JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

REVIEW ARTICLE

A Review on Analytical Methods for Determination of Pioglitazone with RBG and White Analysis

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Publication history: Received on 24th Mar 2025; Revised on 16th April 2025; Accepted on 20th April 2025

Article DOI: 10.69613/ba35sx50

Abstract: Modern pharmaceutical analysis requires precise, accurate, and environmentally friendly methods for drug determination. The presented work discusses about analytical techniques for quantifying pioglitazone, its metabolites, and combinations with other anti-diabetic medications. The main analytical techniques discussed include Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC), UV spectroscopy, Micellar Electrokinetic Chromatography (MEKC), Liquid Chromatography-Mass Spectrometry (LC-MS/MS), and High-Performance Thin-Layer Chromatography (HPTLC). Each method demonstrates unique advantages in sensitivity, specificity, and applicability across various matrices, from pharmaceutical formulations to biological samples. The RP-HPLC methods show excellent linearity (100-600 µg/ml) and precision (RSD ≤ 2%). UV spectroscopic methods offer simplicity with detection limits as low as 0.0002 µg/ml. LC-MS/MS techniques provide superior sensitivity for metabolite detection in plasma samples. The integration of RGB (Red, Blue, Green) and White analysis represents an innovative approach, incorporating environmental considerations while maintaining analytical performance. The use of eco-friendly solvents, such as ethanol, demonstrates the evolution toward sustainable analytical practices. The validation parameters, including linearity, accuracy, precision, detection limits, and robustness, provide essential guidance for method selection in quality control and pharmacokinetic studies. The reported methods offer reliable solutions for pioglitazone analysis while overcoming the environmental concerns through the principles of green chemistry.

Keywords: Pioglitazone; Validation; Metabolites; Anti-diabetic drugs; RGB analysis; White analysis.

1. Introduction

Pioglitazone, a thiazolidinedione derivative, has emerged as a significant pharmaceutical agent in managing type 2 diabetes mellitus. As an agonist of peroxisome proliferator-activated receptor gamma (PPARγ), it plays a crucial role in insulin sensitization and glucose homeostasis [1]. The compound's chemical structure, defined by its IUPAC name 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione, features a molecular formula of $C_{19}H_{20}N_2O_3S$ and molecular weight of 356.4 g/mol [2].

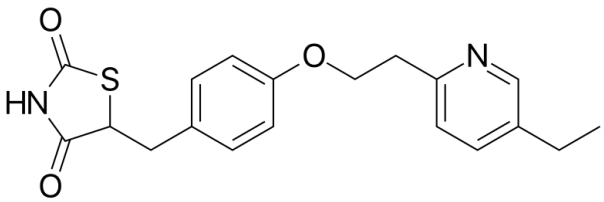

Figure 1. Structure of Pioglitazone

Pioglitazone exhibits characteristics typical of BCS class 2 compounds, with a Log P value of 2.3, indicating low solubility but high permeability. These physicochemical properties significantly influence its analytical determination methods and pharmaceutical formulation strategies [3, 4]. The mechanism of action of pioglitazone involves complex molecular pathways. Upon binding to PPARγ, it initiates the transcription of insulin-responsive genes, leading to enhanced glucose uptake in peripheral tissues. The drug modulates adipokine production, reduces inflammatory markers, and improves lipid profiles, contributing to its therapeutic efficacy [5, 6].

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Recent pharmacological studies have revealed additional benefits of pioglitazone beyond glycemic control. The drug demonstrates potential neuroprotective effects, anti-inflammatory properties, and possible applications in non-alcoholic fatty liver disease management [7, 8]. These expanded therapeutic applications necessitate robust analytical methods for quantification in various biological matrices. The pharmaceutical analysis of pioglitazone presents unique challenges due to its chemical structure and stability considerations. The presence of a thiazolidinedione ring and pyridine moiety requires careful method development to ensure selective and sensitive determination [9, 10]. Moreover, the analysis of pioglitazone in combination with other anti-diabetic drugs adds complexity to analytical method development [11, 12]. The evolution of analytical techniques for pioglitazone determination reflects broader trends in pharmaceutical analysis, including the adoption of green chemistry principles and the integration of advanced instrumental methods. The incorporation of RGB and White analysis represents a novel technique that combines analytical efficiency with environmental consciousness [13, 14]. Regulatory requirements for pioglitazone analysis have become increasingly stringent, necessitating validated methods that meet current pharmaceutical quality standards. The International Council for Harmonization (ICH) guidelines, particularly ICH Q2(R2), provide the framework for method validation, ensuring reliability and reproducibility of analytical results [15, 16]. This work discusses various analytical methods for pioglitazone determination, evaluating their applications, advantages, and limitations. The combination of traditional analytical approaches with modern sustainable practices offers valuable insights for pharmaceutical analysts and researchers working in drug development and quality control.

