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Research

Rp- Hplc Method Development And Validation For Estimation Of Rosiglitazone In Bulk And Pharmaceutical Tablet Dosage Form

Thota Shivaranjani*[1], K. Chaitanya Prasad[1], P. Vedavahini[1], B. Sudhakar[1], K. Radhika[1]

[1]*Department Of Pharmaceutical Analysis, Samskruti College Of Pharmacy In Ghatkesar, Telangana. 501301.*

*Author for Correspondence: Thota Shivaranjani

Email: shivaranjinithota12@gmail.com

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Abstract

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Rosiglitazone in its pure form as well as in tablet dosage form. Chromatography was carried out on Apollo C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol (100%v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 265nm. The retention time of the Rosiglitazone was 3.379 min. The method produce linear responses in the concentration range of 25-125ppm of Rosiglitazone. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Rosiglitazone, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

INTRODUCTION TO HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice 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.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.[6]

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

Based on Principle of Separation

Liquid/solid chromatography (Adsorption)

LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.[3]

Liquid/Liquid chromatography (Partition Chromatography)

LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.[1,2]

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions. [3.16,17]

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers. [3,15]

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity. [3]

Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength. [3]

Based on the scale of operation

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC.

Based on type of analysis

Qualitative analysis: Which is used to identify the compound,detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample.[3]

MATERIALS AND METHODS

Rosiglitazone(Pure Drug)-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 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 0.75ml of the above Rosiglitazone 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 Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol in proportion 100% v/v.

Optimization of Column

The method was performed with various columns like ODS column, Symmetry columns. Apollo C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape at 0.9ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	35ºC
Column	:	Apollo C18 (4.6 x 150mm, 5µm)
Mobile phase	:	Methanol (100% v/v)
Flow rate	:	0.9ml/min
Wavelength	:	265nm
Injection volume	:	10µl
Run time	:	10min

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 1000ml (100%) of HPLC Methanol in a volumetric flask and use as diluent.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol (100% V/V)

Column : Apollo C18 (4.6 x 150mm, 5µm)

Column temperature : 35ºC
Wavelength : 265nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 10min

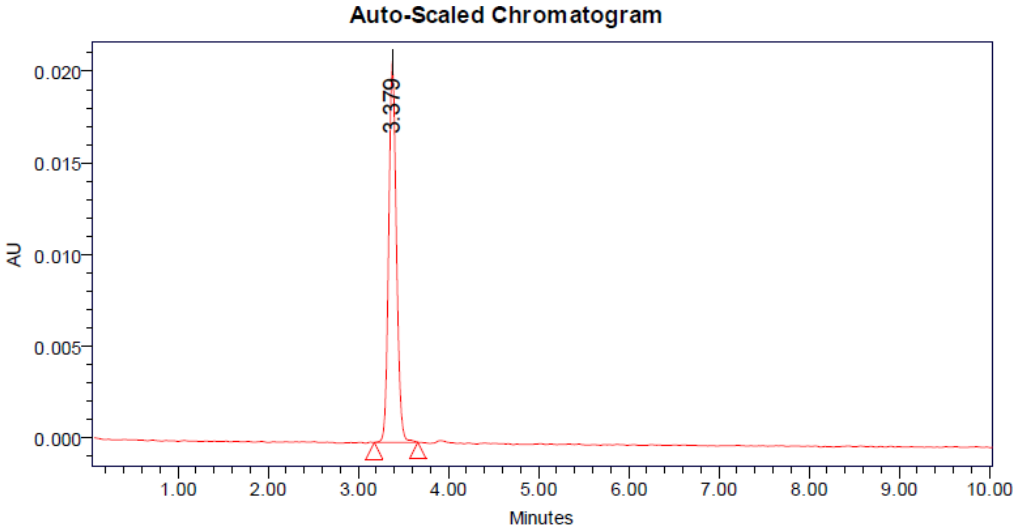


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Rosiglitazone	3.379	121849	20181	1.12	7482

Observation

In this above trail it was observed that proper elution of sample peak. And it shows proper baseline, tailing and plate count in the chromatogram. So it's an optimized chromatogram.

