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UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PIOGLITAZONE IN BULK AND SUSTAINED-RELEASE TABLET DOSAGE FORM

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ABSTRACT

Pioglitazone is an orally administered insulin-sensitizing thiazolidinedione agent developed for the treatment of type 2 diabetes mellitus. It works by activating the nuclear peroxisome proliferator-activated receptor gamma (PPAR-γ), which increases the transcription of proteins involved in glucose and lipid metabolism. These proteins enhance the post-receptor actions of insulin in the liver and peripheral tissues, leading to improved glycaemic control without increasing endogenous insulin secretion. In placebo-controlled clinical trials, monotherapy with pioglitazone at doses of 15–45 mg/day has been shown to reduce glycosylated haemoglobin (HbA1c) levels in patients with type 2 diabetes mellitus. Additionally, combining pioglitazone 30 mg/day with pre-existing therapy such as metformin, or adding pioglitazone 15 or 30 mg/day to treatments with sulphonylureas, insulin, or voglibose, has demonstrated significant reductions in HbA1c and fasting blood glucose levels in patients with poorly controlled diabetes. Pioglitazone has also been associated with improvements in serum lipid profiles in randomized placebo-controlled studies. The drug has generally been well tolerated in adult patients of all ages. Oedema has been reported during monotherapy, and pooled data indicate that hypoglycaemia occurs in approximately 2–15% of patients when pioglitazone is added to sulphonylurea or insulin therapy. No cases of hepatotoxicity have been reported.

INTRODUCTION

Pioglitazone (commonly sold as Actos) is an oral anti-diabetic medication from the thiazolidinedione (TZD) class used to manage type 2 diabetes by enhancing insulin sensitivity. Approved in 1999, it functions as a PPARγ agonist that reduces hepatic glucose production and increases glucose uptake in muscles. It is taken once daily, often in combination with metformin or insulin, to improve glycemic control [1, 2].

Drug Profile

Pioglitazone is an oral anti- diabetic medication used to manage blood sugar levels in adults with type 2 diabetes. It is usually prescribed alongside diet and exercise. It belongs to thiazolidinedione [TZD] class. It is used often in combination
ith metformin , sulfonylureas, [or] insulin . It works by increasing the body's sensitivity to its own insulin, particularly in muscle, faand liver tissues. It acts by improves insulin sensitivity, lowers blood sugar levels and reduces liver fat [3].

Chemical formula

$C_{19}H_{20}N_2O_3S$

MECHANISM OF ACTION:Pioglitazone works by activating the Peroxisome Proliferator-Activated Receptor-gamma (PPARγ), a nuclear receptor, primarily in fat, muscle, and liver tissues, which improves the body's sensitivity to insulin, decreases

glucose production by the liver, and increases glucose uptake by peripheral tissues,effectively lowering blood sugar in Type 2 diabetes. It's an insulin sensitizer, meaning it helps your own insulin work better rather than increasing insulin secretion, by regulating genes involved in glucose and lipid metabolism [4-6].

MATERIALS & METHOD

Pioglitazone, commonly used as Pioglitazone Hydrochloride is an oral anti-hyperglycemic agent in the thiazolidinedione class. Its chemical analysis and formulation involve specific reagents, solvents, and excipients to handle its poor aqueous solubility (BCS Class II).

Instrument

Pioglitazone primarily refers to the analytical instruments used for its identification, quantification, and quality control, as well as the manufacturing equipment for itsformulation.The most common instrumental technique is High-Performance Liquid Chromatography (HPLC) [7].

METHOD DEVELOPMENT [8-9]

Preparation of stock solution

The preparation of a stock solution of Pioglitazone Hydrochloride (a BCS Class-II drug) primarily involves dissolving the powder in an organic solvent due to its low aqueous solubility. Methanol is the most commonly used solvent for creating a standard stock solution.

Common Method for 1000 g/mL (1 mg/mL) Stock Solution

1. Weighing: Accurately weigh 10 mg (or 100 mg for higher volume) of Pioglitazone HCl.
2. Transfer: Transfer the weighed powder into a 10 mL (or 100 mL) volumetric flask.
3. Dissolution: Add a small amount of methanol (approximately 5-7 mL) to the flask. Sonication may be used to enhance solubility.
4. Volume Adjustment: Make up the volume to the mark with methanol.
5. Final Concentration: This results in a 1000 g/mL (1000 ppm) stock solution.

