-3] and in combination [4, 5]. Pioglitazone, an orally hypoglycemic drug belongs to thiazolidinedione group, which reduce insulin resistance [6-8]. It is used to control NIDDM (non-insulin-dependent diabetes mellitus, sugar diabetes) called type 2 diabetes. It presented officially in IP and has agonistic activity at peroxisome proliferator activated receptors (PPAR) in target tissues for insulin action such as liver, skeletal muscle and adipose tissue. [9]. There are few studies were reported for quantitation of Pioglitazone alone and combined with other drugs which includes bioanalytical methods using human plasma[10, 11] (12,13) and rat plasma[12], RP-HPLC[13-17], spectrophotometric methods[18-27]. Alogliptin and Pioglitazone are oral diabetes medicines. Alogliptin and pioglitazone were used in combination to control blood glucose levels. So they are very effective in the management of diabetes II. This form was approved by FDA in 2013 [28]. Different methods could be found for the quantitation of alogliptin and Pioglitazone simultaneously in the same dosage form including UV spectrophotometric methods as 1st order derivative 2nd order derivative, area under curve and dual wavelength methods, chromatographic HPTLC method and RP-HPLC [28-40]. No method is available in the pharmacopoeias; hence there is a chance for a simple study for analysis of selected drugs in combined formulations. Attempts were done to develop simple, precise and accurate quantitative methods for estimation of the selected drugs simultaneously and apply on their formulations. As a simple method of estimation is a pre-requisite for the marketing of most of the combination dosage forms, a RP-HPLC stability indicating method had been developed and validated for the estimation of titled drugs simultaneously. Multi-ingredients formulations are useful in controlling of various ailments where in the intake of large number of dosage forms are avoided. Chemical structures of Pioglitazone and alogliptin were obtained in **Figure 1**.

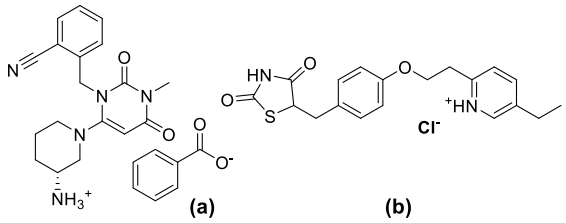

Figure 1: Chemical structures of alogliptin benzoate (a) and pioglitazone HCl(b).

Here, RP-HPLC method was used due to its unique characters, as it is environmentally and economically simpler than other methods [41-50]. The priority of the proposed study is that the estimation of alogliptin and Pioglitazone can be done in presence of each other and in presence of their degradation products on RP-HPLC system with UV detection without the need for prior derivatization. Regarding the results, we might use the suggested stability indicating study for quality control purposes. To evaluate the reproducibility and applicability of the proposed study, validation was carried out regarding ICH guidelines [51].

1. Experimental

1.1. Instruments

1.2. *Apparatus*

High performance liquid chromatography, (Beckman Coulter, Japan)

1.2.1. Reagents, standards, and materials.

Alogliptin benzoate and Pioglitazone HCl working standard were provided by El Obour Modern Pharmaceutical Industries – (El Obour City - Cairo) with purities of 99.98%. Tablets of piogliptal are obtained from local Market, methanol and acetonitrile are of HPLC grade, 0.1N HCl and Sodium dihydrogen phosphate are of analytical grade.

1.3. Instrumentation and HPLC conditions

The HPLC system (Beckman Coulter, Japan) composed of auto-sampler with HPLC Pumps, Dual lamp Absorbance Detector and In-Line Degasser ISA Card. LC solution soft-ware was used to perform data acquisition. The HPLC resolution and estimation were

preceded on Hypersil BDS C18 column (250 mm x 4.6 mm internal diameter, 5 µm particle size), with a 271 nm UV detector and mobile phase consisted of sodium phosphate pH 3.6, and acetonitrile (60:40; v/v at flow rate 1 ml/min. 0.05M sodium phosphate prepared by adding 1.6 gm of sodium dihydrogen phosphate to 1000 ml distilled water in volumetric flask and complete to the mark. the pH was adjusted by Metrohm 827 pH meter (Switzerland) at 3.6 using 0.1 M orthophosphoric acid then filtered by 0.45 mm filter before use.

