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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND ALOGLIPTIN IN BULK AND DOSAGE FORM

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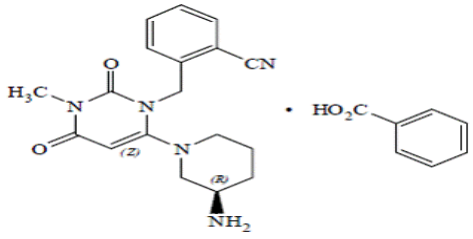
ABSTRACT

A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Pioglitazone and Alogliptin in bulk and dosage form**.** The method has been validated as per the guidelines of ICH. The separation is achieved on BDS hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size) column with flow rate 1.0 mL per minute in isocratic mode using Buffer pH 3.5: Methanol (70:30) as mobile phase. Column oven temperature is maintained at 25°C and observations are recorded at 271 nm. The linearity range was found to be in the range of 3.75-18.75µg/ml for Pioglitazone and 6.25-31.25 µg/ml for Alogliptin. Correlation co-efficient for calibration curve of Pioglitazone and Alogliptin was found to be 0.9997 and 0.9993 respectively. The method is simple, accurate, reproducible and short and can be used for simultaneous analysis of Pioglitazone and Pioglitazone.

INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample. (Chemistry 111 Lab: Intro to Spectrophotometry. Spectrophotometry 2005: E1-8) Pioglitazone is a selective, orally bioavailable inhibitor of the enzymatic activity of dipeptidylpeptidase-4 (DPP-4). Chemically, Pioglitazone is prepared as a benzoate salt, which is identified as 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-3, 4-dihydropyrimidin- 1(2H)-yl} methyl) benzonitrile monobenzoate. Its Molecular formula & Molecular weight are $C_{18}H_{21}N_5O_2 \bullet C_7H_6O_2$ & 461.51 respectively. The structural formula is:



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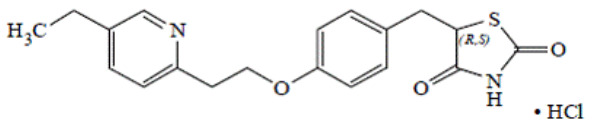
Pioglitazone benzoate is a white to off-white, crystalline powder, containing one asymmetric carbon in the amino-piperidine moiety. It is soluble in dimethylsulfoxide, sparingly soluble in water and methanol, slightly soluble in ethanol, and very slightly soluble in octanol and isopropyl acetate. Pioglitazone is an oral antihyperglycemic agent that acts primarily by decreasing insulin resistance. Chemically, pioglitazone is prepared as hydrochloride salt, which is identified as (±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy] phenyl] methyl]-2,4-thiazolidinedionemonohydrochloride. Its Molecular formula & Molecular weight are $C_{19}H_{20}N_2O_3S$ HCl & 392.90 respectively.

The structural formula is



Pioglitazone hydrochloride is an odorless white crystalline powder that contains one asymmetric carbon in the thiazolidinedione moiety. The synthetic compound is a racemate and the two enantiomers of pioglitazone interconvert in vivo. It is soluble in N, N dimethylformamide, slightly soluble in anhydrous ethanol, very slightly soluble in acetone and acetonitrile, practically insoluble in water, and insoluble in ether. (OSENI: http://www.rxlist.com/oseni-drug.htm) But up to now there is no HPLC methods develop for simultaneous

estimation Pioglitazone and Pioglitazone Analysis of the drug is important for development of drugs in their formulation and their use in therapies, for which we require standard analytical procedures. (Jajow Swapna *et al.,* 2012; Effat *et al.,* 2008; Vinod *et al.,* 2011; Dhirender *et al.,* 2011; Ramzia *et al.,* 2012; Gadapa and Upendra, 2013; Madhukar *et al.,* 2011; Lalit and Navpreet, 2011; Bhat *et al.,* 2008; Sane *et al.,* 2004; Karthik *et al.,* 2008; Narsimha *et al.,* 2011; Surendra and Sravani, Ravi, 2012; Sonali *et al.,* 2012; Ramzia *et al.,* 2012; Srinivasulu *et al.,* 2010; Adukondalu *et al.,* 2011; Pranshu 2012; Vijaya *et al.,* 2012) The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity and range, Robustness (Ludwig, 2007; International Conference on Harmonisation, Guidance for industry in; Q2B Validation on Analytical Procedures: Methodology, 1996). As quality control process is not static some form of validation/verification should continue till the validated procedure is in use. It should not be a concept that once the method is initially developed and validated it is forgotten.