Alternative Methods Based on Application:

- For HPLC (Mobile Phase): Dissolve 30 mg in 10 mL of dimethylformamide (DMF) or methanol, then dilute to 100 mL with the mobile phase (e.g., ACN:Buffer).
- For UV Spectroscopy (Acidic Media): Dissolve 100 mg of Pioglitazone in a small quantity of 0.1 N HCl, then make up to 100 mL with 0.1 N HCl (1000 mg/100mL).
- For In-Vitro Release (pH 7.4 Buffer): Dissolve 100 mg in 40 mL of methanol, then make up to 100 mL with phosphate buffer pH 7.4.

Preparation of working standard solution

The preparation of a working standard solution of Pioglitazone involves creating a highly concentrated stock solution followed by dilution to a lower, usable range for analytical techniques such as UV-Spectrophotometry or HPLC. Pioglitazone hydrochloride is commonly soluble in methanol, acetonitrile, and dimethyl sulfoxide (DMSO).

1. Preparation of Standard Stock Solution (1000 µg/mL)

- Accurately weigh 10 mg (or 100 mg, depending on scale) of Pioglitazone Hydrochloride working standard.
- Transfer to a 10 mL (or 100 mL) volumetric flask.Add 5–7 mL of HPLC-grade methanol (or a mixture of acetonitrile/methanol) to dissolve the drug.
- Sonicate for 5–10 minutes to ensure complete dissolution.
- Make up to the mark with the same solvent to achieve a 1000 µg/mL (1 mg/mL) stock solution.

2. Preparation of Working Standard Solution

Working solutions are generally prepared in the linear range of 1–50 µg/mL for UV or similar ranges for HPLC, often using the mobile phase as a diluent.

- Pipette 0.5 mL to 5 mL of the 1000 µg/mL stock solution into a 100 mL volumetric flask.
- Dilute to the mark with mobile phase (e.g., Methanol:Water 75:25 or Acetonitrile:Buffer) to obtain working concentrations (e.g., 5 µg/mL to 50 µg/mL).

3. Alternative Method (Dilution by Volume)

- Pipette 1 mL of the 1000 µg/mL stock solution into a 10 mL volumetric flask.
- Dilute to the mark with solvent to make a 100 µg/mL intermediate solution.
- Further dilute (e.g., 1 mL to 10 mL) to achieve a 10 µg/mL working solution.

Determination of λ max

The standard stock solution of pioglitazone was diluted suitably to get a concentration of 10µg/ml andscanned within the range of 271nm.

Experimental Procedure of Pioglitazone

Experimental procedures for Pioglitazone focus primarily on HPLC analysis, dissolution studies, or formulation development. A standard RP-HPLC method involves using a C18 column, a mobile phase of acetonitrile and ammonium acetate (often pH 4.1-4.5) and sample preparation using solvents like methanol or dimethylformamide.

1. Analytical Quantification (RP-HPLC)

The most common experimental procedure for determining Pioglitazone in bulk or tablet form involves

2. Solubility Enhancement Experiments

To improve its low aqueous solubility, researchers often use Solid Dispersion or Cyclodextrin Complexation.

- Kneading Method: Drug and cyclodextrin are mixed in a 1:1 molar ratio, made into a paste with aqueous ethanol, triturated for 15–20 minutes, and dried.

- Spray Drying: Pioglitazone is dissolved in an ethanol-acetone mixture, mixed with a carrier, and processed through a Mini spray drier with controlled inlet/outlet temperatures (e.g., 65°C/55°C).

3. Bioanalytical Procedures (Plasma Analysis)

- **Extraction:** Liquid-liquid extraction using Ethyl Acetate or Solid Phase Extraction (SPE) with C18 cartridges.
- **Internal Standards:** Rosiglitazone or Piroxicam are commonly used as internal standards to ensure accuracy.
- **Sample Processing:** Plasma proteins are precipitated using Methanol or Acetonitrile, followed by centrifugation (e.g., 4000 rpm for 10 min).

4. Dissolution Testing

Dissolution experiments follow USP guidelines to ensure therapeutic efficacy.

