Journal of Pharmacy & Allied Medicine

JPAM, 2(2), 82-92, 2024
https://doi.org/10.58985/jpam.2024.v02i02.24
ISSN (Print): 3005-2726

*Review Article*

# Analytical techniques for determination of metformin-thiazolidinediones combination antidiabetic drug

**Imad Osman Abu Reid*, Sayda Mohamed Osman** and **Somia Mohammed Bakheet**

International University of Africa, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Khartoum, Sudan.

***Article Information***
*Received:* 06 December 2024
*Revised:* 19 December 2024
*Accepted:* 24 December 2024
*Published:* 27 December 2024

***Academic Editor***
Prof. Dr. Christian Celia

***Corresponding Author***
Prof. Dr. Imad Osman Abu Reid
E-mail: iabureid@hotmail.com
Tell: +00249123565660

***Keywords***
Analysis, combination, metfor-min, review, thiazolidine-diones, dosage form.

## Abstract

Diabetes mellitus (DM) remains a significant global health challenge, causing considerable mortality and morbidity annually. Type 2 DM is the most prevalent form of the disease, and fixed-dose combinations of oral anti-diabetic drugs are essential for its management. Their use creates a substantial demand for analytical methods capable of handling the diverse matrices, compounds, and physicochemical complexities involved in the quantitative analysis of these drug combinations. This article reviews current analytical methods for quantitatively determining metformin combined with thiazolidinediones in various marketed formulations. The review covered the period from 2005 to the present. The most commonly used methods include high-performance liquid chromatography (HPLC), spectrophotometric techniques, high-performance thin-layer chromatography (HPTLC), and capillary zone electrophoresis (CZE). Recent trends indicate a preference for HPLC (44.8%) due to its higher sensitivity, resolution potential, reduced sample and reagent consumption, and shorter analysis times. This shift underscores the industry's move towards more efficient and precise analytical techniques.

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a collection of metabolic disorders characterized by persistently high blood glucose levels due to problems with insulin secretion and/or function [1]. It has emerged as the third most serious chronic non-communicable disease threatening global health, following cardiovascular diseases and cancer [2]. Diabetes inflicts chronic damage on various organs, including the eyes, heart, and kidneys, severely affecting patients' lives and quality of life, and placing a significant financial burden on families and individuals [3, 4].

Oral antidiabetic drugs are easy to take, which encourages strong adherence among T2DM patients. Effective management of T2DM involves a comprehensive approach that includes lifestyle modifications, medication, and regular blood glucose monitoring [5, 6]. Metformin, a biguanide, has been utilized as an oral hypoglycemic agent and is considered the first-line treatment for T2DM for over 50 years. However, as the disease progresses, due to insufficient dosing, poor adherence, or other conditions that affect glucose metabolism, metformin alone often fails to maintain satisfactory blood glucose levels [7]. In such situations, clinicians may enhance treatment by increasing the metformin dose or adding another T2DM medication to the regimen to achieve better glycemic control.

Metformin, a biguanide agent, acts primarily as an insulin sensitizer. Its primary clinical site of action is in the liver, improving hepatic insulin sensitivity and as a result, decreasing hepatic gluconeogenesis.

Metformin may also increase both hepatic and splanchnic glucose utilization. Metformin also has a significant effect on peripheral insulin sensitivity, primarily in muscle and modestly at adipocyte by phosphorylation and activation of AMP-activated protein kinase [8]. Therefore, reductions in blood sugar with metformin occur in the setting of reduced insulin levels and theoretically reduced beta-cell work. There appears to be little or no direct mechanistic effect of the drug, however, on beta-cell function or apoptosis. Metformin is negligibly protein bound and is primarily renally cleared, proportionate to declines in creatinine clearance. Therefore, dosage reduction or therapeutic restriction may be required in the aging or renally impaired patient [9].

Troglitazone, the first thiazolidinedione approved for clinical use, was withdrawn from the market three years after its approval due to a high incidence of liver injury, including cases of acute liver failure. The thiazolidinediones (TZDs) currently available on the market, rosiglitazone and pioglitazone are potent agonists of the peroxisome proliferator-activated receptor (PPAR)-gamma. This receptor is a ligand-activated transcription factor crucial for glucose and lipid metabolism. Additionally, TZDs demonstrate modest activation of PPAR-alpha, another transcription factor involved in lipid metabolism and fat oxidation. PPAR-gamma receptors are highly expressed in the vasculature, adipocytes, and macrophages, indicating that thiazolidinediones may also significantly impact non-metabolic processes such as inflammation and atherogenesis.

The fixed-dose combination (FDC) of thiazolidine-diones and metformin brings together two insulin sensitizers to improve insulin sensitivity [10]. This combination has been proposed as an alternative to increasing the dose of metformin for patients with T2DM who are not achieving adequate glycemic control [11]. The rationale behind combining these agents lies in their complementary mechanisms of action. Together, thiazolidinediones and metformin improve glycemic control by reducing insulin resistance and enhancing insulin sensitivity without causing hypoglycemia.

Additionally, it has been observed that increasing the number of medications can make it more challenging for patients to adhere to their treatment regimens. In contrast, using a single tablet containing fixed doses of both drugs, which have distinct mechanisms, can reduce the number of medications needed. This approach ensures better glycemic control and improves overall patient compliance. This paper aims to review and analyze the literature on the analytical methods for the determination of metformin combinations with thiazolidinediones in pharmaceutical dosage forms. To our knowledge, a review on this topic is not yet published.