Optimized Chromatogram (Sample)

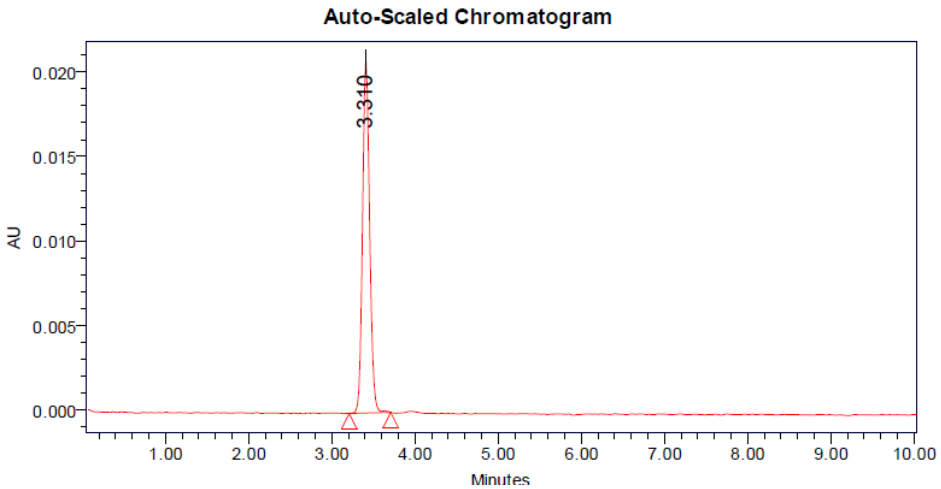


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Rosiglitazone	3.310	128472	20183	1.13	6927

Theoretical plates must be not less than 2000, Tailing factor not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosiglitazone	3.379	123249	20635	7434	1.10
2	Rosiglitazone	3.303	123324	20587	7483	1.09
3	Rosiglitazone	3.322	124060	20690	7550	1.10
4	Rosiglitazone	3.327	124322	20883	7636	1.10
5	Rosiglitazone	3.310	123689	20774	7575	1.10
Mean			123728.8			
Std. Dev.			462.9349			
% RSD			0.374153			

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

Assay (Sample)

Table 4: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Rosiglitazone	3.310	123689	20774	1.10	7575	2
2	Rosiglitazone	3.398	123350	20725	1.09	7499	3
3	Rosiglitazone	3.388	122444	206632	1.11	7545	1

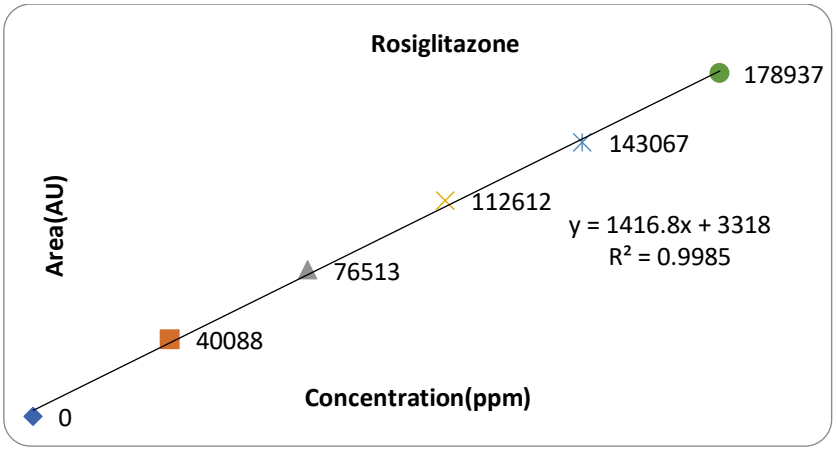
$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Rosiglitazone in pharmaceutical dosage form was found to be 99.4 %.