- **Apparatus:** USP Type II (Paddle) is typically used at 50–100 rpm.
- **Medium:** 0.1 N HCl (to simulate gastric fluid) or phosphate buffer (pH 7.4) is used to observe the drug release pattern over 12–24 hours.

Method Validation

Method validation of Pioglitazone is commonly performed using reversed-phase high-performance liquid chromatography (RP-HPLC) or UV spectroscopy, adhering to ICH guidelines. Key parameters include linearity (typically 1–20 µg/mL to 5-50 µg/mL), precision, accuracy, specificity, and robustness, ensuring accurate determination of the drug in bulk and tablet formulations.

Common HPLC Conditions

- **Column:** C18 (e.g., Hypersil BDS, Inertsil).
- **Mobile Phase:** Acetonitrile : Phosphate Buffer (e.g., 60:40 or 55:45).
- **Flow Rate:** 1.0 ml/min

Retention Time: Approximately 4.7–8.0 minutes [10-11.

RESULTS AND DISCUSSION:

Linearity

A calibration curve was plotted using concentration (on x-axis) against absorbance at 271nm (on y-axis) from the graph linearity regression co-efficient yintercept was calculated.

Table 1: Linearity studies of Pioglitazone in Bulk form

Table :2: Linearity studies of Pioglitazone in Tablet Dosage form

Concentration	Absorb
1	0.1
1	0.3
2	0.5
2	0.7
3	0.9

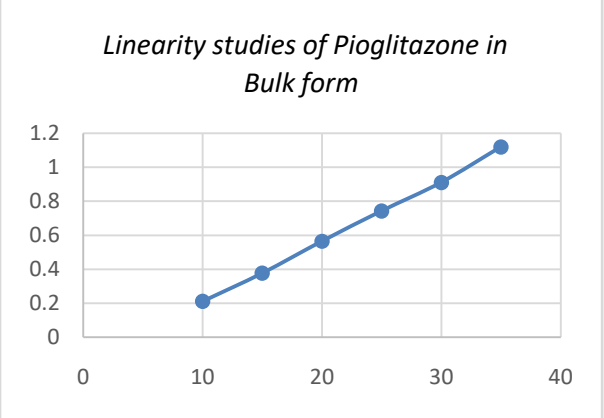

Fig 1: Linearity studies of Pioglitazone in Bulk form

The linearity regression co-efficient was more than 0.99 and hence the method was said to obey Beer's law and there is a linear and proportional relationship exists between concentration and absorbance.

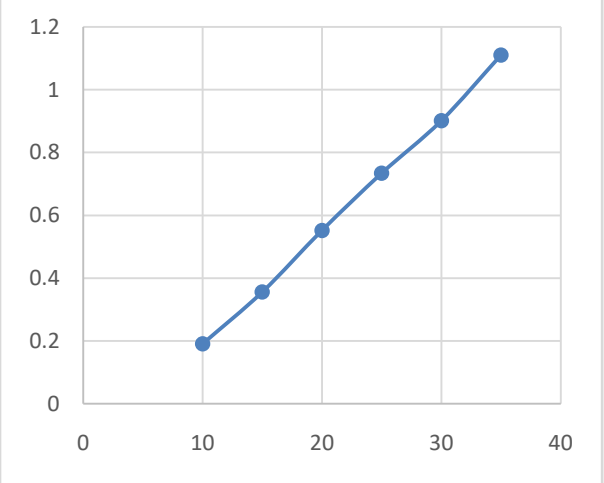

Fig 2: Linearity studies of Pioglitazone in Tablet Dosage Form

Accuracy

The accuracy of the method was demonstrated by a recovery experiment performed at three different levels: 75%, 100%, and 125%. Three solutions of different concentrations, namely 11.25 µg/mL, 15 µg/mL, and 18.75 µg/mL, were prepared and analyzed on the same day, and the absorbance was recorded.

According to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, the %RSD value should not exceed 2%.