## 2. Materials and methods

We conducted comprehensive literature searches in PubMed, Google Scholar, Web of Science; using the term "determination of metformin and glitazones combination or determination of metformin and thiazolidinediones combination". The search covered the period from 2005 to the present.

## 3. Results and discussion

*3. Analysis techniques*

*3.1 Official methods*

For the determination of MET and PIO combination in tablets, the United States Pharmacopeia (USP) [12] employs high-performance liquid chromatography with an octacylsilane column (6.0 mm × 15 cm; 5 µm) and a mobile phase made of: 7.2 g/L of sodium dodecyl sulfate in a mixture of 0.05 M monobasic ammonium phosphate and acetonitrile (1:1). Metformin is detected at 255 nm, whereas pioglitazone is detected at 225 nm. The mobile phase is pumped at a flow rate of 1 mL/minute.

*3.2 Separation methods*

*3.2.1 High-performance liquid chromatography (HPLC)*

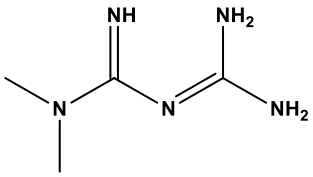
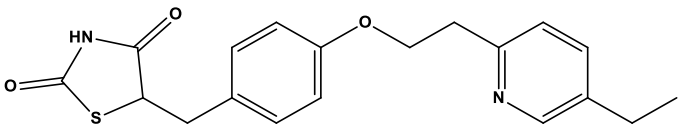
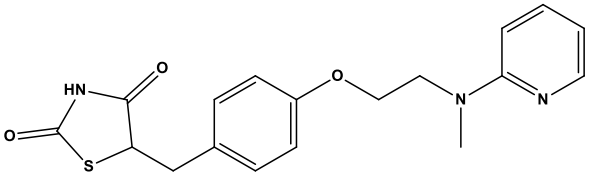
Reversed-phase high-performance liquid chromate-graphy (RP-HPLC) is extensively used for quantifying MET and thiazolidinedione combinations in pharmaceutical dosage forms. Most established methods employ isocratic elution with reversed-phase columns ($C_8$ or $C_{18}$) and mobile phases composed of mixtures of organic solvents and buffers, with carefully adjusted pH levels Analyzing combination drug products containing two or more active pharmaceutical ingredients (APIs) presents several challenges for RP-HPLC.

These challenges include difficulties with analyte retention, separating impurities, distinguishing excipients, maintaining peak shape, and ensuring



https://doi.org/10.58985/jpam.2024.v02i02.24

**Table 1.** Structure and Log P (octanol/water) of metformin, pioglitazone and rosiglitazone

| Compound | Chemical structure | Log P | Ref. |
|---|---|---|---|
| Metformin |  | - 2.6 | [15] |
| Pioglitazone |  | 3.31 | [16] |
| Rosiglitazone |  | 2.78 | [16] |

column longevity [13].

The polarity and ionization potential of molecules play a crucial role in their retention on organic reverse-phase high-performance liquid chromatography (RP-HPLC) columns [14]. When active pharmaceutical ingredients (APIs) in a combination drug exhibit substantial differences in polarity, it often necessitates the development of multiple chromatographic methods. This method development and validation process can be both resource-intensive and time-consuming. A typical example of this challenge is observed in drug combinations that include metformin and thiazolidinediones. Metformin, being highly polar, contrasts significantly with thiazolidinediones, which have medium-to-low polarity, as reflected by their respective log P values in Table 1.

Under reversed-phase conditions, due to the significant difference in polarity between the combined compounds, metformin is expected to elute very early, near or with the solvent front. In contrast, the retention of thiazolidinediones (PIO and ROS) varies depending on the pH of the mobile phase and the amount of the organic modifier used. To evaluate the effect of chromatographic conditions on the retention of metformin, we calculated the retention factor (k) according to the guidelines set by the United States Pharmacopeia (USP) [12]. Only methods [22, 23] satisfied the USP requirements ($2 < k < 10$), while in methods [18, 25-27, 29], metformin eluted slightly away from the solvent front. In the remaining methods [19, 20, 24, 28], metformin eluted very close to the dead time, resulting in a k value approaching zero.

The challenge of metformin's low affinity for reversed-phase packing material is further complicated by the presence of thiazolidinediones. At a retention time where metformin achieves adequate retention (i.e., $k > 2$), the thiazolidinediones elute at unacceptably long times. To address this issue, ion-pairing reagents such as sodium dodecyl sulfate and hexane sulfonic acid [23, 25]

have been employed to enhance metformin's retention. El-Zaher et al. [28] successfully tackled the retention challenges of metformin and pioglitazone by using a cyanopropyl column, an approach similar to the one followed by Reid [30-32] and Gedawy et al. [33, 34] for the determination of combinations containing compounds of different polarities. This approach allowed for effective separation of metformin from thiazolidinediones and moved the metformin peak away from the solvent front. A description of the reported HPLC methods is given in Table 2.

