JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

METHOD DEVELOPMENT AND VALIDATION OF PIOGLITAZONE BY USING RP-HPLC

Kondappagari Srikanth Reddy*[1], Rajasekharan. S [2]

1. Department of Pharmaceutical Analysis, Research Scholar, Institute of Pharmaceutical Sciences and Research centre, Bhagwant University, Ajmer, Rajasthan, India.

2. Department of Pharmacology, AL AZHAR college of pharmacy ,Perumpilichira, Thodupuzha, Kerala, India.
Email:srikanth.pharma2018@gmail.com

ABSTRACT*:* A method was set up for synchronous estimation of a Pioglitazone by RP-HPLC system. The chromatographic conditions were viably created for the unit of Pioglitazone by using Inertsil - ODS C18(250 x 4.6 mm, 5µ), stream is 1.0 ml/min, convenient stage extent was Methanol: Buffer (70:30), recognizable proof wave length was 254 nm.

Key words : Pioglitazone RP-HPLC, Acetonitrile, Methanol, Water.

1. INTRODUCTION TO HPLC

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today.[1]In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production.[2] HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products.[3]

The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants.[4]High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability.[5] HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. The technique of HPLC has following features.[6]

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase

2. Pioglitazone

Pioglitazone is a selective agonist at peroxisome proliferators-activated receptor-gamma (PPARγ) in target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPARγ increases the transcription of insulin-responsive genes involved in the control of glucose and lipid production, transport, and utilization. Through this mechanism, pioglitazone both enhances tissue sensitivity to insulin and reduces the hepatic production of glucose (i.e. gluconeogenesis) - insulin resistance associated with type 2 diabetes mellitus is therefore improved without an increase in insulin secretion by pancreatic beta cells.

3.MATERIALS AND METHODS

Instruments-Instruments:

- HPLC –Waters Model NO.2695 series Compact System Consisting of Inertsil-C18 ODS column.
- UV spectrophotometer (Systronics)
- Electronic balance (SARTORIOUS)
- Sonicator (FAST CLEAN)

Substances containing chemicals:

- Methanol HPLC Grade.
- Buffer(KH2PO4)Hplc Grade.

Raw Equipment(Unprocessed Materials):

Pioglitazone is working standard.

Stock Solution Preparation:Take 100mg Pioglitazone working standard in 100ml V.F add methanol sonicate it 30minets,(That is 1000ppm solution).

Further Dilution (or) Trails Solution: Take 10ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets(That 100ppm solution)

DEVELOPMENT OF AN HPLC METHOD:

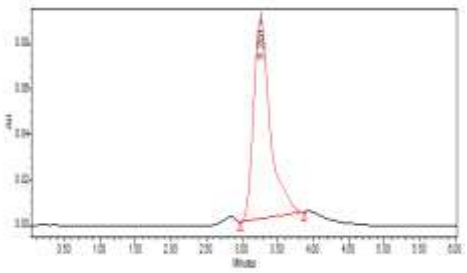
The goal of this study was to improve the assay technique for simultaneous quantification of Pioglitazone on literature surveys. As a result, the trials detailed below show how the optimization was accomplished.

Trail: 1

Step Mobile: Degassed Methanol: Water 65:35 .

Chromatographic Conditions:

Flow rate: 1.0ml / min
Column: Inertsil-C18, plate ODS
Wave longitude detector :254 nm
Tempo in the column: Ambient
Size of injection: 20µl
Time to run : 6min
Retention time:**3.261**



S.NO	Name of the peak	Retention time(min)
1.	Pioglitazone	3.261

Fig1:Chromatogramoftrail1

Inference : Got noise base line and peak tailing.

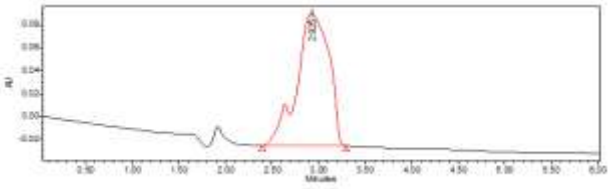
Trail: 2

Mobile Phase: Degassed Acetonitrile and methanol in the ratio of 20:80 V/V.