MATERIALS AND METHODS

Chromatographic methods offer an advantage in terms of sensitivity and selectivity. These methods can be used for routine analysis of dosage forms where two or more drugs are present together. HPLC method was developed for simultaneous estimation of Pioglitazone and Alogliptin.

Selection of Mobile Phase

After assessing the solubility of drugs in different solvents as well on the basis of literature survey, the standard solution of Pioglitazone and Alogliptin were injected into the HPLC system by using different solvent systems. Different mobile phases were tried in order to find the best conditions for the separation of both the drugs. It was found that Buffer pH 3.5: Methanol (70:30) give satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase was determined to be Buffer pH 3.5: Methanol (70:30) which show in Figure 1.

Selection of Detection Wavelength

The Detection of wavelength was done in UV SIMADZU 1800 instrument. The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study standard drug solutions of 10µg/ml Pioglitazone and 10 µg/ml Alogliptin were, therefore, prepared in solvent mixtures of mixture of Buffer pH 3.5: Methanol (70:30) This drug solution was than scanned in the UV region of 200-400 nm and the spectrum was recorded 271nm which is shown in Figure 2.

Optimized Chromatographic Conditions

The HPLC used for the method was SPD-20AT UV DETECTOR to optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation. Buffer preparation (pH3.5), Mobile phase: Buffer pH 3.5: Methanol (70:30) Flow rate: 1.0 mL/min, Wavelength: 271nm, Column: BDS hypersil C_{18}, 250mm × 4.6mm, 5µ(particle size) and Injection volume: 20 micro liter

Preparation of standard solutions

Preparation of mobile phase

700 ml of Buffer (pH 3.5) & 300ml Methanol were mixed and used as mobile phase. Use mobile phase as a diluents.

Preparation of STD Stock solution of Pioglitazone

Stock solution of Pioglitazone: 10mg of Pioglitazone was taken as working standard into a 100ml volumetric flask. Added 60ml mobile phase and dissolve, make up volume with mobile phase (100 µg/ml)