*3.2.2 Thin-layer high performance liquid chromatography (HPTLC)*



https://doi.org/10.58985/jpam.2024.v02i02.24

The HPTLC separation of metformin (MET) and pioglitazone (PIO) is typically performed on silica gel 60F254 precoated plates using various chromatographic conditions. Dharmamoorthy et al. [35] employed a mobile phase of toluene, methanol, and triethylamine (6:4:0.1 v/v/v). The method had a working range of 50-300 µg/mL for MET and 1.5-9.0 µg/mL for PIO, with limits of detection (LOD) of 10 ng/spot and 0.5 ng/spot, respectively. Analytes were detected at 230 nm.

Khorshid et al. [36] used a mobile phase consisting of toluene, methanol, and acetic acid (5:5:0.5 v/v/v) to separate MET and PIO in the presence of the impurity melamine. The method's working range was 3-12 µg/band for MET, 3-20 µg/band for PIO, and 0.5-5 µg/band for melamine. Detection was performed at 240 nm.

Modi et al. used a mobile phase of butanol, 1,4-dioxane, and glacial acetic acid (5:3:2 v/v/v). The method exhibited a linear range of 2000-20000 ng/band for MET and 60-600 ng/band for PIO, with detection limits of 629.89 ng/band and 8.51 ng/band, respectively. Analytes were detected at 226 nm [37].

The selection of detection wavelengths was clearly justified and supported by the overlain spectra of the analytes in references [36] and [37]. However, in reference [35], the chosen detection wavelength is not consistent with the overlain spectra, which suggest 250 nm as the more suitable wavelength for detection. The methods were thoroughly validated for specificity, linearity, limit of detection and quantitation, accuracy, precision, and robustness. They were successfully applied to the simultaneous determination of MET and PIO in tablets.

*3.2.3 Capillary zone electrophoresis (CZE)*

Metformin and pioglitazone were analyzed using capillary zone electrophoresis (CZE) with a 75 mmol/L phosphate buffer containing 30% acetonitrile (ACN) at pH 4.0. The conditions included a 10-second hydrodynamic injection time at 0.5 psi, a separation voltage of 25 kV, and a column temperature of 25°C. Detection was carried out at 210 nm. The method showed a linear range of 10-80 µg/mL for MET and 10-100 µg/mL for PIO, with limits of detection (LOD) of 0.091 µg/mL and 0.277 µg/mL, respectively [38]. The method's suitability was confirmed through validation for specificity, linearity, stability, accuracy, limit of detection and quantitation, precision, and robustness, as well as by comparing it for the analysis of the two drugs combination with a previously reported high-performance liquid chromatography method.

Yardımcı et al. [39] reported the determination of metformin (MET) and rosiglitazone (ROS) using capillary electrophoresis with a running buffer of 25 mM acetate at pH 4.0 and a fused-silica capillary column (80.5 cm × 75 µm i.d., effective length 72.0 cm). The applied voltage was +25.0 kV, and detection was performed at 203 nm. The method demonstrated a linear range of 1.0–12.0 µg/mL for ROS and 100.0–1200.0 µg/mL for MET, with a limit of detection (LOD) of approximately 0.5 µg/mL for both analytes. During optimization, various electrolyte parameters such as pH, buffer concentration and type, and the type and concentration of the organic modifier were studied. The effects of applied voltage, separation temperature, and injection time were also evaluated. The optimized method was then validated for specificity, linearity, stability, accuracy, limit of detection and quantitation, precision, and robustness. Their method was then successfully applied to the simultaneous determination of MET and ROS.

*3.3 Spectrophotometric methods*

Analytical chemists face considerable challenges when analyzing and controlling multicomponent formulations through direct spectrophotometry, primarily due to overlapping spectral bands. To address this, various spectrophotometric methods have been developed to resolve mixtures of compounds with overlapping spectra. The success of these techniques largely depends on the degree of spectral overlap and the number of components present in the mixture. When the overlap between analytes is minimal, methods involving simple mathematical manipulation of spectral data are employed [40]. However, for mixtures with extensive overlap, complete spectral analysis is used to determine all components [41]. The accuracy of these determinations can be enhanced by carefully selecting wavelength ranges, which helps reduce collinearity or spectral overlap [42]. Table 3 gives the details of the spectrophotometric methods used.


https://doi.org/10.58985/jpam.2024.v02i02.24

**Table 2.** High performance liquid chromatographic methods used for the analysis of metformin and thiazolidinediones combinations.