1.4. Preparation of standard solutions

Stock solutions were prepared by Accurately weigh 136 mg of Alogliptin benzoate and 132.24 mg of Pioglitazone HCl standard in 50 ml volumetric flask and dissolve with enough solvent solution, complete the volume to the mark, mixed, completed the volume with water then filtered by 0.45 m filters to get a solution of 0.272 and 0.264 mg/ml of Alogliptin benzoate and Pioglitazone HCl, respectively. The working solution was prepared by withdraw 1 ml of each and dilute to reach 10 ml with mobile phase into 10 ml volumetric flask.

1.5. Preparation of Sample Solutions

1.6. Twenty tablets were weighed, powdered and transferred a portion of powder equal to 136 mg of alogliptin benzoate and 132.24 mg of pioglitazone HCl into 50 ml volumetric flask dissolve in sufficient quantity of solvent solution, sonicate for 5 minutes and complete to volume with solvent solution withdraw 1 ml and dilute to 10 ml with mobile phase in 10 ml volumetric flask filtered by 0.45 m filter to get a solution with concentration of 0.272 and 0.264 mg/ml of alogliptin benzoate and pioglitazone HCl, respectively.

1.7. Procedure

For SS: Working solutions of 6.25-18.75 ug/ mL alogliptin and 11.25-33.75 ug/mL pioglitazone were got by diluting the stock solutions using the mobile phase. 20 uL aliquot of each sample was automatically injected into the column three times and the results were recorded, the peak areas were determined, and calibration curve was constructed by plotting drug concentration against the mean peak area.

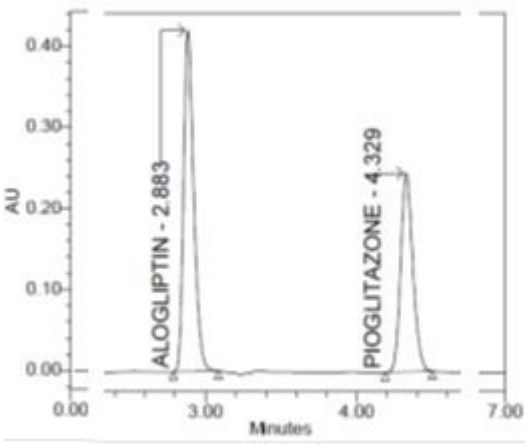

Figure 2: Typical HPLC chromatogram of alogliptin and pioglitazone

For combined tablet dosage form: inject an equal quantity (about 20 µ1) of the Working solution and the assay sample into the system separately, record the chromatogram and measure the responses for the major peaks. The concentrations of alogliptin and pioglitazone in thier tablets dosage form were determined by using calibration curves or via regression equations.

1.8. Validations

1.9. Accuracy

Accuracy estimated by applying of the proposed study to synthetic mixtures of the drug components to which known quantities of analyte have been spiked within the range of the calibration curve. Accuracy should be assessed using at least three concentrations with average recovery percent ranging from 98% to 102% of spiked drug.

1.10. Precision

Precision of the proposed method is the degree of agreement among results of individual test when it applied repeatedly, and it is expressed as the relative standard deviation RSD of a series of measurements. It should be assessed using at least six quantitations at 100% of the test concentration. Obtained Relative Standard Deviation percent (RSD %) are within the accepted range (NMT 2%) indicating that the method is Precise and repeatable.

1.11. Linearity, LOD and LOQ

The linearity represents the ability to get results that are directly proportional to the concentration of drug in samples within specific range. A minimum of five concentrations should be used. If appears to be a linear relationship, calculate regression coefficient and y-intercept which should be higher than 0.99 and near to zero respectively. Linearity of the method represents its ability to get a direct relationship between the obtained results and the given concentration of the drug. At the optimized HPLC conditions, linearity was obtained by injection of sample mixtures series of alogliptin and pioglitazone at five different levels; 50, 75, 100, 125 and 150 % of the selected concentration range. Plotting the peak area against the selected concentration was used estimate the calibration curves of each sample. The Slope (b), intercept (a) and correlation r^2 were estimated. LOD was determined from the formula; LOD = 3 σ /SD, where (σ) is SD of the intercept and (S) is the slope of regression equation. LOQ was calculated similarly from the equation LOQ = 10 σ/S.