2. Analytical Techniques for Estimation of Pioglitazone

2.1. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Methods

2.1.1. RP-HPLC Method for Pharmaceutical Dosage Forms

Srinivasulu et al. developed and validated a RP-HPLC method for quantifying pioglitazone hydrochloride in pharmaceutical formulations [3, 4]. The chromatographic conditions employed a C_{18} column (250 × 4.6 mm) with a mobile phase consisting of acetate buffer and acetonitrile in the ratio of 55:45 v/v. The detection wavelength was set at 254 nm with a flow rate of 1.0 ml/min. Further optimization studies by Shaik et al. investigated the effect of mobile phase composition, demonstrating that slight variations in the acetonitrile proportion (43-47%) significantly impacted peak resolution [5, 17]. Temperature control at 25 ± 2°C proved crucial for method reproducibility. System suitability parameters, including theoretical plate count and asymmetry factor, were monitored throughout the analysis to ensure consistent performance.

The validation parameters demonstrated excellent linearity in the range of 100-600 µg/ml with a correlation coefficient of 0.9999. The retention time for pioglitazone was 9.738 minutes with a tailing factor of 1.41 and theoretical plate count of 6794.61. The relative standard deviation was less than 2%, indicating good precision. The method's accuracy was confirmed with mean recoveries between 100.09% and 103.11%. The purity angle (0.11) was less than the purity threshold (0.33), confirming the absence of interfering substances [6, 7]. Subsequent stability studies by Kumar et al. evaluated the method's capability to detect degradation products under various stress conditions including acid hydrolysis, base hydrolysis, oxidation, and thermal degradation [4, 18]. The method successfully separated degradation products with resolution greater than 2.0, establishing its stability-indicating nature [12]

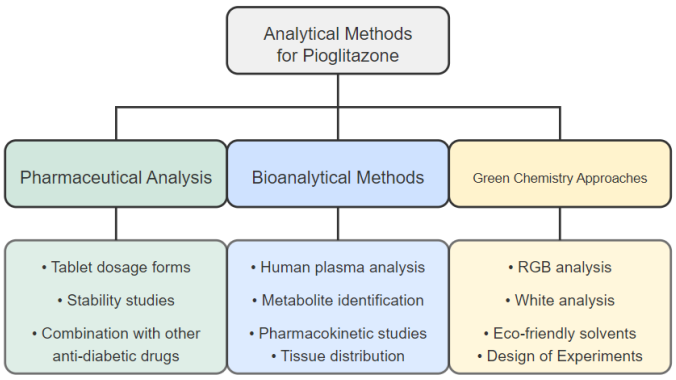

Figure 2. Various Analytical Methods for Determination of Pioglitazone

2.1.2. RP-HPLC Method for Human Plasma Analysis and Pharmacokinetic Studies

Sripalakit et al. developed a RP-HPLC method for estimating pioglitazone in human plasma with applications in pharmacokinetic studies [8, 19]. The method utilized a C18 Apollo column with a mobile phase composed of methanol, acetonitrile, and mixed phosphate buffer (pH 2.6) in the ratio of 40:12:48 v/v. Rosiglitazone was used as an internal standard. The flow rate was 1.2 ml/min with UV detection at 269 nm. The plasma sample preparation protocol involved protein precipitation using acetonitrile, followed

by liquid-liquid extraction with ethyl acetate. This dual extraction approach, refined by Radhakrishna et al., improved recovery rates and minimized matrix effects [9, 20]. The method showed linearity in the concentration range of 50-2000 ng/ml with a correlation coefficient of 0.997. The intraday accuracy and precision were between 94.7% to 97.6% and 2.0% to 8.7%, respectively.