| No. | Metformin + | Column | Mobile Phase | Detection λ (nm) | Working range (µg/mL) | LOD (µg/mL) | Ref |
|---|---|---|---|---|---|---|---|
| 1 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | acetonitrile: phosphate buffer (pH 5.0) (50:50 v/v) at a flow rate of 1 mL/min. | 258 | 20.0-80.0 MET 0.5-3.5 PIO | 5.0 MET 10.0 PIO | [17] |
| 2 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | acetonitrile: 0.01M sodium dihydrogen phosphate (60:40) and the flow rate was maintained at 1 mL/min | 228 | 20.0-120.0 MET 0.6-3.6 PIO | 2.38 MET 0.09 PUO | [18] |
| 3 | Pioglitazone tablets | $C_{18}$ (10 cm x 4.6 mm, 5 µ) | methanol: 25 mM $KH_2PO_4$ (pH 4.9) ( 75:25 v/v, at flow rate of 2.7 mL/min | 210 | 1–100 for both | 0.25 MET 0.5 PIO | [19] |
| 4 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | acetonitrile: $KH_2PO$ buffer (pH 3) ( 50:50 v/v) at flow rate of 1 mL/min | 238 | 40-240 MET 12-72PIO | NA | [20] |
| 5 | Pioglitazone tablets | $C_{18}$ (15 cm x 4.6 mm, 5 µ) | $Na_2HPO_4$: acetonitrile and triethylamine (660:340:1) and adjusted pH to 7.10, at flow rate of 1 mL/min | 225 | 8.5-70.0 MET 6.0-90 PIO | 0.2 PIO 0.1 MET | [21] |
| 6 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | methanol: phosphate buffer, pH 6.5 containing (50:50 V/V) 0.01 M sodium dodecyl sulphate, at flow rate 1.5 mL/min | 270 | 7.5-22.5 PIO 425-1275 MET | 0.01 PIO 0.05 MET | [22] |
| 7 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ), 35°c | methanol: 0.1 $KH_2PO_4$ (pH4.5) (30:70 v/v), at flow rate 1 mL/min | 240 | 0.1-0.3 MET 0.01-0.03 PIO | 0.125 MET 0.026 PIO | [23] |
| 8 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | methanol: water (45:55 v/v) containing 0.2% (w/v) n-heptanesulfonic acid and 0.2% (v/v) triethylamine, at flow rate 1 mL/min | 265 | 100-750 MET 5-30 PIO | 0.464 MET 0.317 PIO | [24] |
| 9 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | methanol:10 mM phosphate buffer (pH 3.0) (94:6, v/v), at 1 mL/min | 230 | 0.5–100 for both | 0.16 For both | [25] |



https://doi.org/10.58985/jpam.2024.v02i02.24

**Table 2.** *(Continued)*

| No. | Metformin + | Column | Mobile Phase | Detection λ (nm) | Working range (µg/mL) | LOD (µg/mL) | Ref |
|---|---|---|---|---|---|---|---|
| 10 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | phosphate buffer and acetonitrile in the ratio of 35:65 at pH 3.4, The flow rate was 1.5 ml/min | 228 | 50-100 MET 20-180 PIO | 1.52 MET 1.02 PIO | [26] |
| 11 | Pioglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | acetonitrile: 0.01M $NaH_2PO_4$ (60: 40 v/v). The flow rate was 1 mL/min | 228 | 20-120 MET 0.6 - 3.6 PIO | 2.38 MET 0.09 PIO | [27] |
| 12 | Pioglitazone tablets | CN (25 cm x 4.6 mm, 5 µ) | acetonitrile: 0.02 M $KH_2PO_4$ (pH 3.17; 50:50, v/v). The flow rate was 1 mL/min | 220 | 0.175-350 MET 0.125–250 PIO | 0.003 MET 0.019 PIO | [28] |
| 13 | Rosiglitazone tablets | $C_{18}$ (25 cm x 4.6 mm, 5 µ) | 0.02 M ammonium dihydrogen phosphate buffer (pH 4.5): acetonitrile (65:35v/v) , at a flow rate of 1.0 mL min | 230 | 20- 70 MET 12-32 ROS | 1.100 MET 0.725 ROS | [29] |

Given the lack of detection limit data for many reported methods, the evidence suggests that among the spectrophotometric techniques for determining MET in combination with PIO, the area under the curve, dual wavelength methods [46], and the Q ratio method [49] are the most sensitive. These methods demonstrate better detection limits and wider linear ranges compared to other available approaches. Furthermore, they are easy to use and do not require sophisticated instruments or expensive reagents.

Two first derivative methods [50, 52] and a simultaneous equation method [51] were reported for the determination of MET and ROS combination, the first derivative method [52] demonstrated greater sensitivity than the other two, with a LOD of 0.165 and 0.050 µg/mL for ROS and MET, respectively.

Chemometric methods, such as classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS, have also been employed for the determination of MET and PIO in eye drops [47]. While these techniques are effective in resolving overlapping spectral bands, their practicality for routine analysis is limited compared to simpler approaches as these techniques require specialized equipment and computer software.

*3.4 Electrochemical methods*

Selective electrodes based on polyvinyl chloride (PVC) membranes were developed for the determination of pioglitazone hydrochloride (PIO) and metformin hydrochloride (MET) using ion association complexes with sodium tetraphenyl borate (TPB) and ammonium reineckate (RNC). The electrodes, fabricated with PVC and plasticized using dibutyl sebacate, were paired with a double-junction Ag/AgCl reference electrode. Optimal stability was observed within pH ranges of 3.5–5.5 for PIO-TPB, 3.0–4.5 for PIO-RNC, 4–9 for MET-TPB, and 4–6 for MET-RNC, with response times of 30–40 seconds for PIO sensors and 20–30 seconds for MET sensors. The sensors demonstrated linear behavior, with PIO measurable between $3.162 \times 10^{-5}$ M to $1 \times 10^{-2}$ M and MET between $1 \times 10^{-3}$ M to $1 \times 10^{-1}$ M, achieving Nernstian slopes of ~26.74 mV/decade for PIO-TPB and ~51.56 mV/decade for MET-TPB, along with correlation coefficients of 0.999. These electrodes were successfully applied to laboratory-prepared mixtures, accurately quantifying PIO in the presence of up to 83.3% of its acid degradant and PIO-MET mixtures without interference.