1.12. Specificity:

Demonstration of specificity was achieved by mixing the drug with appropriate quantity of excipients and confirming that the results weren't affected by the presence of them. This step also should include samples shelved under relevant stress conditions as Acid, base hydrolysis, and oxidation to illustrate the ability of methods to assess the drug with its degradation products (stability indicating method). Obtained degradation products should be separated from the major peak (Resolution factor, R, greater than 1.5) to indicate the stability indicating properties of analytical method.

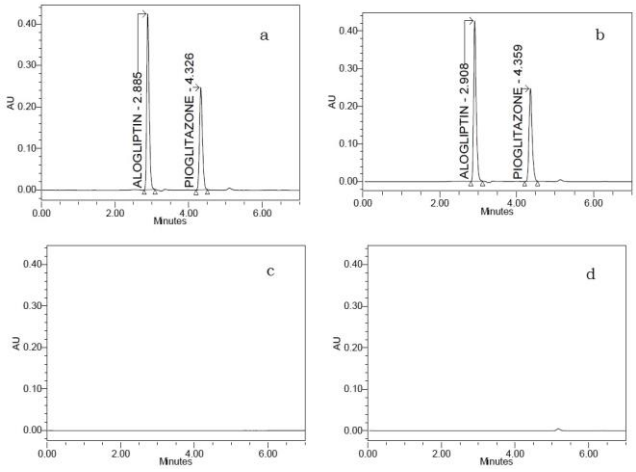

Figure 3: Chromatogram of (a) alogliptin and pioglitazone working standard solution (b) tablet sample solution (c) Placebo blank (d) mobile phase blank.

Table 1: Analytical performance characteristics and acceptable criteria.

Parameters	Acceptance criteria	Reference
Linearity and range	$r^2 \geq 0.99$	ICH
Accuracy	Recovery percent between 98% and 102%	ICH
Precision	Relative standard deviation lower than 2%	ICH
Specificity	No interference	ICH
Ruggedness	Results reproducible under a variety of conditions (RSD% ≤ 2%)	USP
Robustness	Results unaffected with small variations in method parameters (RSD% ≤ 2%)	USP

1.13. Ruggedness

The ruggedness of a study is get by analyze aliquots from homogeneous lot by different drugs and in different days. The grade of reproducibility of a method is a function of the assay variables. This reproducibility under normal conditions compared to the precision to get a measure of the ruggedness of this method.

1.14. Robustness

The robustness of a method is its ability to be not affected by small variations in its parameters as temperature, wavelength, and change in mobile

phase. This reproducibility under the normal parameters compared to the precision to get a measure of the robustness of the analytical method.

1.15. Stability Studies

To check the stability indicating properties of the presented chromatographic method, stress degradation procedures were studied. The dosage forms were subjected to oxidation, thermal and photo degradation and mixed with acid, alkali.

1.16. Acid and alkali hydrolysis

Powdered tablets equivalent to 45 mg of pioglitazone and 25 mg of alogliptin were put in a 100 mL volumetric flask, mixed with 10 mL of 0.1 N hydrochloric acid (acid) or 10 mL of 0.1 N sodium hydroxide (alkali) and sonicated for 30 min. then neutralized with an equivalent amount of acid (sodium hydroxide) or base (f hydrochloric acid) to that of the previously added. The flask was diluted to the mark with mobile phase.

1.17. Oxidative degradation

Powdered tablets equivalent to 45 mg of pioglitazone and 25 mg of alogliptin were put in 100 mL volumetric flask, mixed with 10 mL of 30% hydrogen peroxide solution and sonicated for 30 min. then, the samples was completed up to 100 mL with mobile phase.

Table 2: Results of Alogliptin benzoate in piogliptal tablets.

Sample #	Conc. (mg/ml)	Percent to working concentration	Response	Average response
1	0.136	50 %	1906210 1904881 1904300	1905130.3
2	0.204	75 %	2871167 2869832 2868807	2869935.3
3	0.272	100 %	3796239 3794190 3793565	3794664.7
4	0.34	125 %	4779714 4777150 4777620	4778161.3
5	0.408	150 %	5666530 5662819 5661597	5663648.7
Regression coefficient (r^2)				0.9998

1.18.

