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QBD-Oriented Assay Method Development for the Estimation of Rosiglitazone and Pioglitazone in Bulk and Formulated Dosage Forms

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Abstract:
This study presents the Quality by Design (QbD)-driven development and validation of a robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Rosiglitazone and Pioglitazone in bulk and pharmaceutical formulations. Employing a Box-Behnken Design (BBD) via Design Expert software, critical method parameters—flow rate, buffer pH, and organic phase ratio were systematically optimized. Statistical validation through ANOVA confirmed the model's significance, particularly in enhancing resolution and minimizing tailing. The method was validated in accordance with ICH Q2(R1) guidelines, demonstrating excellent system suitability, accuracy (98–102% recovery), precision (%RSD < 2%), linearity (R^2 > 0.999), sensitivity (acceptable LOD and LOQ), and robustness under varied analytical conditions. The resolution between the two analytes exceeded the threshold of 2, ensuring effective separation. These results affirm the method's reliability, reproducibility, and suitability for routine quality control of Rosiglitazone and Pioglitazone in pharmaceutical dosage forms.
Keywords: Quality by Design (QbD), RP-HPLC, Rosiglitazone, Pioglitazone, ICH Q2(R1) guidelines, LOD, LOQ, robustness, ANOVA

Introduction

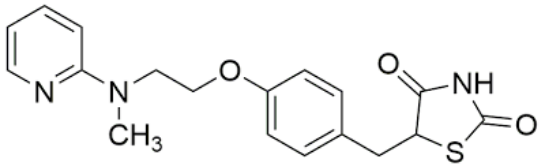

Fig.1: Rosiglitazone

Basic Information
IUPAC Name: (RS)-5-[4-[2-(methyl(pyridin-2-yl)amino)ethoxy] benzyl] thiazolidine-2,4-dione1
Molecular Formula: $C_{18}H_{19}N_3O_3S1$
Molecular Weight: 357.43 g/mol1
Melting Point: 122 to 123 °C1
pKa: Approximately 6.81
Category: Antidiabetic (Thiazolidinedione)1
Solubility: Poorly soluble in water1

Description
Rosiglitazone is an antidiabetic drug in the thiazolidinedione class. It works as an insulin sensitizer, helping to improve blood sugar control in adults with type 2 diabetes mellitus12.

Mechanism of Action
Rosiglitazone binds to peroxisome proliferator-activated receptor gamma (PPARγ) in fat cells, making the cells more responsive to insulin. This action helps to lower blood glucose levels.

Pharmacodynamics
Rosiglitazone enhances insulin sensitivity in muscle and adipose tissue and inhibits hepatic gluconeogenesis, leading to improved glycemic control.

Pharmacokinetics
Absorption: Well absorbed from the gastrointestinal tract.
Distribution: Widely distributed in body tissues.
Metabolism: Metabolized in the liver primarily by CYP2C81.
Route of Elimination: Primarily excreted in the urine (64%) and feces (23%).
Protein Binding: Approximately 99.8%.
Half-Life: Around 3 to 4 hours.

Uses: Primary Use: Treatment of type 2 diabetes mellitus.
Other Uses: Often used in combination with other antidiabetic agents.

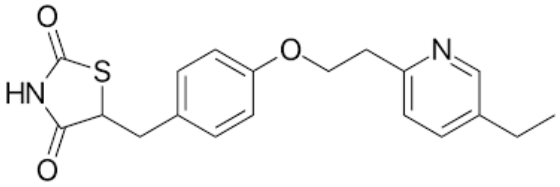

Fig.2: Pioglitazone

Basic Information
IUPAC Name: (RS)-5-[4-[2-(methyl(pyridin-2-yl) amino) ethoxy] benzyl] thiazolidine-2,4-dione1
Molecular Formula: $C_{21}H_{20}N_2O_3S1$
Molecular Weight: 356.46 g/mol1
Melting Point: 182-183 °C1
pKa: Approximately 5.91
Category: Antidiabetic (Thiazolidinedione)
Solubility: Poorly soluble in water1

Description
Pioglitazone is an oral antidiabetic medication used to improve blood sugar control in adults with type 2 diabetes mellitus. It is not used for treating type 1 diabetes.

Mechanism of Action
Pioglitazone works by increasing the sensitivity of liver, fat, and muscle cells to insulin. This helps the body use insulin more effectively, reducing blood sugar levels.

Pharmacodynamics

Pioglitazone enhances insulin sensitivity by activating peroxisome proliferator-activated receptor gamma (PPARγ), which regulates the transcription of insulin-responsive genes involved in glucose and lipid metabolism.

Pharmacokinetics

Absorption: Well absorbed from the gastrointestinal tract.
Distribution: Widely distributed in body tissues.
Metabolism: Metabolized in the liver primarily by CYP2C8 and CYP3A4.
Route of Elimination: Primarily excreted in the urine (15-30%) and feces (55%) as metabolites.
Protein Binding: Approximately 99%.
Half-Life: Around 3 to 7 hours.
Uses: Primary Use: Treatment of type 2 diabetes mellitus.
Other Uses: Often used in combination with other antidiabetic agents.

