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Research

A simple, robust and specific reverse phase-hplc method development and validation for estimation of rosiglitazone and glimepiride in active pharmaceutical ingredient and its pharmaceutical dosage form

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Abstract

An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Rosiglitazone and Glimepiride in bulk and combined pharmaceutical tablet dosage forms. Rosiglitazone and Glimepiride were separated by using a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size; Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of Methanol: 0.1% Orthophosphoric acid (64:36% v/v). The flow rate was set to 1ml/min with the responses measured at 224nm. The retention time of Rosiglitazone and Glimepiride was found to be 2.808min and 3.880min respectively with resolution of 5.68. Linearity was established for Rosiglitazone and Glimepiride in the range of 20-100µg/ml for Rosiglitazone and 60-140µg/ml for Glimepiride with correlation coefficient 0.999. The percentage recovery was found to be is 100.30% for Rosiglitazone and 100.21% for Glimepiride respectively. Validation parameters such as specificity, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present and in combined tablet dosage form.

Keywords: Rosiglitazone and Glimepiride, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Analytical chemistry[1] is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography

HPLC

HPLC[3] is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved .The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed, efficient ,accurate and highly resolved method of separation.

For the recent study Clonazepam and Propranolol was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages

- Speed many analysis can be accomplished in 20min (or) less.
- Greater sensitivity (various detectors can be employed).
- Improved resolution (wide variety of stationary phases).
- Re usable columns (expensive columns but can be used for many analysis).
- Ideal for the substances of low viscosity.
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour).
- Precise and reproducible.
- Integrator itself does calculations.
- Suitable for preparative liquid chromatography on a much larger scale.

HPLC components

The essential components[4] of a complete HPLC system are solvent delivery system (Pump), detector, fixed volume injector loop or auto sampler, solvent reservoirs, packed column, data system and recorder. A schematic of a simplified HPLC system is shown in Fig 1.

Column

The column is probably the heart of HPLC system. The development of this column technology leads to the evolution of the HPLC instrumentation systems used today. The conventionally used HPLC columns are particle packed columns. The key of column selection when previous separation is not available resides in knowing the chemistry of the sample. Columns should never be dry. A dry column will eventually have voids because the packing will shrink away from the wall, which would result in band broadening. Before running a sample in HPLC the column should be equilibrated. Usually column equilibrium is achieved after passage of 10 – 20 column volumes of the new mobile phase through the column. Insufficient column equilibrium usually leads to retention difference.

Pump

The solvent delivery system or as it is commonly called the pump includes two major types, constant volume or flow and constant pressure. Constant volume pumps are mechanically driven systems, most commonly using screw driven syringes or reciprocating pistons. On the other hand, constant pressure pumps are driven or controlled by gas pressure.

Injector or Auto sampler

Samples are usually introduced by syringe injection via a manual injector into the mobile phase stream or by the use of an auto sampler. The important aspects in sample introduction are precise and reproducible injections. This is especially important with quantitative analysis where the reproducibility of the peak response is dependent on the precision of the sample introduction. Direct syringe injection through a manual injector was the first popular method of sample introduction. As HPLC instrumentation evolved, many auto sampler techniques were applied so that sample introduction has become more precise and rapid.

Detector

HPLC detectors include ultraviolet-visible, fluorescence, electrochemical, refractometer, mass spectrometer and others. The UV visible absorption detector is the most widely used detector in liquid chromatography, since most organic compounds show some useful absorption in the UV region. This detector is fairly universal in application, although sensitivity depends on how strongly the sample absorbs light at a particular wavelength.

Solvent reservoir

Different containers are used as a solvent delivery system reservoir. The best material from which the containers are made is glass. Plastic containers are not recommended as it leads to plasticizer leaching. The container should be covered to prevent solvent evaporation. The tubing from the reservoir can be made of stainless steel or Teflon, and both are satisfactory.

Data handling and analysis

Data handling in HPLC is as important to the success of any experiment or analysis as any other components in the system. It is part of good HPLC techniques to properly label and document the analytical results. The advanced computer softwares used now in data handling and analysis allow easy recording and storage of all chromatographic data.

Normal phase chromatography

In normal phase mode the stationary base (eg; silica gel) is polar in nature and the mobile phase is non polar. In this technique, non polar compound travel faster and are eluted first. This is because less affinity between solute and stationary phase and take more time to elute.