1.19. Thermal and photo degradation

Powdered tablets equivalent to 45 mg of pioglitazone and 25 mg of alogliptin were

Table 3: Results of Pioglitazone HCl in piogliptal Tablets.

Sample #	Conc. (mg/mL)	Percent to working concentration	Response	Average response
1	0.132	50 %	1669804 1669181 1668473	1669152.7
2	0.198	75 %	2533758 2532156 2530602	2532172
3	0.264	100 %	3356282 3355696 3353107	3355028.3
4	0.33	125 %	4205978 4202319 4205401	4204566
5	0.396	150 %	5022528 5019585 5015199	5019104
Regression coefficient (r^2)				0.9999

heated at 110 °C for 30 min in oven and left at direct sun light for up to 48 h for thermal degradation and photo degradation respectively. After the specified time, the powder was cooled, transferred to a 100 mL volumetric flask, dissolved in 30 mL of mobile phase and diluted to volume with the mobile phase to get a concentration of22.5 µg/mL (pioglitazone) and 12.5 µg/mL (alogliptin). The solutions were filtered by 0.45 µm membrane filter. A volume of 20 µL injected into the system and the results recorded.

2. Results and discussion

2.1. Method development

The aim of this study was to get a sensitive, reliable, and robust RP-HPLC method for the estimation of pioglitazone and alogliptin in their dosage form simultaneously. During method optimization, three columns were tried as Hypersil BDS C18 column (250 mm x 4.6 mm internal diameter, 5 µm particle size) and Zorbax C8 column (150 mm x 4.6 mm internal diameter, 5 µm particle size at a temperature of 30. Best results (good symmetrical sharp peak, acceptable tailing factor and resolution) were obtained with the later one. Hence, BDS C18, (250x 4.6 mm) at a temperature of 30 was used to develop the method.

Various mobile phases include 0.1 % Orthophosphoric acid, NaH_2PO_4, KH_2PO_4 and ammonium acetate with Methanol at different ratios were tried. Also, flow rate and pH were tried, and the responses were recorded. After a series of procedures, highly symmetrical and sharp peaks of pioglitazone and alogliptin with high resolution were achieved at pH 3.6 by using phosphate buffer and acetonitrile (60:40 (v/v)) as mobile phase at a flow rate of 1.0 mL/min. The pioglitazone and alogliptin in the specified mobile phase have good absorption at 271 nm, which was therefore selected for the proposed method. Figure 2 shows a typical HPLC chromatogram of pioglitazone and alogliptin using the optimized HPLC conditions.

2.2. *Method validation*

The developed RP-HPLC method was validated using ICH guidelines (35).

2.3. *System suitability test*

To get acceptable resolution and reproducibility of the method, suitability parameters as% RSD of peak area, % RSD of retention time, USP tailing factor and USP plate count were checked. To test the system suitability, standard solution of alogliptin- 12.50 µg/mL and pioglitazone 22.50 µg/mL was injected five times into the HPLC system. The results (Table 1) demonstrate the method suitability.

2.4. *Precision*

System repeatability data showed that RSD % was 0.23 and 0.18 for alogliptin and pioglitazone, respectively.

2.5. *Specificity*

chromatograms of mobile phase alone (blank), placebo blank, working solution of alogliptin 12.50 µg/mL and pioglitazone 22.50 µg/ mL and tablet sample solution of alogliptin 12.50 µg/mL and pioglitazone 22.50 µg/mL were injected into the HPLC system and evaluated to determine Specificity.

The chromatograms are shown in Figure 3. The chromatograms of working standard and tablet sample, placebo and mobile phase did not obtain any peaks other than that of alogliptin and pioglitazone. The specificity of the method was confirmed.

2.6. *Accuracy*

The data of accuracy studies were shown in Table 4; recovery results were within the accepted range.

2.7. *Linearity*

The mean peak area Plotted against concentration got the linear relationship within the range 6.25-18.75 µg/mL for alogliptin and 11.25-33.75 µg/mL for pioglitazone.

2.8. *Limit of detection (LOD) and limit of quantification (LOQ)*

Table 4: Results of accuracy of Pioglitazone HCl in piogliptal tablets.