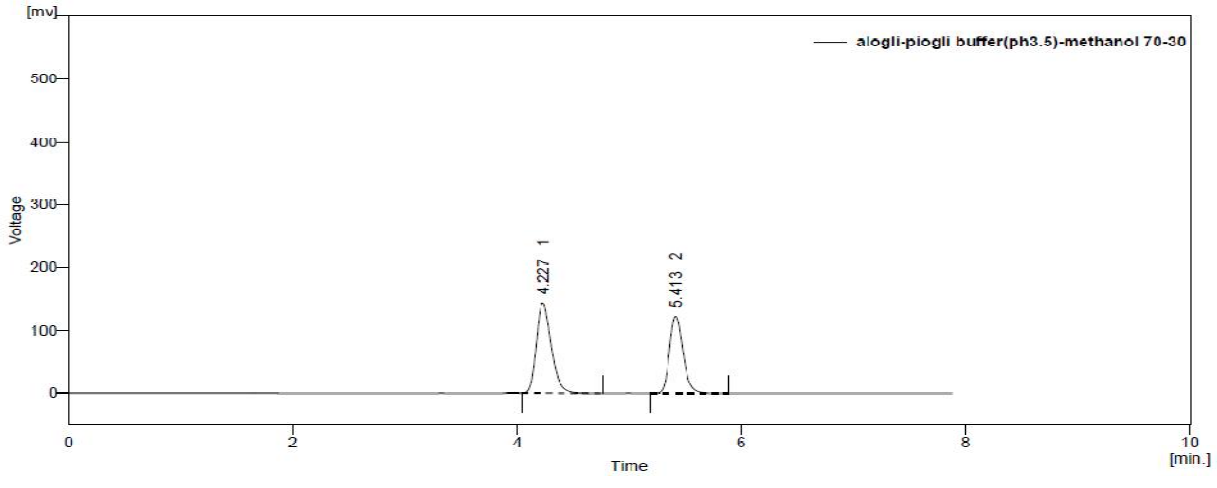


Figure 1. Selection of Mobile Phase

Preparation of STD Stock solution of Alogliptin

Stock solution of Alogliptin: 10mg of Alogliptin was taken as working standard into a 100ml volumetric flask. Add 60ml mobile phase and dissolve, make up volume with mobile phase (100 µg/ml)

Calibration curve for the 3.75-18.75µg/ml Pioglitazone and 6.25-31.25µg/ml Alogliptin

Appropriate volume of aliquots from standard Pioglitazone and Alogliptin stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 3.75, 7.5,11.25,15 &18.75 µg/ml Pioglitazone and 6.25, 12.5, 18.75,25 & 31.25 µg/ml Alogliptin. Each of these mixed standard solutions was chromatographed for 10 minutes run time using mobile phase at 271nm at flow rate of 1 ml/min. The graphs were plotted for peak area vs. concentration for both the drugs. Data is recorded in Table 1 and Figure 3, 4 and 5.

Table 1. STD curve data for Alogliptin and Pioglitazone

Pioglitazone		Alogliptin	
Concentration (µg/ml)	Concentration (µg/ml)	Concentration (µg/ml)	Peak Area* (mAU*S)
3.75	663.593	6.25	760.046
7.5	996.688	12.5	1141.598
11.25	1335.11	18.75	1529.179
15	1646.079	25	1855.363
18.75	1962.437	31.25	2247.677

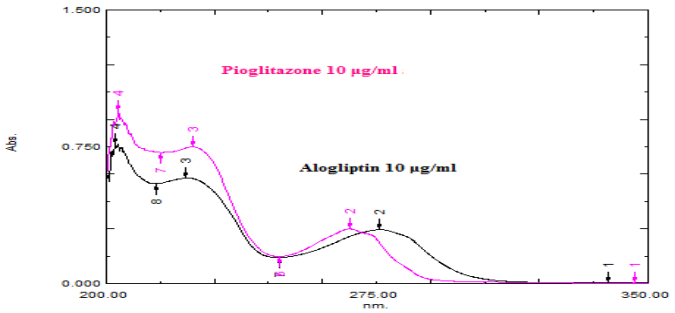


Figure 2. Detection of wavelength 271nm

Analysis of marketed formulation

Solution-1: Sample was taken equivalent to 10mg of Pioglitazone (10ml sample) into a 100ml volumetric flask. Added 60ml of mobile phase and shaked for 15 minutes to dissolve. Made up with mobile phase. Filtered this solution with 0.45micron membrane filter Solution-2: solution-1 was diluted into a 10ml volumetric flask to get 11.25 µg/ml Pioglitazone and 18.75 µg/ml Alogliptin.

The prepared sample solution was chromatographed for 10 minutes run time using mobile phase at 271nm at flow rate of 1 ml/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated by fitting peak area responses into the equation of the straight line representing the calibration curves for Pioglitazone and Alogliptin. And result shown in Table 2 and Figure 6.

Table 2. Analysis of marketed formulation

Drugs	Label Claim (mg)	Amount Found (mg)	%
PIOGLITAZONE	15	15.20	103.3
ALOGLIPTIN	25	25.44	101.7

Validation of proposed HPLC method

System suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. System suitability test was carried out to verify that the analytical system is working properly to give accurate and precise results. Standard solution (15µg/ml Pioglitazone and 25µg/ml Alogliptin) was injected and the chromatogram was recorded in Table 3 and Figure 7.