Reverse phase chromatography

The popularity of reversed phase liquid chromatography is easily explained by its unmatched simplicity, versatility and scope. Neutral and ionic analytes can be separated simultaneously. Retention in RPLC is believed to occur through nonspecific hydrophobic interaction of the solute with the stationary phase. The near universal application of RPLC stems from the fact that almost all organic compounds have hydrophobic regions in their structure and are capable of interacting with the stationary phase.

A decrease in the polarity of the mobile phase leads to a decrease in retention. It is also generally observed in RPLC that branched chain compounds are retained to a lesser extent than their straight chain analogues and that unsaturated compounds are eluted before their fully saturated analogs. A wide variety of RP-HPLC columns are available. Most columns are silica based. Silica offers good mechanical stability. A typical stationary phase is formed by chemically bonding a long-chain hydrocarbon group to porous silica. Typical ligands are n-octadecyl (C18), n-octayl (C8), n-butyl (C4), diphenyl (C2), and cyano propyl.

Parameters affecting separation[6]:

Separation in reversed phase chromatography is affected by stationary phase type and column length. It is also affected by organic solvent type and percentage in the mobile phase and by mobile phase pH. Flow rate could also affect separation in reversed phase chromatography; however it is usually limited by the developed backpressure. Moreover temperature of the column also has an effect on separation.

MATERIALS AND METHODS

Rosiglitazone (Pure)-Sura labs, Glimepiride (Pure)-Sura labs, Water and Methanol for HPLC-Lichrosolv (Merck), Acetonitrile for HPLC-Merck/

HPLC METHOD DEVELOPMENT
TRAILS
Preparation of standard solution

Accurately weigh and transfer 10 mg of Rosiglitazone and Glimepiride working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.6ml of Rosiglitazone and 1ml of Glimepiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: 0.1% Orthophosphoric acid in proportion 64:36 v/v respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, X terra and ODS column. Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 38ºC
Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Flow rate : 1ml/min
Wavelength : 224nm
Injection volume : 20µl
Run time : 7.0minutes

Method validation
Preparation of mobile phase
Preparation of mobile phase

Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

(Optimized Condition)

Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)
Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Flow rate : 1 ml/min
Wavelength : 224 nm
Column temp : 38ºC
Sample Temp : Ambient
Injection Volume : 20 µl
Run time : 7 minutes

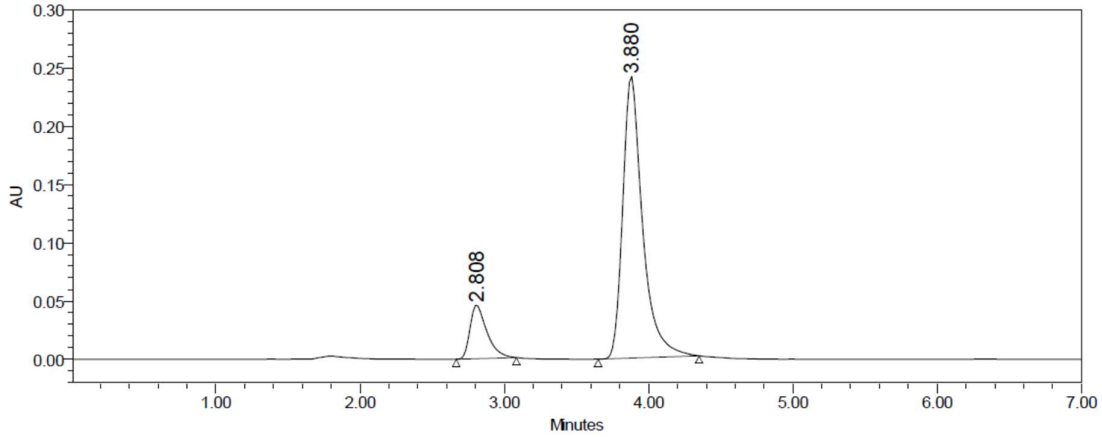

Fig 1: Chromatogram for Trail 5

Table 1: Peak Results for Trail 5

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Rosiglitazone	2.808	65258	4326		1.08	5685.4
2	Glimepiride	3.880	8659854	659823	5.68	1.42	6895.7

From the above chromatogram it was observed that the Rosiglitazone and Glimepiride peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.
Retention time of Rosiglitazone–2.808min
Retention time of Glimepiride – 3.880 min

Assay (Standard)

Table 2: Showing assay standard Results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Rosiglitazone	2.813	65684	4365		1.08	5632.4	1
2	Glimepiride	3.886	8659824	659824	5.69	1.42	6859.2	1
3	Rosiglitazone	2.813	65985	4329		1.09	5682.3	2
4	Glimepiride	3.886	8645872	658266	5.68	1.43	6824.1	2
5	Rosiglitazone	2.813	65784	4426		1.08	5692.8	3
6	Glimepiride	3.886	8657847	6589412	5.69	1.43	6895.4	3