The method also showed high precision and accuracy when applied to pharmaceutical formulations, with recovery rates averaging 99–100%, and in spiked


https://doi.org/10.58985/jpam.2024.v02i02.24

**Table 3.** Spectrophotometric methods used for the determination of metformin and thiazolidinediones combinations

| No. | Metformin+ | Technique | Wavelengths nm | Solvent | LOD (µg/mL) | Linear range (µg/mL) | Ref. |
|---|---|---|---|---|---|---|---|
| 1 | Pioglitazone in tablets | Simultaneous equation | 225 PIO<br>237 MET | Methanol | 0.9 PIO<br>0.4 MET | 2-20 PIO<br>2-10 MET | [43] |
| | | Absorption correction | 267 PIO<br>237 MET | | 3.0 PIO<br>0.4 MET | | |
| 2 | Pioglitazone in tablets | Difference | 228.1 PIO<br>228.2 MET | 0.1M NaOH<br>0.1 M HCl | NA | 0.1-0.5 for both drugs | [44] |
| 3 | Pioglitazone in tablets | Second order derivatives | 247.5 MET<br>279.5 PIO | 0.1 M NaOH | NA | 5-30 MET<br>2.5 -15 PIO | [45] |
| 4 | Pioglitazone in tablets | Zero order | 268 PIO | Methanol | | 3-25 PIO | [36] |
| | | Double divisor | 254 MET | | NA | 10-45 MET | |
| | | Isoabsorptive point | 255 MET | | | 10-50 MET | |
| 5 | Pioglitazone in tablets | Area under curve | 228-238 MET<br>265-275 PIO | Methanol | 0.252 MET<br>0.266 PIO | 2-10 MET<br>10-50 PIO | [46] |
| | | Dual wavelength | 235 and 266 MET<br>216 and 241.5 PIO | | 0.251 MET<br>0.234 PIO | | |
| 6 | Pioglitazone in tablets | First derivative | 247 MET<br>280 PIO | Methanol | NA | 5-30 MET<br>10-90 PIO | [47] |
| | | Ratio derivative | amplitudes at 238 nm and 248.6 nm | | | 5-30 MET | |
| | | Q ratio | MET<br>268 PIO<br>254.6 nm ($A_{iso}$) | | | 5-90 PIO<br>5-100 MET | |
| | | CLS<br>PCR<br>PLS-2 | 200-300 | | | | |
| 7 | Pioglitazone in tablets | First derivative | 269 PIO<br>231 MET | Dimethyl formamide and hydrochloric acid | NA | 0.5-4.5 PIO<br>16.5-150 MET | [48] |
| | | Q ratio | 247.5 nm ($A_{iso}$)<br>231 MET | | | | |
| 8 | Pioglitazone in tablets | Q ratio | 231($A_{iso}$)<br>269 PIO | Methanol | 0.167 MET<br>0.201 PIO | 5-30 MET<br>2-12 PIO | [49] |
| 9 | Rosiglitazone in tablets | First derivative | 312 ROS<br>247 MET | Water: ethanol (50:50) | 0.214 ROS<br>0.257 MET | 10-90 MET<br>2-18 ROS | [50] |


https://doi.org/10.58985/jpam.2024.v02i02.24

**Table 3.** *(Continued)*

| No. | Metformin+ | Technique | Wavelengths nm | Solvent | LOD (µg/mL) | Linear range (µg/mL) | Ref. |
|---|---|---|---|---|---|---|---|
| 10 | Rosiglitazone in tablets | Simultaneous equation | 228 MET 241 ROS | 0.01M HCl | NA | 4- 32 | [51] |
| 11 | Rosiglitazone in tablets | First order derivative | 334 ROS 227 MET | Methanol: water (50:50) | 0.165 ROS 0.050 MET | 5.0–50.0 ROS 1.0–10.0 MET | [52] |

human plasma samples, yielding recoveries between 98–101%, confirming its applicability for routine analysis in pharmaceuticals and biological matrices [53].

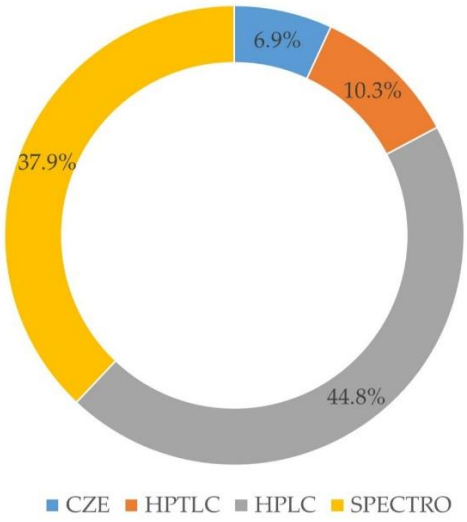



**Figure 1.** The % ratio of the analytical methods used for the determination of metformin/thiazolidinediones combinations

## 4. Conclusions

This review provides a comprehensive overview of commonly used analytical methods for determining metformin in combination with thiazolidinediones. It covers a wide range of methods described for these combinations in various bulk and pharmaceutical dosage forms, spanning the literature from 2005 to the present. The review highlights the primary distinction between chromatographic and non-chromatographic techniques. As shown in Fig. 1, high-performance liquid chromatography (HPLC) methods are the most prevalent, accounting for 44.8% of all techniques used for these compounds. Spectrophotometric methods follow at 37.9%, thin-layer chromatography (TLC) at 10.3%, and capillary zone electrophoresis (CE) at 6.9%. This distribution proves primacy of HPLC (44.8%) and confirm general trends moving towards more sensitive methods, with higher resolution potential, consuming small quantities of samples and reagents and requires less analysis time. Future research could focus on optimizing analytical methods following the quality-by-design approach and developing more methods using eco-friendly solvents for determining combinations with corticosteroids in topical pharmaceutical preparations.