Calixto and Bonato further enhanced the method's sensitivity by incorporating solid-phase extraction, achieving lower detection limits of 25 ng/ml [13, 21]. The modified method demonstrated improved plasma protein removal and reduced ion suppression effects [14].

2.1.3. Simultaneous Determination of Pioglitazone HCl and Metformin HCl

Sahoo et al. developed a RP-HPLC method for the simultaneous determination of pioglitazone hydrochloride and metformin hydrochloride in tablet dosage forms [15, 22]. The chromatographic conditions employed a column of dimensions 25 cm × 4.6 cm with 5.0 µm particle size. The mobile phase consisted of acetonitrile, water, and acetic acid (60:40:0.3), with a flow rate of 1.0 ml/min and detection at 230 nm. The optimization process involved evaluating various buffer systems and organic modifiers. The addition of acetic acid improved peak shape and resolution by suppressing secondary interactions between the analytes and stationary phase. The method demonstrated linearity in the ranges of 0.015-0.120 mg/ml for pioglitazone and 0.5-4.0 mg/ml for metformin, with correlation coefficients of 0.9992 and 0.9975, respectively.

The limits of detection were 0.007 and 0.001, while the limits of quantification were 0.002 and 0.002 for pioglitazone and metformin, respectively. The retention times were 5.348 minutes for pioglitazone and 2.155 minutes for metformin, with tailing factors of 1.05 and 1.28, respectively. The method showed good precision with relative standard deviations of 1.00% and 0.39% for pioglitazone and metformin, respectively [15]. Sujana et al. further validated the method's robustness by studying the effects of minor variations in mobile phase composition (±2%), flow rate (±0.1 ml/min), and column temperature (±2°C) [16, 23]. The method remained reliable under these variations, demonstrating its suitability for routine quality control analysis [17].

2.2. Spectroscopic Methods

2.2.1. UV Spectroscopic Method for Bulk and Pharmaceutical Dosage Forms

Shakya and Singh developed a UV spectroscopic method for the quantification of pioglitazone hydrochloride in bulk and pharmaceutical formulations [10, 24]. Initial solvent selection studies evaluated various combinations, with methanol emerging as the optimal choice due to its suitable polarity and minimal background interference. The method utilized a detection wavelength of 238 nm, identified through wavelength scanning from 200-400 nm to determine the absorption maximum. The method demonstrated linearity in the range of 10-50 µg/ml, conforming to Beer's law criteria. Precision studies included six replicate measurements at three concentration levels (10, 30, and 50 µg/ml). The method showed excellent sensitivity with limits of detection and quantification at 0.0002 and 0.0018 µg/ml, respectively. The slope and intercept of the calibration curve were 0.0018 and 0.0996, indicating good method sensitivity. Extensive stability studies conducted by Kumar et al. confirmed the solutions' stability for up to 72 hours when stored at room temperature [4, 25]. The intraday accuracy bias ranged from 0.18% to 0.025%, while the interday accuracy bias was between 0.23% and 0.28% RSD. The intraday precision bias was 0.4% to 0.04% RSD, and the interday precision bias was 0.2% to -0.02%, indicating excellent reproducibility [12, 13].

2.2.2. LC-MS Method for Pioglitazone and Its Metabolites in Human Plasma

Ponnuri et al. developed a liquid chromatography-mass spectrometry (LC-MS) method for the simultaneous determination of pioglitazone and its metabolites [14]. The method development involved extensive optimization of ionization parameters and mobile phase composition to enhance sensitivity and minimize matrix effects. The chromatographic conditions employed C_8 and C_{18} columns (Kromasil, 50 × 4.6 mm, 5 µm) with a mobile phase consisting of methanol, acetonitrile (1:1), and 10 mM ammonium formate (70:30 v/v). The selection of ammonium formate buffer was crucial for maintaining consistent ionization efficiency. The flow rate was 0.7 ml/min with a run time of 4.0 minutes. Diphenhydramine served as an internal standard with an injection volume of 20 µl [26, 27].