Assay (Sample)

Table 3: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Rosiglitazone	2.799	66859	4458		1.09	5785.4	1
2	Glimepiride	3.863	8756854	669585	5.69	1.43	6956.7	1
3	Rosiglitazone	2.799	66258	4462		1.10	5789.5	2
4	Glimepiride	3.861	8769582	663598	5.68	1.44	6945.2	2
5	Rosiglitazone	2.799	66435	4438		1.09	5784.1	3
6	Glimepiride	3.863	8754985	668548	5.69	1.44	6927.7	3

Table 4: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Rosiglitazone	60mg	59.84	99.68%
2	Glimepiride	500mg	499.63	99.46%

The retention time of Rosiglitazone and Glimepiride was found to be 2.808mins and 3.880mins respectively. The % purity of Rosiglitazone and Glimepiride in pharmaceutical dosage form was found to be 99.68% and 99.46% respectively.

PRECISION

Table 5: Results of method precision for Rosiglitazone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rosiglitazone	2.808	65898	4365	5682.2	1.08
2	Rosiglitazone	2.808	65487	4375	5628.6	1.09
3	Rosiglitazone	2.808	65324	4395	5649.7	1.08
4	Rosiglitazone	2.808	65982	4328	5638.4	1.09
5	Rosiglitazone	2.808	65248	4371	5698.3	1.08
6	Rosiglitazone	2.808	65734	4391	5682.7	1.09
Mean			65612.17			
Std. Dev			304.8425			
% RSD			0.464613			

Table 6: Results of method precision for Glimepiride

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Glimepiride	3.880	8659824	658784	6859.4	1.42	5.68
2	Glimepiride	3.880	8658547	657489	6824.6	1.43	5.69
3	Glimepiride	3.880	8659824	652368	6829.3	1.42	5.68
4	Glimepiride	3.880	8659875	658745	6892.7	1.43	5.69
5	Glimepiride	3.880	8658745	658213	6875.2	1.42	5.68
6	Glimepiride	3.880	8659862	652354	6859.8	1.42	5.69
Mean			8659446				
Std. Dev			623.2924				
% RSD			0.007198				

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

INTERMEDIATE PRECISION/RUGGEDNESS

Table 7: Results of Intermediate precision for Rosiglitazone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rosiglitazone	2.808	66895	4468	5784.2	1.09
2	Rosiglitazone	2.808	66986	4523	5835.1	1.09
3	Rosiglitazone	2.808	66258	4475	5864.4	1.10
4	Rosiglitazone	2.808	66457	4514	5864.6	1.09
5	Rosiglitazone	2.808	66539	4489	5784.9	1.10
6	Rosiglitazone	2.808	66298	4565	5748.5	1.10
Mean			66572.17			
Std. Dev			304.536			
% RSD			0.457452			

Table 8: Results of Intermediate precision for Glimepiride

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Glimepiride	3.882	8758568	669583	6982.4	1.43	
2	Glimepiride	3.882	8756982	665984	6935.3	1.44	5.69
3	Glimepiride	3.882	8746925	665345	6984.7	1.44	
4	Glimepiride	3.882	8723654	665325	6952.8	1.43	5.70
5	Glimepiride	3.882	8754982	669852	6898.9	1.44	
6	Glimepiride	3.882	8754698	665874	6976.5	1.43	5.69
Mean			8749302				
Std. Dev			13188.56				
% RSD			0.150738				

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

Table 9: Accuracy (recovery) data for Rosiglitazone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	35921.67	30	30.134	100.446%	100.30%
100%	70894.33	60	60.205	100.341%	
150%	105654.7	90	90.093	100.103%	

- The % Recovery for each level should be between 98.0 to 102.0%.