## Authors' contributions

All the authors contributed equally for writing the content.

## Acknowledgements

The authors don't have anything to acknowledge.

## Funding

This work was not financed by any agency other than the authors.

## Availability of data and materials

All relevant data are within the paper and its supporting information files. Additional data will be made available on request according to the journal policy.

## Conflicts of interest

The authors declare no conflict of interest.

## References

1. Biadgo, B.; Melku M.; Abebe, S.M.; Abebe, M. Hematological indices and their correlation with



https://doi.org/10.58985/jpam.2024.v02i02.24

fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. Diabetes Metab Syndr Obes. 2016, 17, 91-99. https://doi. org/10.2147/ DMSO.S97563.

2. Seo, D.H.; Kang, S.; Lee, Y.H.; Ha, J.Y.; Park, J.S.; Lee, B.W.; Kang, E.S.; Ahn, C.W.; Cha, B.S. Current management of type 2 diabetes mellitus in primary care clinics in Korea. Endocrinol. Metabol. 2019, 34(3), 282-90. https://doi.org/10.3803/enm.2019.34.3.282
3. Maruthur, N.M.; Tseng, E.; Hutfless, S.; Wilson, L.M; Suarez-Cuervo, C.; Berger, Z.; Chu, Y.; Iyoha, E.; Segal, J.B.; Bolen, S. Diabetes medications as monotherapy or metformin-based combination therapy for type 2 diabetes: A systematic review and meta-analysis. Ann. Int. Med. 2016, 164(11), 740-51. https://doi.org/10.7326/ m15-2650
4. Sudlow, A.; Le, Roux, C.W.; Pournaras, D.J. Review of multimodal treatment for type 2 diabetes: combining metabolic surgery and pharmacotherapy. Ther. Adv. Endocrinol. Metabol. 2019, 10, 2042018819875407. https://doi: 10.1177/2042018819875407
5. Reusch, J.E.; Manson, J.E. Management of type 2 diabetes in 2017: getting to goal. JAMA. 2017, 317(10), 1015-6. https://doi.org/10.1001/jama.2017.0241
6. Pernicova, I.; Korbonits, M. Metformin—mode of action and clinical implications for diabetes and cancer. Nat. Rev. Endocrinol. 2014, 10(3),143–56. https://doi.org/10.1038/nrendo.2013.256
7. Kirpichnikov, D.; McFarlane, S.I.; Sowers, J. Metformin: An update. Annals Int. Med. 2002, 137(1), 25–33. https://doi.org/10.7326/0003-4819-137-1-200207020-00009
8. Waugh, J.; Keating, G.M.; Plosker, G.L. Pioglitazone: A review of its use in type 2 diabetes mellitus. Drugs. 2006, 66(1), 85–109. https://doi.org/10.2165/00003495-200666010-00005
9. DeFronzo, R.A.; Inzucchi, S.; Abdul-Ghani M.; Nissen S.E. Pioglitazone: The forgotten, cost-effective cardioprotective drug for type 2 diabetes. Diab. Vasc. Dis. Res. 2019, 16(2), 133–43. https://doi.org/10.1177/1479164118825376
10. Davidson, M.B.; Peters, A.L. An overview of metformin in the treatment of type 2 diabetes mellitus. Am. J. Med. 1997, 102(1), 99-110. https://doi: 10.1016/s0002-9343(96)00353-1
11. Charpentier, G. Oral combination therapy for type 2 diabetes. Diabetes/metabolism research and reviews. 2002, 18(S3), S70-6. https://doi.org/10.1002/dmrr.278
12. The United States Pharmacopoeia 43, The National Formulary, 26th Edn., U.S. Pharmacopeial Convention, Washington, DC, electronic edition, 2024.
13. Kumar, D.; Kumar, A.; Kumar, V.; Raj, A.; Rai, R.; Baliyan, V.; Kumar, N. A comprehensive review on analytical method development using RP-HPLC and recent advances in pharmaceutical applications. J. Res. Appl. Sci. Biotechnol. 2023, 2(2),53-60. https://doi.org/10.55544/jrasb.2.2.9
14. Schoenmakers, P.J.; van, Molle S.; Hayes, C.M.; Uunk, LG. Effects of pH in reversed-phase liquid chromatography. Anal. Chim. Acta. 1991,250,1-9. https://doi.org/10.1016/0003-2670(91)85058-z
15. https://pubchem.ncbi.nlm.nih.gov/compound/metformin#section=LogP accessed on 17/11/2024
16. Giaginis, C.; Theocharis, S.; Tsantili-Kakoulidou, A. Investigation of the lipophilic behaviour of some thiazolidinediones: Relationships with PPAR-γ activity. J. Chromatog. B. 2007, 857(2), 181-7. https://doi.org/10.1016/j.jchromb.2007.07.013
17. Alexandar, S.; Diwedi, R.; Chandrasekar, M.J. A RP-HPLC method for simultaneous estimation of metformin and pioglitazone in pharmaceutical formulation. Res. J. Pharm. Biol. Chem. Sci. 2010, 1(4), 858-66.
18. Rao, S.V.; Gollu, S.R.; Krishna, A.G.; Ameer, S.K.; Sekar, K.C. Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride in their Bulk and formulation dosage forms by using RPHPLC method. Orient. J. Chem. 2011, 27(3),1221.
19. Abdelmonem, A.A.; Ragab, G.H.; Hashem, H.A.; Bahgat, E.A. High-performance liquid chromatographic and spectrophotometric determinations of pioglitazone-HCl either alone or in combination with metformin-HCl. J. Liq. Chromatog. Rel. Technol. 2012, 35(19), 2706-2723. https://doi.org/10.1080/10826076.2011.637278
20. Swapn, J.; Madhu, C.; Srivani, M.; Sumalatha, M.; Nehalatha, Y.; Anusha, Y. Analytical method development and method validation for the simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride in tablet dosage form by RP-HPLC. Asian J. Pharm. Anal. 2012, 2(3), 85-9.
21. Srinivas, P.; Venkataramana, K.; Srinivasa, R.J.; Srinivasa, R.N. Simultaneous determination of metformin and pioglitazone tablets in pharmaceutical dosage form by RP-HPLC method. IJPCBS. 2012, 2(1), 104-109.
22. Elrefay, H.; Ismaiel, O.; Hassan, W.; Shalaby, A. Development and validation of a stability-indicating HPLC-UV method for the determination of pioglitazone hydrochloride and metformin hydrochloride in bulk drug and combined dosage form. Asian J. Pharm. Clin. Res. 2013, 116-120.