Table 3. Summary of HPLC method for Pioglitazone & Alogliptin

Parameter	Pioglitazone	Alogliptin
Wavelength detection(nm)	271 nm	
Beer's law limit (µg/ml)	3.75-18.75µg/ml	6.25-31.25 µg/ml
STD CURVE		
Regression equation	y = 129.88x + 21.95	y = 98.374x + 31.162
r^2	0.9997	0.9993
Slope (m)	86.4815	59.0626
Intercept (c)	3.6661	9.9408
System suitability	15µg/ml	25µg/ml
	Retention time:4.3	Retention time:5.9
	Theoretical Plate: 4553	Theoretical Plate:7270
	Tailing factor:1.51	Tailing factor:1.39
	Resolution:6.072	Resolution:6.072
Solvent suitability	15µg/ml	25µg/ml
	1639.351±6.87377	1847.817±7.68048
PRECISION	INTRA DAY	INTRA DAY
	667.287±4.92341	762.502±8.09129
	1340.708±4.32638	1542.404±9.25622
	1986.012±5.26315	2264.610±8.46238
	INTERDAY	INTER DAY
	658.0637±2.05046	751.386±6.17723
	1316.046±22.1408	1498.324±20.0276
	1968.73±22.61689	2250.645±31.61368
Repeatability	11.25µg/ml	18.75 µg/ml
	1331.850±13.5782	1519.703±10.75045
ACCURACY	99.84±1.11727	101.47±1.06943
	100.77±0.55371	101.09±1.04028
	100.15±1.12414	101.46±0.40498
Robustness	11.25µg/ml	18.75 µg/ml
	Flow rate +2	Flow rate +2
	1349.226±11.4710	1544.644±14.3774
	Flow rate -2	Flow rate -2
	1297.713±14.7463	1481.031±26.9563
	M.P. +2	M.P. +2
	1346.750±12.0087	1543.811±15.8198
	M.P. -2	M.P. -2
	1320.334±9.9267	1512.775±10.7315
	pH +2	pH +2
	1365.049±4.3056	1551.828±15.3551
	pH -2	pH -2
	1278.743±7.2648	1459.302±17.5576
LOD	0.1398	0.5554
LOQ	0.4239	1.68