	Sample #	Conc. mg/mL	Percent to working conc.	Response	Average response	Recovery %	Acceptable Recovery % limits
Authen	:	0.264	100%	3330768	3329646	-	98%-102%

			3327986		
			3330184		
			1649332		
1	0.132	50%	1648952		99 %
			1647433	1648572.3	
			3301003		
2	0.264	100%	3300510		99.1 %
			3299065	3300192.7	
			4978901		
3	0.396	150%	4978016		99.7%
			4974642	4977186.3	

Table 5: The robustness results for alogliptin and pioglitazone.

Parameter	**Drug**		**Condition 1**	**Condition 2**	**Condition 3**	
			269 nm	271 nm	273 nm	
wavelength		Average	3803837	3786391.3	3778379.3	
(3 replicate)	**alogliptin**	SD	2137.8	2324.4	831.1	
		RSD%	0.056%	0.061%	0.022%	
		Average	3344656.7	3351368	3389795	
	pioglitazone	SD	1263.1	1594.9	1545.1	**Acceptable limits RSD% ≤ 2%**
		RSD%	0.038%	0.048%	0.046%	
			Condition 1(39 :61)	Condition 2(40:60)		
Mobile phase		Average	3780060.7	3851399		
(3 replicate)	**alogliptin**	SD	1442.2	1450.8		
		RSD%	0.038 %	0.038%		
		Average	3323811.3	3338328		
	pioglitazone	SD	1721.1	1636.8		
		RSD%	0.052 %	0.049 %		

Table 6: Chromatographic results for Alogliptin benzoate and its degradation product used to assess the stability indicating properties of the proposed HPLC method.

	Alogliptin			
	Standard Rt.	**Degradation product Rt..**	**Resolution**	**Acceptable limit**
Acid hydrolysis	2.25	1.65	2.45	Resolution; not less than 1.5
Base hydrolysis	2.26	1.73	2.04	
Chemical	2.26	1.71	2.53	

Oxidation				

Table 7: Chromatographic results for Pioglitazone HCl and its degradation product used to assess the stability indicating properties of the proposed HPLC method.

	Pioglitazone HCl			
	Standard Rt.	**Degradation product Rt..**	**Resolution**	**Acceptable limit**
Acid hydrolysis	4.6	5.73	9.04	Resolution; Not less than 1.5
Base hydrolysis	4.75	5.9	12.3	
Chemical Oxidation	4.7	5.88	4.66	

LOD and LOQ were calculated regarding ICH guidelines. The LOD for alogliptin and pioglitazone was 0.047 and 0.085 µg/mL, respectively. Results of the regression statistics obtained for alogliptin and pioglitazone are presented in Tables 2 and 3.

2.9. Ruggedness

The reproducibility compared to the precision under the normal conditions to obtain a measure of the ruggedness of the analytical method. Analyst to analyst and Day to Day results were within the acceptable limits RSD% ≤ 2 for alogliptin and pioglitazone.

2.10. Robustness

The robustness of this method is a measure of its capacity to remain unaffected by small variations in method parameters as wavelength changes and change in mobile phase. Obtained results were within the acceptable limits RSD% ≤ 2 for alogliptin and pioglitazone as in table 5.

2.11. Stability Studies

The tablet sample was subjected to acid hydrolysis, alkali hydrolysis and chemical oxidation, resulted in degradation for alogliptin and Pioglitazone HCl with production of detectable degradation products with resolution factors higher than 1.5 as in tables 5 and 6.

These results confirm the validity of this method for determination of Alogliptin benzoate and Pioglitazone HCl in Tablets and valid as stability indicating method.

3. Conclusion

The study concerned with the analytical method described for determination of alogliptin benzoate and pioglitazone HCl in Tablets. The analytical method was tested for Linearity, accuracy, precision, specificity, ruggedness and robustness. Obtained results of alogliptin benzoate and pioglitazone HCl showed that the method is linear with regression coefficient (r^2) equal 0.9998 and 0.9999 within working concentration range 50% to 150%. It was found also accurate within the acceptable limits (98% to 102%). The method was tested for specificity and found selective to the active constituent with no interference with excipients or degradation products indicating that the method is valid as stability indicating method. The described HPLC method is valid for determination of Alogliptin benzoate and Pioglitazone HCl in Tablets and valid as stability indicating method.

Conflict of Interest

The authors declare and state that this research was conducted in the absence of any potential or source for conflict of interest.

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