Materials and Methods

Preparation of buffer and mobile phase:

Preparation of Phosphate buffer PH-3.5:

To prepare Sodium phosphate buffer solution, by adding 6.4gm of phosphate buffer in 1000ml water. Adjust this solution to ph 3.5 by using Ortho Phosphoric Acid.

Preparation of mobile phase:

Mix a mixture of above 350ml of Phosphate buffer (35%) and MEOH 650ml (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Phosphate buffer pH-3.5: MEOH (35:65) ratio.

Validation parameters:

Assay:

Standard Solution Preparation:

Accurately weigh and transfer 16.67mg of Rosiglitazone and 25mg Pioglitazone working standard into a 25ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.9ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (60ppm, 90 ppm).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 16.67mg of Rosiglitazone and 25mg Pioglitazone equivalent weight of the sample into a 25ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.9ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (60ppm, 90 ppm).

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for the Rosiglitazone and Pioglitazone peaks**.**

Calculation: (For Rosiglitazone and Pioglitazone)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC= Label Claim mg/ml.

Results and Discussion

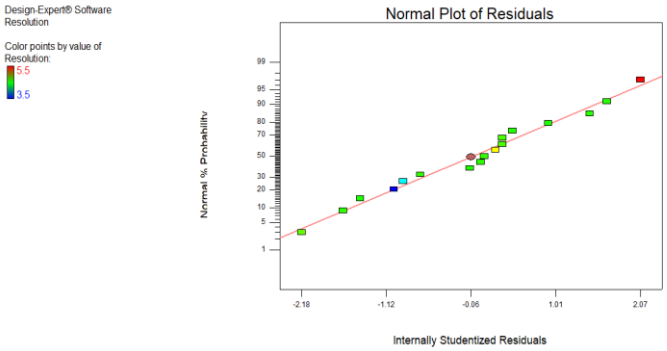

Fig.3: Normal plot of Residuals for Pioglitazone and Rosiglitazone

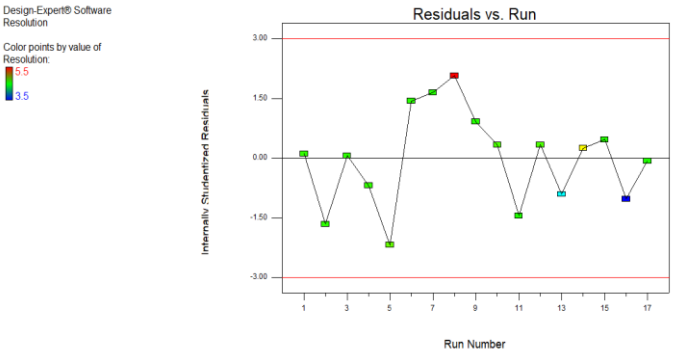

Fig.4: Residuals vs. Run for Pioglitazone and Rosiglitazone

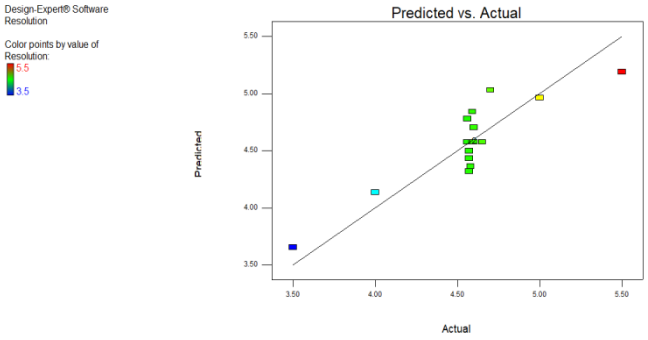

Fig.5: Predicted vs. Actual for Pioglitazone and Rosiglitazone

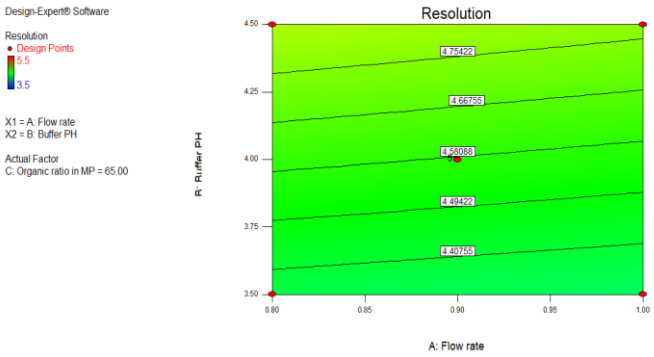

Fig.6: Resolution for Pioglitazone and Rosiglitazone

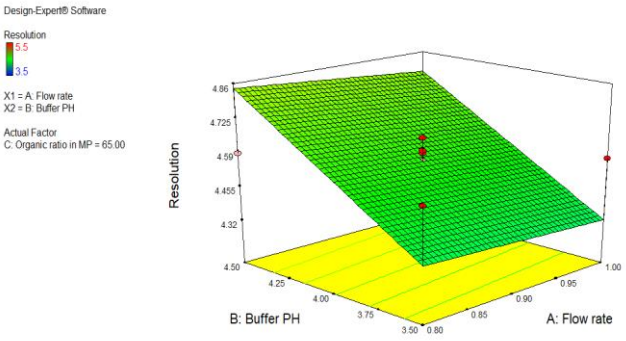

Fig.7: 3D Surface for Pioglitazone and Rosiglitazone

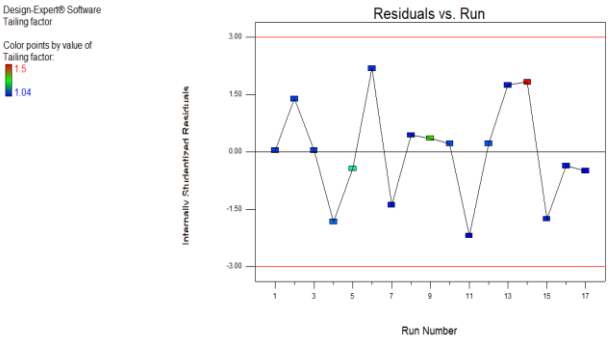

Fig.8: Residuals vs. Run for Pioglitazone and Rosiglitazone

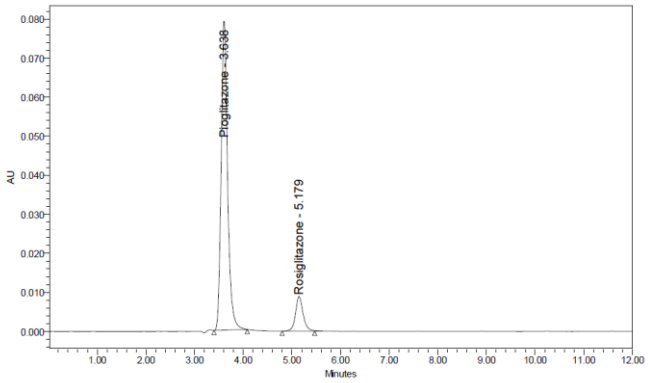

Fig.9: Chromatogram for system suitability

Optimized Chromatographic Conditions

Equipment : High performance liquid chromatography equipped with Auto Sampler and PDA detector
Column : YMC ODS (4.6*250mm, 5µm)
Buffer : Phosphate buffer
PH : 3.5
Mobile phase : 35% buffer: 65% Methanol