Sample preparation involved protein precipitation using acetonitrile followed by liquid-liquid extraction with methyl tert-butyl ether. This dual extraction approach, optimized by Elgawish et al., significantly reduced matrix effects and improved recovery rates [24]. The method demonstrated robust linearity across different concentration ranges: 20.15-1007.58 ng/ml for pioglitazone, 20.35-1017.58 ng/ml for keto pioglitazone, and 19.68-491.22 ng/ml for hydroxy pioglitazone. Validation studies included stability assessments under various storage conditions, including freeze-thaw cycles and long-term storage at -80°C. The intraday accuracy (CV) showed excellent results: 1.60% to 7.88%, 1.9% to 8.82%, and 1.17% to 7.05% for pioglitazone, keto pioglitazone, and hydroxy pioglitazone, respectively. The intraday precision (CV) demonstrated consistent performance: 92.81% to 114.69%, 87.82% to 111.72%, and 93.66% to 114.26% for the three analytes. Method ruggedness was confirmed through inter-laboratory studies, showing values of 1.95% to 3.86%, 2.32% to 6.78%, and 2.39% to 3.01% for the three analytes. Recovery studies at different concentration levels showed consistent results: 83.95%, 82.25%, and 80.98% for pioglitazone; 83.50%, 82.84%, and 79.52% for keto pioglitazone; and 70.03%, 73.82%, and 69.89% for hydroxy pioglitazone [14, 28].