https://doi.org/10.58985/jpam.2024.v02i02.24

23. Pallapolu, A.; Aneesha, A. Novel RP-HPLC method development and validation of metformin and pioglitazone drugs in pure and pharmaceutical dosage forms. Indian J. Res. Pharm. Biotechnol. 2013, 1(5), 711.
24. Peraman, R.; Peruru, K.K.; Yiragam, P.R.; Gowra, C.S. Stability indicating RP-HPLC method for Simultaneous determination of metformin hydrochloride and pioglitazone hydrochloride in dosage form. Malaysian J. Pharm. Sci. 2014, 12(1), 33-46.
25. Mohamed, A.M.; Mohamed, F.A.; Ahmed, S.; Mohamed, Y.A. An efficient hydrophilic interaction liquid chromatographic method for the simultaneous determination of metformin and pioglitazone using high-purity silica column. J. Chromatog. B. 2015, 997, 16-22. http://dx.doi.org/10.1016/j.jchromb.2015.05.032
26. Agrawal, U.K.; Govindasamy, J. Development and validation of RP-HPLC method for simultaneous estimation of metformin and pioglitazone. Indian J. Pharm. Biol. Res. 2017, 5(01), 48-50. https://doi.org/10.30750/ijpbr.5.1.7
27. Alwesabi, A.S; Mukkanti, K.; Shekar, K.C. Analytical method development and method validation of metformin hydrochloride and pioglitazone hydrochloride in bulk and their pharmaceutical dosage forms by RP-HPLC. Anal. Chem. An Indian J. 2011, 10(12), 771-775.
28. El-Zaher, A.A.; Elkady, E.F.; Elwy, H.M.; Saleh, M.A. Simultaneous determination of metformin, glipizide, repaglinide, and glimepiride or metformin and pioglitazone by a validated LC method: Application in the presence of metformin impurity (1-cyanoguanidine). J. AOAC Int. 2016, 99(4), 957-63. https://doi.org/10.5740/jaoacint.15-0291.
29. Mittan, D.S.; Dwivedi, S.C.; Kushnoor, A. Development and validation of a RP-HPLC method for simultaneous estimation of rosiglitazone and metformin in bulk and tablet dosage form. Eur. J. Anal. Chem. 2011, 6(3), 189-196.
30. Abu Reid, I.O. Concurrent chromatographic determination of pseudoephedrine and loratadine from combination syrups and tablets. Fut. J. Pharm. Sci. 2021, 1-8. https://doi.org/10.1186/s43094-021-00287-3
31. Reid, I. Experimental design optimized chromatographic determination of pseudoephedrine and cetirizine in bulk and tablets. Hacettepe Univ. J. Fac. Pharm. 2022, 42(3), 165-74. https://doi.org/10.52794/hujpharm.1058051
32. Reid, I.O. Experimental design optimized chromatographic determination of pseudoephedrine and fexofenadine in bulk and tablets. Pharm. Chem. J. 2023, 56(10),1426-32. https://doi.org/10.1007/s11094-023-02808-1
33. Gedawy, A.; Al-Salami, H.; Dass, C.R. Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. J. Food Drug Anal. 2019, 27(1), 315-22. https://doi.org/10.1016/j.jfda.2018.06.007
34. Gedawy, A.; Al-Salami, H.; Dass, C.R. Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions. Saudi Pharm. J. 2020, 28(3), 362-8. https://doi.org/10.1016/j.jsps.2020.01.017.
35. Dharmamoorthy, G.; Nandini, Y.; Anupama, M.; Nandhini, M.S. Development and validation of stability indicating HPTLC method for the simultaneous estimation of metformin hydrochloride and pioglitazone in pharmaceutical dosage form. YMER. 2022, 21(8), 612-626.
36. Khorshid, A.; Abdelhamid, N.S.; Abdelaleem, E.A.; Amin, M.M. Simultaneous determination of metformin and pioglitazone in presence of metformin impurity by different spectrophotometric and TLC-densitometric methods. SOJ. Pharm. Pharm. Sci. 2018, 5(3), 1-8. https://doi.org/10.15226/2374-6866/5/3/00183
37. Modi, D.K.; Patel, B.H. Rapid and sensitive simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride in tablet formulation by HPTLC method. J. Liq. Chromatog. Rel. Technol. 2013, 36(5), 618-27. https://doi.org/10.1080/10826076.2012.673202
38. Al Thikrallah, M.K.; Idris, A.M.; Elbashir, A.A.; Elgorashe, R.E.; Buzid, A.; Alnajjar, A.O. Development of capillary zone electrophoresis method for the simultaneous separation and quantification of metformin and pioglitazone in dosage forms; and comparison with HPLC method. Molecules. 2023, 28(3), 1184. https://doi.org/10.3390/molecules28031184.
39. Yardımcı, C.; Özaltın, N. Method development and validation for the simultaneous determination of rosiglitazone and metformin in pharmaceutical preparations by capillary zone electrophoresis. Anal. Chim. Acta. 2005, 549(1-2), 88-95. https://doi.org/10.1016/j.aca.2005.06.014
40. Beckett, A.H.; Stenlake, J.B. Practical Pharmaceutical Chemistry. part II, 4th Edn., London; Bloomsbury Publishing, pp281-306. 2001.
41. Esbensen, K.H.; Swarbrick, B. Multivariate Data Analysis. 6th Edn. CAMO Software AS: Norway, 2018.
42. Rossi, D.T.; Pardue, H.L. Effects of wavelength range on the simultaneous quantitation of polynuclear aromatic hydrocarbons with absorption spectra. Anal. Chim. Acta. 1985, 175,153-161. http://dx.doi.org/10.1016/S0003-2670(00)82727-0.