Table 10: Accuracy (recovery) data for Glimepiride

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4276302	50	50.208	100.416%	100.21%
100%	8484717	100	100.148	100.148%	
150%	10160609	150	150.091	100.060%	

- The percentage recovery was found to be within the limit (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Linearity

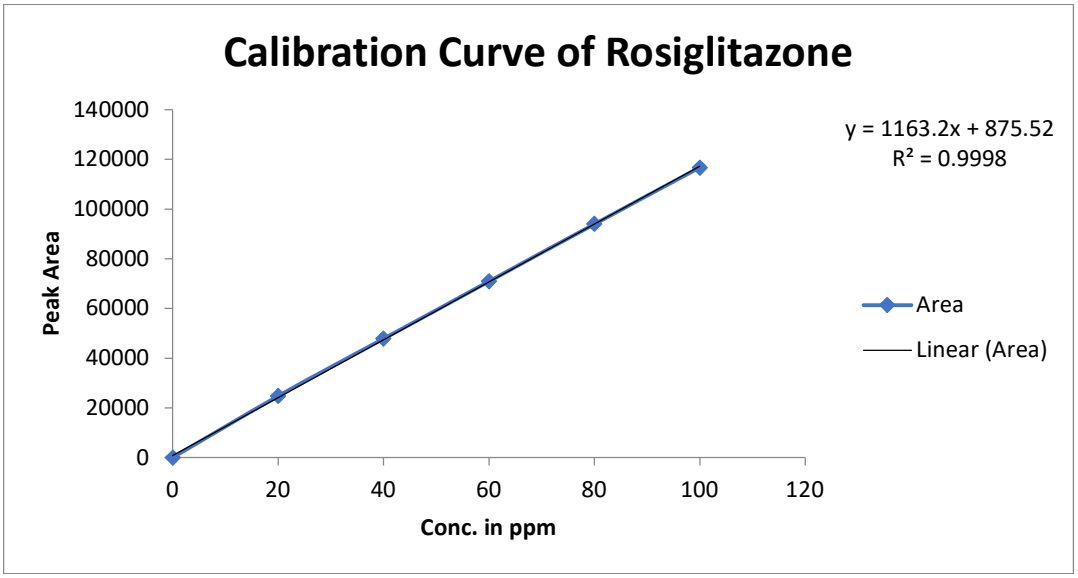

Fig 2: Calibration graph for Rosiglitazone

Linearity Results: (for Rosiglitazone)

S.No.	Linearity Level	Concentration (ppm)	Area
1	I	20	24759
2	II	40	47859
3	III	60	70898
4	IV	80	93985
5	V	100	116698
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.999.

Linearity Results: (for Glimepiride)

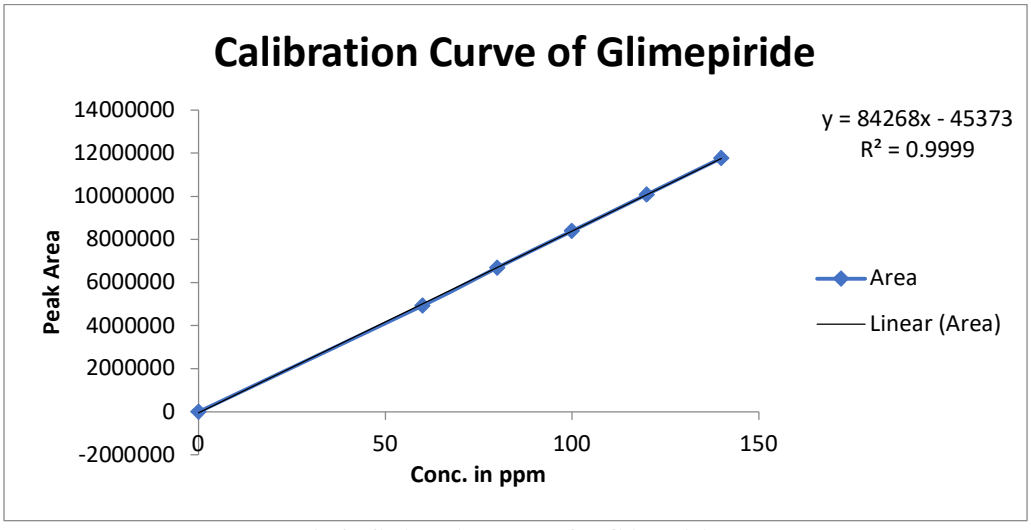

Fig 3: Calibration graph for Glimepiride

S.No.	Linearity Level	Concentration(ppm)	Area
1	I	60	4928578
2	II	80	6687842
3	III	100	8389878
4	IV	120	10085847
5	V	140	11769854
Correlation Coefficient			0.999

- Correlation coefficient should be not less than 0.99.

Robustness

Table 11: System suitability results for Rosiglitazone

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5895.3	1.12
2	*Actual	5685.4	1.08
3	10% more	5964.2	1.16

Table 12: System suitability results for Glimepiride

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6785.2	1.46
2	*Actual	6895.7	1.42
3	10% more	6982.4	1.49

CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Rosiglitazone and Glimepiride in bulk and tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Rosiglitazone and Glimepiride.

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