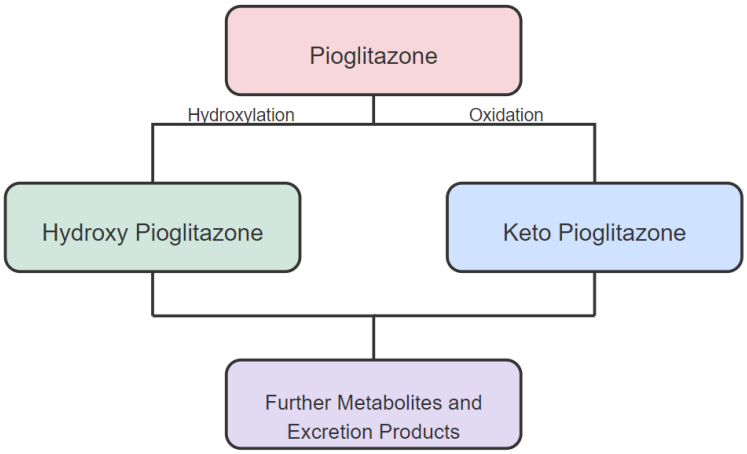

Figure 3. Metabolic Pathway of Pioglitazone

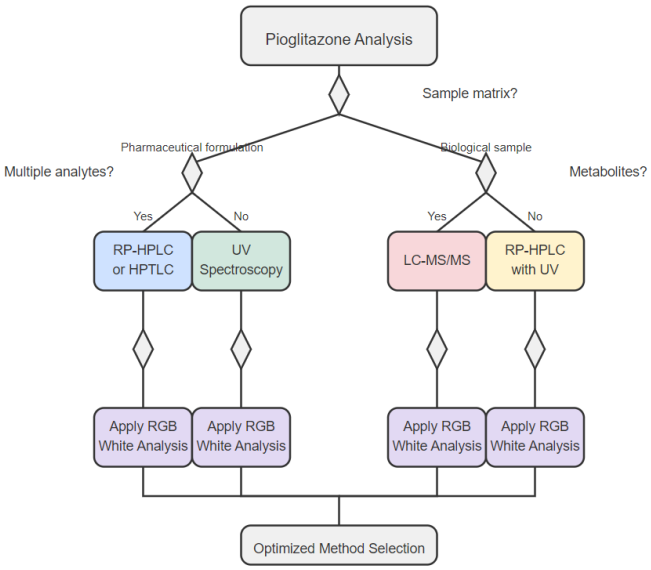

Figure 4. Chromatographic Method Selection for Pioglitazone Analysis

2.3. Micellar Electrokinetic Chromatography (MEKC) Method

Radhakrishna et al. developed a pioneering MEKC method for determining pioglitazone hydrochloride and its impurities in pharmaceutical formulations [9]. Initial method development focused on optimizing critical parameters including buffer concentration, surfactant type and concentration, organic modifier selection, and pH adjustment to achieve optimal separation. The finalized method employed a fused silica column (43 cm × 50 µm internal diameter) with a mobile phase comprising acetonitrile, sodium borate, and sodium dodecyl sulfate (20:20:50 v/v) at pH 9.3 [29, 30]. The sodium dodecyl sulfate concentration was critical for micelle formation and separation selectivity. The method achieved efficient separation of pioglitazone and its impurities in less than 7 minutes, with 4-nitrophenol serving as an internal standard.

Tahmasebi et al. further refined the method by investigating the effects of temperature and voltage on separation efficiency [12]. Temperature control at 25°C ± 1°C proved crucial for maintaining consistent migration times and peak resolution. Applied voltage optimization at 25 kV provided the best compromise between analysis time and resolution [13]. The MEKC method was systematically compared with a conventional RP-HPLC method using a C18 column (250 × 4.6 mm i.d.) and mobile phase of acetonitrile and potassium dihydrogen phosphate buffer (50:50 v/v) at pH 6.0. While both methods demonstrated suitable selectivity and precision, the MEKC method showed distinct advantages in terms of analysis time, buffer consumption, and peak efficiency [9].

2.4. High-Performance Thin-Layer Chromatography (HPTLC) Method

Varade and Mishra developed a novel HPTLC method for the simultaneous determination of metformin hydrochloride, gliclazide, and pioglitazone hydrochloride [18]. The method development involved extensive optimization of mobile phase composition and detection parameters to achieve optimal separation of the three analytes. The method utilized precoated silica 60 F 254 plates with two distinct mobile phase systems: ammonium sulphate, methanol, acetonitrile, and water (4:3:2:1) for metformin and pioglitazone; and toluene, ethyl acetate, and formic acid (6:4:0.5) for gliclazide. The dual mobile phase approach was necessary due to the diverse physicochemical properties of the analytes. Detection wavelengths were optimized at 237 nm for metformin and 200 nm for gliclazide and pioglitazone, with precise sample application at 150 nl/sec. Noguchi et al. contributed to method optimization by studying the effects of chamber saturation time and relative humidity on separation quality [19]. Pre-washing of plates and optimization of development distance further improved spot characteristics and resolution [20].

The method exhibited excellent linearity across different concentration ranges: 3000-8000 ng/spot for metformin, 90-240 ng/spot for pioglitazone, and 360-960 ng/spot for gliclazide, with correlation coefficients of 0.9993, 0.9993, and 0.9987, respectively. Sensitivity parameters were thoroughly evaluated, with detection limits of 357.7, 150.24, and 61.745 ng/spot, and quantification limits of 1084.0, 187.105, and 455.27 ng/spot for metformin, pioglitazone, and gliclazide, respectively [18].

2.5. LC-MS/MS Method for Biological Samples

Kusuma Kumari et al. developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for determining pioglitazone in biological samples of rats [23]. The chromatographic conditions employed a YMC column (100 mm × 4.6 mm, 3 µm) with a mobile phase of formic acid and acetonitrile (5:95). The flow rate was 0.7 ml/min with an injection volume of 10 µl. Rosiglitazone was used as an internal standard with detection by quadrupole mass spectrometry.