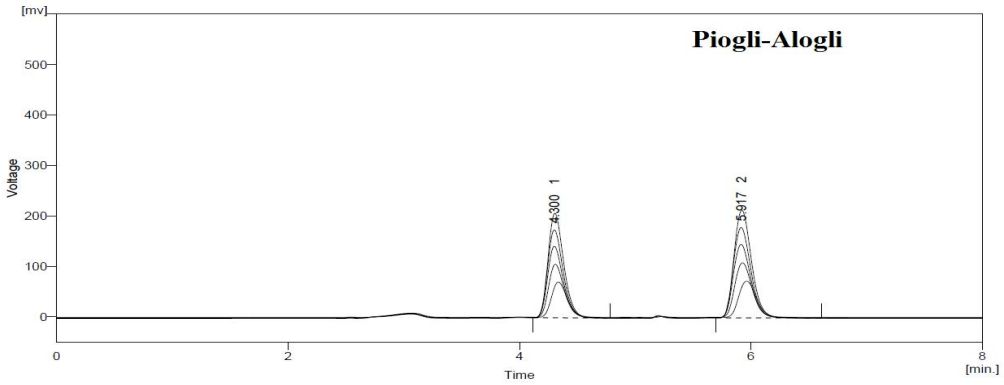


Figure 3. STD curve linearity

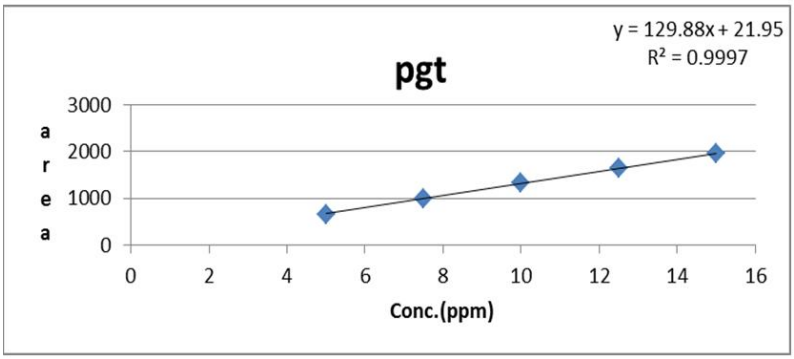


Figure 4. STD curve for Pioglitazone

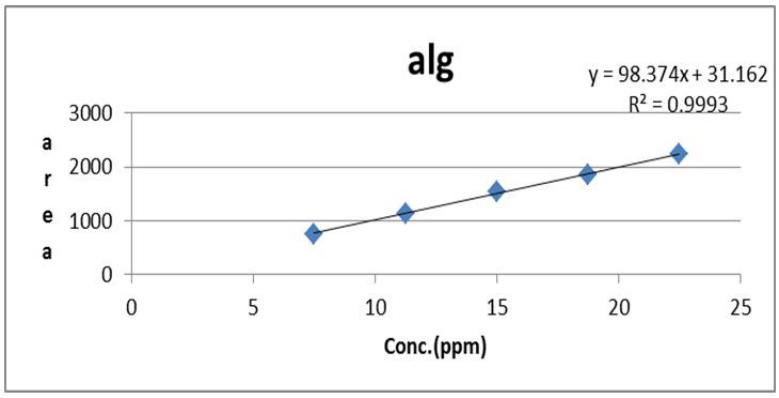


Figure 5. STD curve for Alogliptin

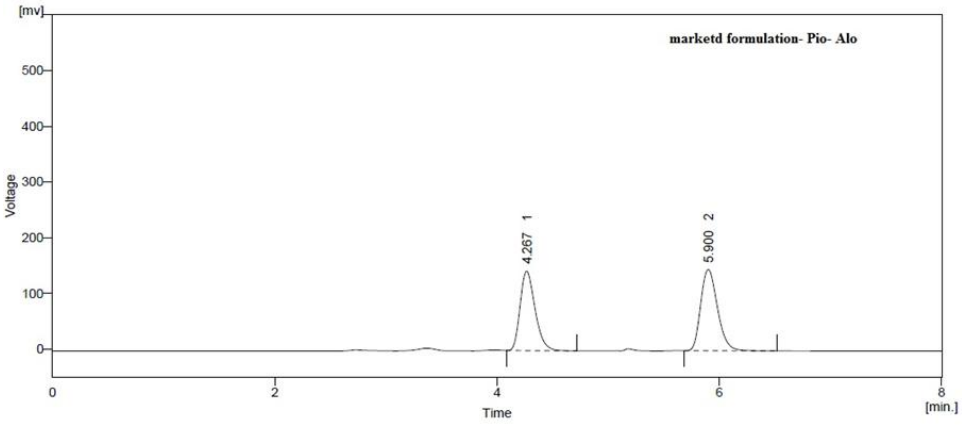


Figure 6. Analysis of marketed formulation

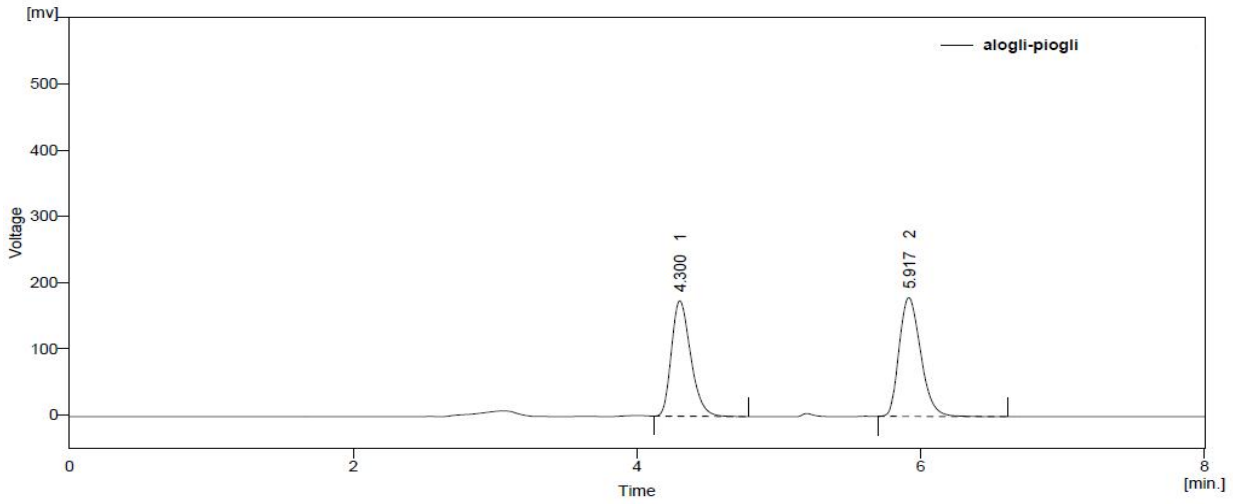


Figure 7. System suitability

Acceptance Criteria

The % RSD for area response obtained from six replicate injections of Standard solution should be ≤ 2.0 %, Tailing factor should be ≤ 2.0, Theoretical should be ≥ 2000 and Resolution should be ≥ 2.0 in Standard solution.

Solvent suitability

Recorded in Table 3.

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. The linearity response was determined by analyzing independent levels of concentrations in the range of 3.75-18.75µg/ml and 6.25-31.25µg/ml for Pioglitazone and Alogliptin respectively six times. Peak area of each solution was measured.

Precision

Repeatability

6 replicates of standard mixture solution having and Pioglitazone (15µg/ml) and Alogliptin (25µg/ml) were prepared and chromatograms were recorded and RSD was calculated and shown in Table 3.

Intraday precision

Standard solutions containing 3.75, 11.25 & 18.75µg/ml Pioglitazone and 6.25, 18.75 & 31.25 µg/ml Alogliptin were analyzed 3 times on the same day. Chromatogram of each sample was recorded. SD and RSD were calculated and shown in Table 3.

Interday precision

Standard solutions containing 3.75, 11.25 & 18.75µg/ml Pioglitazone and 6.25, 18.75 & 31.25 µg/ml Alogliptin were analyzed 3 times on three different days. Chromatogram of each sample was recorded. SD and RSD were calculated and shown in Table 3.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery Pioglitazone and Alogliptin by standard addition method.

Preparation of sample solution for % recovery