Flow rate : 1.0 ml per min
Wavelength : 240 nm
Injection volume : 20 µl
Run time : 12 min.

Table.2: Results of system suitability parameters

S.No	Name	RT(min)	Area(µV sec)	Height (µV)	USP tailing	Resolution	USP plate count
1	Pioglitazone	3.638	**244125**	**77290**	**1.20**	**4.90**	**4012**
2	Rosiglitazone	5.179	**33618**	**8244**	**1.11**		**3021**

Table 3: Results of Assay for Rosiglitazone and Pioglitazone

	Label Claim (mg)	% Assay
Rosiglitazone	4mg	99.4
Pioglitazone	15mg	100.4%

Table 4: Analytical performance parameters of Rosiglitazone and Pioglitazone

Parameters	Rosiglitazone	Pioglitazone
Slope (m)	563.31	2723.5
Intercept (c)	316.05	2369.9
Correlation coefficient (R^2)	0.9994	0.9993

Table 5: Results of Precision for Rosiglitazone and Pioglitazone

Injection	Area of Rosiglitazone	Area of Pioglitazone
Injection-1	321751	244156
Injection-2	321144	242321
Injection-3	322651	244051
Injection-4	323514	244393
Injection-5	324984	244671
Injection-6	320124	244051
Average	322361.3	243940.5
Standard Deviation	1740.747	828.4959
%RSD	0.5	0.3

Table 6: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	CONC
Rosiglitazone	57	167	2.93	1.22µg/ml
Pioglitazone	57	162	2.84	0.19µg/ml

Table 7: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	CONC
Rosiglitazone	57	566	9.93	4.12µg/ml
Pioglitazone	57	550	9.65	0.64µg/ml

Conclusion

The present study focused on the Quality by Design (QbD)-oriented development and validation of a robust, accurate, and reliable RP-HPLC method for the simultaneous estimation of Rosiglitazone and Pioglitazone in bulk and formulated dosage forms. Utilizing a Box-Behnken Design (BBD) within Design Expert software, the method was optimized by assessing the effects of critical variables such as flow rate, buffer pH, and organic ratio in the mobile phase. The statistical analysis through ANOVA confirmed the significance of the model, particularly in improving resolution and tailing factor. A comprehensive validation, as per ICH Q2(R1) guidelines, demonstrated that the method met all acceptance criteria across various parameters including system suitability, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and robustness.

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