Chromatographic Conditions:

Flow rate: 1.0ml / min
Column: Inertsil-C18, plate ODS
Wave longitude detector :254 nm
Tempo in the column: Ambient
Size of injection: 20µl
Time to run : 6min
Retention time:2.925

Fig 2: Trial 2 chromatogram:



S.NO	Name of the peak	Retention time(min)
1	Pioglitazone	2.925

Inference: Got more assymetry

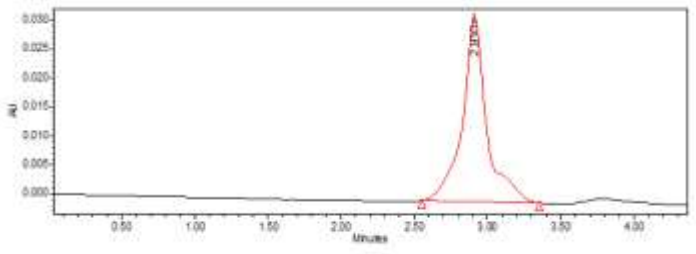
Trail: 3

Mobile Phase: Degassed Acetonitrile and Water in the ratio of 35:65 V/V.

Chromatographic Conditions:

Flow rate: 1.0ml / min
Column: Inertsil-C18, plate ODS
Wave longitude detector : 254 nm
Temp in the column: Ambient
Size of injection: 20µl
Time to run : 6min
Retention time:2.910

Fig 3: Trial3 chromatogram



S.NO	Name of the peak	Retention time(min)
1	Pioglitazone	2.910

Inference :Got Bad Peak.

4.RESULTS AND DISCUSSIONS

ADVANCED METHOD (OPTIMIZED METHOD)

Mobile Phase: Methanol: Buffer (70:30) V/V. Sonicate it 30minets, Filter this mobile phase through 0.45micron filter paper.

Preparing phosphate buffer with pH 3.4: Weighed and transferred 2.7218 g of KH2PO4 into a 1000ml beaker, dissolved later and diluted to 1000ml with HPLC water and balanced to pH 3.4 with ortho phosphoric acid.

Optimized Method Stock Solution Preparation:Take 100mg Pioglitazone working standard in 100ml V.F add methanol sonicate it 30minets,(That is 1000ppm solution).

Further Dilution (or) Optimized Method Solutions Preparation: Take 4ml of above solution in 100ml V.F add Methanol up to mark sonicate it 10minets(That 40ppm solution).

Chromatographic conditions:

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18}(250 x 4.6 mm,5 µ)
Mobile Phase	Methanol:Buffer (70:30)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	6 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254 nm
Drug RT (min)	3.351min

VALIDATION DATA

SYSTEM SUITABILITY: A Standard solution was prepared by using Pioglitazone working standard as per test method and was injected Five times into the HPLC system.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Pioglitazone retention times and peak areas.

TABLE-1: Data of System Suitability

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.351	1115623.12	11035	1.012

2	3.353	1115684.35	11042	1.016
3	3.355	1115601.99	11054	1.023
4	3.352	1115674.56	11038	1.014
5	3.353	1115688.56	11045	1.019
Mean	3.3528	1115654.51	11042	1.016
SD	0.001483	39.5337	-------	-------
% RSD	0.044239	0.00352	-------	-------

PRECISION:

Repeatability:

a. System precision: Standard solution prepared as per test method and injected five times.

b. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

TABLE-2 Data of Repeatability (System precision)

	Injection	**Peak Areas of Pioglitazone**	**%Assay**
Concentration 40ppm	1	1115589.45	100.16
	2	1115601.05	100.17
	3	1115596.58	100.16
	4	1115608.89	100.17