*https://doi.org/10.58985/jpam.2024.v02i02.24*

43. Sujana, K.; Swathi, R.G; Bhanu, PM.; Reddy, M.S. Simultaneous estimation of pioglitazone hydrochloride and metformin hydrochloride using UV spectroscopic method. J Biomed. Sci. Res. 2010, 2(2), 110-115.
44. Sujana, K.; Abbulu, K.; Souri, O.B.; Archana, B.; Sindu, M.; Rani, G.S. Difference spectrophotometric methods for pioglitazone hydrochloride and metformin hydrochloride. J. Pharm. Sci. Res. 2011, 3(4), 1122.
45. Vichare, V.; Suryawanshi, P.; Bhosale, J.; Dhole, S. Simultaneous estimation of metformin HCl and pioglitazone HCl by second order derivative UV-visible spectrophotometric method in tablet formulation. Asian J. Pharm. Anal. 4(3), 121-4.
46. Kant, R.; Bodla, R.; Kapoor, G.; Bhutani, R. Development and validation of novel spectrophotometric methods for simultaneous estimation of pioglitazone and metformin in bulk and fixed dosage forms by area under the curve and dual wavelength mode. Int. J. Appl. Pharm. 2016, 8(3), 48-53.
47. Hegazy, M.A.; El-Ghobashy, M.R.; Yehia, A.M.; Mostafa, A.A. Simultaneous determination of metformin hydrochloride and pioglitazone hydrochloride in binary mixture and in their ternary mixture with pioglitazone acid degradate using spectrophotometric and chemometric methods. Drug Test. Anal. 2009, 1(7), 339-49. https://doi.org/10.1002/dta.60
48. Goswami, L.; Mukhopadhyay, S.; Durgapal, S. Simultaneous estimation of metformin and pioglitazone by ultraviolet spectrophotometry. Indian J. Pharm. Sci. 2010, 72(4), 508. https://doi.org/10.4103/0250-474x.73940
49. Aravind, A.; Das, P.; Babu, A.K.; George, M. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of metformin hydrochloride and pioglitazone in tablet dosage form. World J. Pharm. Res. 2021,11(1), 898-909.
50. Jadhav, S.D.; Magdum, A.A.; Panchal, R.M.; Koli, P.S. Simultaneous estimation of metformin hydrochloride and rosiglitazone maleate from tablet dosage form by derivative spectroscopic method. Asian J. Pharm. Anal. 2013, 3(3), 94-7.
51. Banarjee, M.; Behera, S.K. Simultaneous estimation of metformin and rosiglitazone in pharmaceutical tablet formulation. World J. Pharm. Res. 2021, 10(4),1325-33
52. Önal, A. Spectrophotometric and HPLC determinations of anti-diabetic drugs, rosiglitazone maleate and metformin hydrochloride, in pure form and in pharmaceutical preparations. Eur. J. Med. Chem. 2009, 44(12), 4998-5005. https://doi.org/10.1016/j.ejmech.2009.09.003
53. El-Ghobashy, M.R.; Yehia, A.M.; Mostafa, A.A. Application of membrane-selective electrodes for the determination of pioglitazone hydrochloride in the presence of its acid degradant or metformin hydrochloride in tablets and plasma. Anal. Lett. 2009, 42(1), 123-40. https://doi.org/10.1016/j.ejmech.2009. 09.003


https://doi.org/10.58985/jpam.2024.v02i02.24