LINEARITY

Table 5: Chromatographic Data For Linearity Study

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	25	40088
66.6	50	76513
100	75	112612
133.3	100	143067
166.6	125	178937



Mefenamic Acid

Concentration µg/ml	Average Peak Area
187.5	2080032
218.75	2452782
250	2821426
281.25	3226009
312.5	3587393

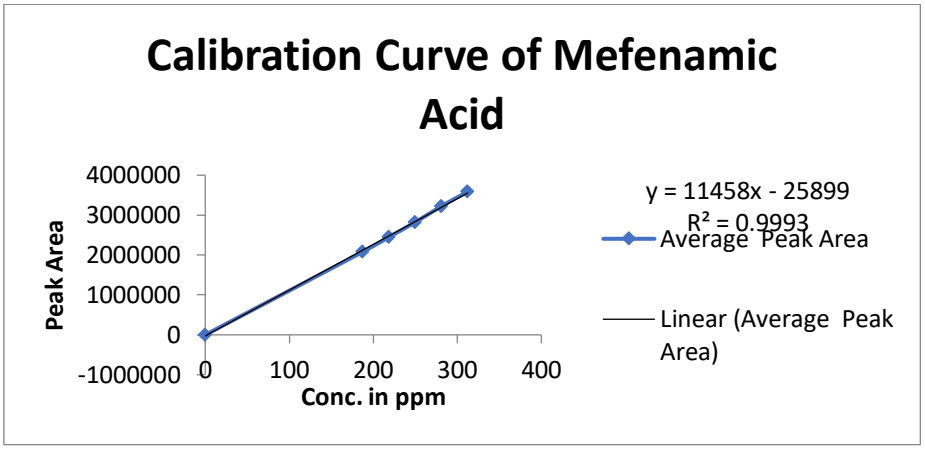


Fig 5: Calibration Graph for Mefenamic Acid

REPEATABILITY

Table 6: Results of repeatability for Rosiglitazone:

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosiglitazone	3.397	122374	20741	7605	1.09
2	Rosiglitazone	3.390	122148	20792	7524	1.10
3	Rosiglitazone	3.384	122845	20969	7592	1.11
4	Rosiglitazone	3.378	121881	20889	7585	1.11
5	Rosiglitazone	3.364	121166	20879	7620	1.09
Mean			122082.8			
Std.dev			622.7445			
%RSD			0.5101			

- %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

Table 7: Results of Intermediate precision for Rosiglitazone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Rosiglitazone	3.371	123187	20953	7547	1.09
2	Rosiglitazone	3.376	122048	20860	7717	1.09
3	Rosiglitazone	3.382	123165	20800	7557	1.09
4	Rosiglitazone	3.359	121599	20877	7584	1.10
5	Rosiglitazone	3.333	120553	20557	7545	1.09
6	Rosiglitazone	3.341	121567	20798	7496	1.09
Mean			122019.8			
Std. Dev.			1020.611			
% RSD			0.836431			

- %RSD of Six different sample solutions should not more than 2

Table 8: Results of Intermediate precision Analyst 2 for Rosiglitazone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
	Rosiglitazone	3.310	123689	20774	7575	1.10
2	Rosiglitazone	3.388	122444	20632	7545	1.11
3	Rosiglitazone	3.378	121881	20889	7585	1.11
4	Rosiglitazone	3.333	120553	20557	7545	1.09
5	Rosiglitazone	3.341	121567	20798	7496	1.09
6	Rosiglitazone	3.396	120695	37100	7830	1.10
Mean			121804.8			
Std. Dev.			1167.887			
% RSD			0.958818			

- %RSD of Six different sample solutions should not more than 2.

ACCURACY

Table 9: The accuracy results for Rosiglitazone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	57296	37.5	37.5	100	99.2%
100%	108656	75	74.8	98.8	
150%	161078.7	112.5	112.4	98.8	

The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 10: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9 mL/min	123249	3.379	7434	1.10
Less Flow rate of 0.8mL/min	132863	3.595	7754	1.12
More Flow rate of 1.0mL/min	114218	3.122	7233	1.09

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Rosiglitazone in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Rosiglitazone was freely soluble in ethanol, methanol and sparingly soluble in water. Water and Acetonitrile was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Rosiglitazone in bulk drug and in Pharmaceutical dosage forms.

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