	5	1115582.65	100.16
Statistical **Analysis**	**Mean**	1115595.72	100.16
	SD	10.1579	0.00091
	% RSD	0.00091	0.00091

TABLE-3:Data of Repeatability (Method precision)

	Injection	**Peak Areas of Pioglitazone**	**%Assay**
Concentration 40ppm	1	1115568.87	100.16
	2	1115590.63	100.16
	3	1115579.42	100.16
	4	1115601.55	100.17
	5	1115595.45	100.16
	6	1115610.62	100.17
Statistical **Analysis**	**Mean**	1115591.09	100.16
	SD	15.09947	0.00135
	% RSD	0.001353	0.00135

Concentration % of spiked level	Area	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	322742.02	20	19.98	99.94	**MEAN**	100.32
50% Sample 2	322769.61	20	20.10	100.52		

ACCURACY:

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Pioglitazone into each volumetric flask for each spike level to get the concentration of Pioglitazone equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Pioglitazone was calculated.

Table 4:

50% Sample 3	322728.59	20	20.10	100.51	**%RSD**	0.3336
100 % Sample 1	645512.85	40	40.09	100.23	**MEAN**	100.42
100 % Sample 2	645489.56	40	40.20	100.51		
100% Sample 3	645530.51	40	40.20	100.52	**%RSD**	0.1651
150% Sample 1	968148.53	60	60.19	100.31	**MEAN**	100.44
150% Sample 2	968125.94	60	60.30	100.50	**%RSD**	0.1104

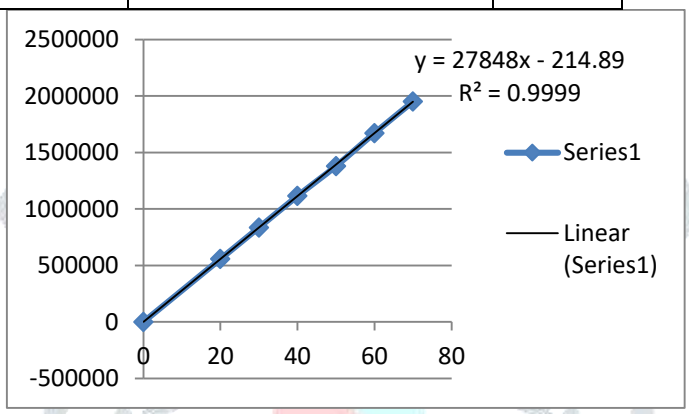
LINEARITY:

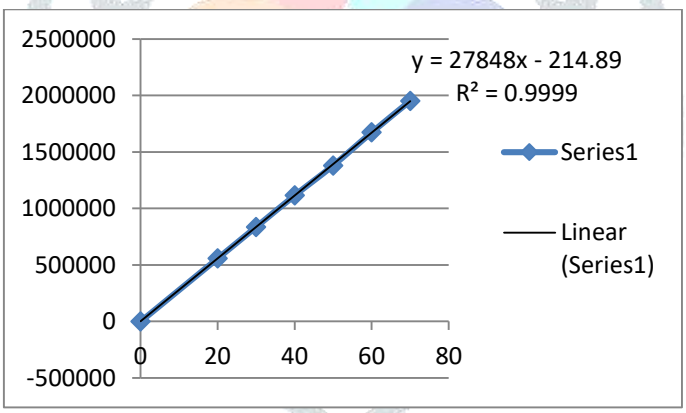
A Series of solutions are prepared using Pioglitazone working standard at concentration levels from 20ppm to 70 ppm of target concentration.

TABLE 5: Data of Linearity

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	27848
20	557827.45	y-Intercept	-214.8

30	836741.48	Correlation Coefficient	0.999
40	1115655.45		
50	1381456.65		
60	1673482.32		
70	1952396.25		





Ruggedness:

System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method.

Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

TABLE: 6

Data on System Variability

System-2

S.NO:	Peak area	Assay % of Linagliptin
1	645488.73	100.22
2	645410.82	100.21
3	645466.89	100.22
4	645419.83	100.21
5	645452.88	100.22
6	645492.21	100.23
Mean	645455.22	100.22
%RSD	0.005302	0.00531

Robustness:

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The systemsuitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow.

Linagliptin was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

TABLE: 7 Data for Effect of variation in flow rate:

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1108456.25	1.111		1115647.14	1.115		1123864.24	1.128
	1108444.66	1.115		1115632.32	1.117		1123888.46	1.130
	1108471.52	1.117		1115639.64	1.115		1123878.23	1.129
	1108462.59	1.121		1115621.35	1.116		1123845.16	1.129
	1108473.19	1.123		1115611.54	1.117		1123854.54	1.128
Avg	1108461.64	1.117	Avg	1115630.39	1.116	Avg	1123866.12	1.129
SD	11.71848	0.0047	SD	14.2029	0.001	SD	17.4834	0.0008
%RSD	0.00105	0.4273	%RSD	0.00127	0.0896	%RSD	0.00155	0.0741

LOD AND LOQ (LIMIT OF DETECTION AND LIMIT OF QUANTITATION):

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3 \times 39.5337}{27848} = 0.00146$$

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10 \times 39.5337}{27848} = 0.0141$$

5. Summary and Conclusion

Different parameters were studied to create the analytical approach. For starters, the maximum absorbance of Pioglitazone was discovered to be 254 nm. The injection volume was set at 20µl, which resulted in a nice peak area. The Inertsil C18 column was employed in this work, and ODS picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 1.0ml/min. Different mobile phase ratios were investigated, however the mobile phase with a Methanol:Buffer (70:30) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase.

The accuracy of both the system and the procedure was determined to be precise and well within range. The correlation coefficient and curve fitting were discovered during the linearity investigation. For both medicines, the analytical approach was shown to be linear throughout a range of 20-70ppm of the target concentration. Both robustness and ruggedness tests were passed by the analytical. The relative standard deviation in both circumstances was excellent.

6. BIBLIOGRAPHY

1. V. Gupta, A.D. K. Jain, N.S. Gill, K. Gupta, Development and validation of HPLC method - a review , Int. Res J Pharm. App Sci., (2012);2(4) 17-25
2. Y. Kazakevich, R. Lobrutto, HPLC for Pharmaceutical Scientists, John Wiley & Sons, New Jersey, 2007.
3. S. Ahuja, H. Rasmussen, Development for Pharmaceuticals,Separation Science and Technology, Elsevier, New York [2007] Vol.8
4. M.S. Azim, M. Mitra, P.S. Bhasin, HPLC method development and validation: A review, Int. Res. J. Pharm. (2013);4(4):39-46.
5. B.V. Rao, G.N. Sowjanya1, A. Ajitha, V.U.M. Rao, Review on stability indicating hplc method development, World Journal of Pharmacy and Pharmaceutical Sciences, (2015);4(8)405-423.
6. M.S. Charde, A.S. Welankiwar, J. Kumar, Method development by liquid chromatography with validation, International Journal of Pharmaceutical Chemistry, (2014);04(02): 57-61.
7. S. Sood, R. Bala, N.S. Gill, Method development and validation using HPLC technique – A review, Journal of Drug Discovery and Therapeutics, 2014; 2(22): 18-24.
8. M.W. Dong, Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006.
9. https://www.derpharmachemica.com/pharma-chemica/rphplc-method-development-and-validation-for-pioglitazone-in-bulk-and-marketed-formulation.pdf
10. https://www.jocpr.com/articles/analytical-method-development-and-validation-of-pioglitazone-hydrochloride-by-rphplc.pdf
11. https://www.ijnrd.org/papers/IJNRD2305785.pdf
12. https://en.wikipedia.org/wiki/Pioglitazone
13. https://go.drugbank.com/drugs/DB01132