An accurately weighed powder was transferred to 100 ml volumetric flask; dissolved and the volume was made up to the mark using mobile phase to prepare 3.75 µg/ml & 6.25 µg/ml Pioglitazone and Alogliptin respectively. The prepared sample solution was chromatographed for 10 minutes using mobile phase at flow rate of 1 ml/min. concentration of Pioglitazone and Pioglitazoneis calculated which is known as pre-analyzed sample. In pre-analyzed sample 80, 100 and 120% of Pioglitazone and Pioglitazonewas spiked. Chromatogram of each spiked solutions was taken and total amount of drug was calculated and from which % recovery was calculated. This is shown in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ are estimated from the set of 6 calibration curves used to determine method linearity.

LOD = 3.3 x (SD / Slope)
LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.
Slope = the mean slope of the 6 calibration curves.
This is shown in Table 3.

Robustness

The robustness of an analytical method was carried out to confirm that the method remained unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution was injected five times for each varied conditions of flow, column temperature, pH, and mobile phase ratio and chromatograms were recorded in table no. 3 Change in Conditions for Robustness like Change in flow rate, M.P. and pH.

RESULTS AND DISCUSSION

High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Pioglitazone and Alogliptin in bulk and dosage form**.** The linearity range was found to be in the range of 3.75-18.75µg/ml for Pioglitazone and 6.25-31.25µg/ml for Alogliptin with using mobile phase Buffer pH 3.5: Methanol (70:30).Correlation co-efficient for calibration curve Pioglitazone and Pioglitazonewas found to be 0.9997 and 0.9993 respectively. The method is Accurate and precise which be used for simultaneous analysis of Pioglitazone and Alogliptin.

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