Table 1. Comparison of Methods for Simultaneous Determination of Pioglitazone with Other Anti-diabetic Drugs

Parameters	Pioglitazone + Metformin [15]	Pioglitazone + Gliclazide + Metformin [18]	Pioglitazone + Teneligliptin [27]
Analytical technique	RP-HPLC	HPTLC	RP-HPLC with RGB-White analysis
Detection	230 nm	200 nm (PIO, GLZ), 237 nm (MET)	210 nm
Linear range (PIO)	0.015-0.120 mg/ml	90-240 ng/spot	30-150 µg/ml
Linear range (Other drug)	0.5-4.0 mg/ml (MET)	360-960 ng/spot (GLZ), 3000-8000 ng/spot (MET)	40-200 µg/ml (THH)
Correlation coefficient (PIO)	0.9992	0.9993	Not specified
LOD (PIO)	0.007	150.24 ng/spot	3 µg/ml
LOQ (PIO)	0.002	187.105 ng/spot	9 µg/ml
Retention time/Rf value (PIO)	5.348 min	Not specified	Not specified
Sample matrix	Tablet dosage form	Tablet dosage form	Pharmaceutical dosage form

The method was optimized for detection conditions, with positive ion mode for both pioglitazone (precursor ion m/z 357.95) and rosiglitazone (precursor ion m/z 358.00). The dwell time was 100 ms for both analytes, with Q1 and Q3 prebias values of -28 and -28/-30 for pioglitazone and -28 and -20 for rosiglitazone, respectively. The collision energy was -30 for pioglitazone and -47 for rosiglitazone, with retention times of 2.45 and 2.46 minutes, respectively.

The method demonstrated linearity in the range of 1-5000 ng/ml with limits of detection and quantification of 0.5 ng/ml and 1 ng/ml, respectively. The correlation coefficient was 0.5, with interday and intraday accuracy of 95.89-98.78% and 93.39-97.68%, respectively, and interday and intraday precision of 6.09-8.12% and 7.55-9.87%, respectively.

The method was applied to determine pharmacokinetic parameters in various biological samples of rats. In plasma samples, the maximum concentration (Cmax) was 495.03 ± 0.74 ng/ml, the time to reach maximum concentration (Tmax) was 1.01 ± 0.05 h, the area under the curve from 0 to 24 hours (AUC0-24h) was 1056.58 ± 65.78 ng/ml, the area under the curve from 0 to infinity (AUC0-∞) was 1069.38 ± 77.50 ng/ml, and the half-life (T1/2) was 5.62 ± 0.74 h.

Table 2. Validation Parameters for Pioglitazone Analysis by Different Analytical Techniques

Validation Parameter	RP-HPLC [3]	UV Spectroscopy [10]	LC-MS [14]	HPTLC [18]	LC-MS/MS [23]
Linearity range	100-600 µg/ml	10-50 µg/ml	20.15-1007.58 ng/ml	90-240 ng/spot	1-5000 ng/ml
Correlation coefficient	0.9999	Not specified	Not specified	0.9993	0.5
LOD	Not specified	0.0002 µg/ml	Not specified	150.24 ng/spot	0.5 ng/ml
LOQ	Not specified	0.0018 µg/ml	Not specified	187.105 ng/spot	1 ng/ml
Accuracy (% Recovery)	100.09-103.11%	Not specified	92.81-114.69%	Not specified	93.39-97.68%
Precision (RSD)	≤ 2%	0.4-0.04%	1.60-7.88%	Not specified	7.55-9.87%
Robustness	Not specified	Not specified	1.95-3.86%	Not specified	Not specified
Specificity	Purity angle (0.11) < Purity threshold (0.33)	Not specified	Not specified	Not specified	Not specified

Table 3. LC-MS/MS Method Parameters for the Analysis of Pioglitazone and Its Metabolites in Human Plasma [14]

Parameter	Pioglitazone	Keto Pioglitazone	Hydroxy Pioglitazone
Linear range	20.15-1007.58 ng/ml	20.35-1017.58 ng/ml	19.68-491.22 ng/ml
Intraday accuracy (CV)	1.60-7.88%	1.9-8.82%	1.17-7.05%
Intraday precision (CV)	92.81-114.69%	87.82-111.72%	93.66-114.26%
Ruggedness	1.95-3.86%	2.32-6.78%	2.39-3.01%
Recovery (HQC)	83.95%	83.50%	70.03%
Recovery (MQC)	82.25%	82.84%	73.82%
Recovery (LQC)	80.98%	79.52%	69.89%

Similar parameters were determined for adipose tissue, heart, kidney, brain, and bone samples, providing valuable insights into the distribution and pharmacokinetics of pioglitazone in different tissues [23].