The formula for calculating %RSD is: %RSD = (SD / Mean) × 100

Table 3: Accuracy studies of Pioglitazone in Bulk form

S.No	Concentration (µg/ml)	Samples	Absorbance at 271 nm	Statistical Analysis
1	75%	15	0.361	Mean = 0.360333 S.D = 0.0015275 %RSD = 0.160227
		15	0.360	
		15	0.360	
2	100%	20	0.561	Mean = 0.561333 S.D = 0.0005774 %RSD = 0.102853
		20	0.561	
		20	0.562	
3	125%	25	0.745	Mean = 0.744667 S.D = 0.001 %RSD = 0.077531
		25	0.744	
		25	0.745	

Table 4: Accuracy studies of Pioglitazone in Tablet Dosage form

S.No	Concentration (µg/ml)	Samples	Absorbance at 271nm	Statistical Analysis
1	75%	15	0.376	Mean = 0.375333 S.D = 0.000577 %RSD = 0.153823
		15	0.375	
		15	0.375	
2	100%	20	0.574	Mean = 0.573333 S.D = 0.000577 %RSD = 0.100701
		20	0.573	
		20	0.573	
3	125%	25	0.764	Mean = 0.763333 S.D = 0.000577 %RSD = 0.075635
		25	0.763	
		25	0.763	

Precision

From the above prepared standard stock solution, 10 ml of the solution was dilutedto 100 ml using methanolto get a concentration of 100 µg/ml. From the above solution 6 ml of solutions were pipetted out into 6 different 100 ml volumetric flasks and the volume was made with distilled water to get the final concentrations of 15 µg/ml. The calculated percentage relativestandard deviation (% RSD) of the results was used to evaluate the method precision.According to ICH guidelines, the %RSD should not be exceeded upto 2%.

Table 5: Precision studies of Pioglitazone in Bulk form

S.No	Samples(µg/ml)	Absorbanceat271 nm	Statistical Analysis
1	20	0.765	Mean = 0.765833 S.D = 0.000983 % RSD = 0.128382
2	20	0.765	
3	20	0.767	
4	20	0.767	
5	20	0.765	
6	20	0.766	

Table 6: Precision studies of Pioglitazone in Tablet Dosage form

S.No	Samples(g/ml)	Absorbanceat271 nm	Statistical Analysis
1	20	0.771	Mean = 0.772167 S.D = 0.000753 % RSD = 0.097488
2	20	0.772	
3	20	0.772	
4	20	0.773	
5	20	0.773	
6	20	0.772	

Pioglitazone in Bulk Form

The method was developed and validated as per ICH guidelines.

The method was validated in terms of Linearity, Precision, Accuracy, LOD, LOQ and Molar absorptivity.

Detection wavelength was selected at 271 nm.

Linearity in response was observed on 5 – 25 µg/ml.

The linearity equation was found to be y = 0.0371x – 0.0174, R2 = 0.9999.

The precision results showed a % RSD = 0.422% at each level, clearly indicating that the method was precise enough for the analysis of Pioglitazone. The accuracy of the method was checked by recovery studies. The LOD = 0.06489 µg/ml and LOQ 0.45151 µg/ml indicate sensitivity of the method. The molar absorptivity was found to be 0.03712 mol-1cm-1.

Pioglitazone in Tabletdosage form

The method was developed and validated as per ICH guidelines. The method wasvalidated in terms of Linearity, Precision, Accuracy, LOD, LOQ and Molar absorptivity. Detection wavelength was selected at 271nm. Linearity in response was observed on 5 – 25 µg/ml. The linearity equation was found to be y = 0.0373x – 0.0207, R2 = 0.9998.

The precision results showed a % RSD = 0.3429% at each level, clearly indicating that the method was precise enough for the analysis of Pioglitazone. The accuracy of the method was checked by recovery studies. The LOD = 0.45151µg/ml and LOQ = 1.36823 µg/ml indicate sensitivity of the method. The molar absorptivity was found to be 0.03734 mol-1cm-1.

CONCLUSION

A Validation UV spectrophotometric method has been developed for the Estimationof pioglitazone in bulk and tablet dosage form. In this proposed method the linearitywas Observed in the concentration range of 5-25µg/ml with correlation coefficientR-0.999 for pioglitazone at 271nm. The developed method was found to be2 simple, accurate, precise, specific, reproducible and linear over the concentration range studies. The proposed method can be used for routine analysis of pioglitazone. The method wasvalidatedasper ICH guideline

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CONFLICT OF INTEREST

Not declared

INFORMED CONSENT AND ETHICAL STATEMENT

Not applicable

AUTHOR CONTRIBUTIONS

Both are contributed equally.

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