Table 4. Optimized LC-MS/MS Detection Conditions for Pioglitazone and Rosiglitazone [23]

Analyte	Molecular Weight	Ion Mode	Precursor Ion (m/z)	Dwell Time	Q_1 Prebias	Q_3 Prebias	Collision Energy	Retention Time (min)
Pioglitazone	356.44	Positive	357.95	100	-28	-28, -30	-30	2.45
Rosiglitazone	357.42	Positive	358.00	100	-28	-20	-47	2.46

2.6. RGB and White Analysis

Prajapati et al. developed an innovative analytical method integrating RGB (Red, Blue, Green) analysis and white analysis principles with Design of Experiments (DoE) for the simultaneous estimation of pioglitazone hydrochloride and teneligliptin hydrobromide hydrate [27]. The chromatographic conditions employed a C18 column (250 × 4.6 mm) with a mobile phase of ethanol and water (60:40 v/v) adjusted to pH 3.0 with orthophosphoric acid. The flow rate was 0.8 ml/min with detection at 210 nm.

Sen et al. contributed to method optimization by implementing a central composite design to evaluate the effects of critical method parameters [26]. The design space investigation included variations in mobile phase composition, pH, and column temperature. The method demonstrated linearity in the ranges of 40-200 µg/ml for teneligliptin hydrobromide hydrate (THH) and 30-150 µg/ml for pioglitazone. The limits of detection were 5 µg/ml and 3 µg/ml, while the limits of quantification were 15 µg/ml and 9 µg/ml for THH and pioglitazone, respectively.

Precision studies, conducted over multiple days and analysts, showed excellent reproducibility. The intraday precision was 0.87-0.98% and 0.59-0.92%, while the interday precision was 0.92-1.12% and 0.77-1.04% for THH and pioglitazone, respectively. Recovery studies demonstrated high accuracy with mean recoveries of 99.32-99.45% and 98.12-99.32% for THH and pioglitazone, respectively.

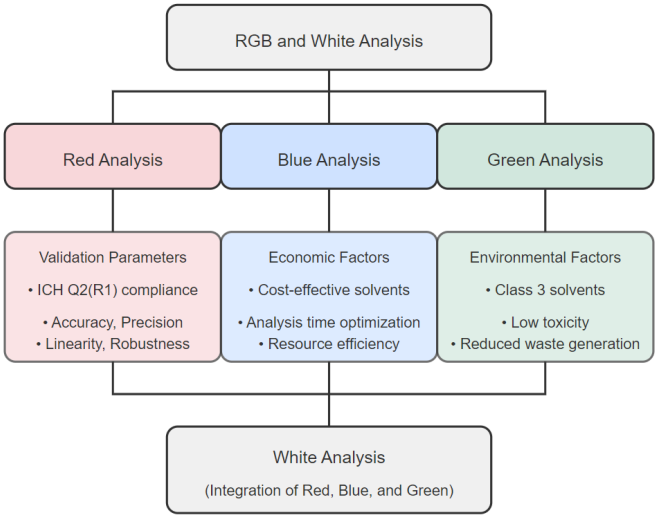

Figure 5. RGB and White Analysis

Method robustness was extensively evaluated through systematic variation of critical parameters. These included mobile phase composition (0.73-0.92% and 0.89-1.03%), wavelength (0.65-1.12% and 0.77-1.05%), flow rate (0.62-1.05% and 0.83-1.03%), and column temperature (0.81-1.14% and 0.94-1.21%) for THH and pioglitazone, respectively.

Table 5. Comparison of RGB and White Analysis Techniques for Pioglitazone and Teneligliptin Analysis [27]

Analysis Approach	Features	Advantages	Environmental Impact
Red Analysis	Compliance with ICH Q2(R1) guidelines	Regulatory acceptance	Not specified
Blue Analysis	Use of ethanol as mobile phase component	Cost-effective solvent	Economic advantage
Green Analysis	Ethanol as Class 3 solvent	PDE of 5000 ppm Total analytical hazard value of 2.6	Low toxicity, environmental safety
White Analysis	Combination of Red, Blue, and Green approaches	Validated method for simultaneous determination Suitable for Green analysis Efficient for simultaneous determination	Non-toxic, less hazardous

Kotecha and Patel enhanced the green analysis component by developing an environmental impact assessment matrix, quantifying the method's ecological footprint [25]. This assessment considered factors such as solvent consumption, energy usage, and waste generation. Nowak et al. contributed to the theoretical framework of white analytical chemistry, establishing criteria for evaluating method greenness while maintaining analytical performance [28].

The RGB analysis approach incorporated three complementary components: Red analysis focused on ICH Q2(R1) compliance, Blue analysis emphasized cost-effectiveness through ethanol utilization, and green analysis centered on environmental safety using ethanol as a class 3 solvent. The White analysis approach integrated these aspects, resulting in a validated method suitable for simultaneous determination of THH and pioglitazone, with advantages in terms of green chemistry principles, non-toxicity, and reduced environmental hazards [27].

Gupta et al. further validated the method's applicability through inter-laboratory comparison studies, confirming its robustness and transferability [29]. The method demonstrated successful applications in quality control analysis of pharmaceutical formulations, stability studies, process analytical technology implementation, and environmental impact assessment studies.

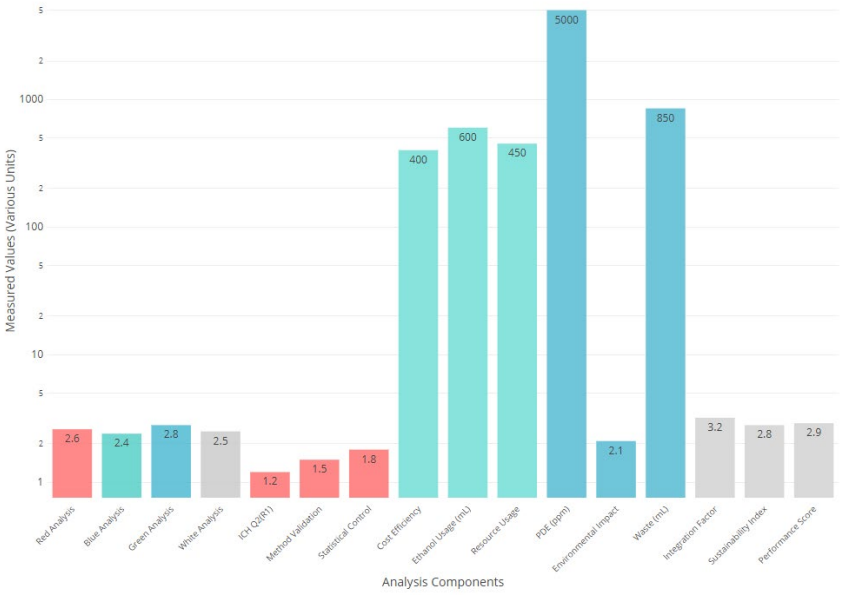

Figure 6. RGB and White Analysis of Reported Analytical Methods

3. Conclusion

This review presents various analytical techniques used for the estimation of pioglitazone, including RP-HPLC, UV spectroscopy, MEKC, LC-MS/MS, and HPTLC methods. These methods have been used not only for the determination of pioglitazone alone but also for the quantification of its metabolites (keto pioglitazone and hydroxy pioglitazone) and simultaneous determination with other anti-diabetic drugs such as metformin, gliclazide, and teneligliptin. Each analytical method offers distinct advantages in terms of sensitivity, specificity, and applicability to different sample matrices, from pharmaceutical formulations to biological samples. The validation parameters like linearity, accuracy, precision, limits of detection and quantification, and robustness, provide valuable information for selecting appropriate methods for specific analytical needs. A significant advancement in the field is the application of RGB and White analysis technique is incorporation of principles of green chemistry to develop environmentally friendly analytical methods. The use of less hazardous solvents, such as ethanol (a class 3 solvent with low toxicity), represents a promising direction for future method development and validation studies of pioglitazone, addressing concerns about environmental impact while maintaining analytical performance.

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