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**Review Paper**

# Development And Validation of a Simple, Precise, Accurate, And Reproducible RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Glimepiride in Combined Dosage Form

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## ABSTRACT

Type 2 diabetes mellitus (T2DM) is frequently managed with combination therapy once metformin monotherapy fails to achieve adequate glycaemic control. Metformin Hydrochloride, a biguanide that improves peripheral insulin sensitivity and suppresses hepatic gluconeogenesis, is commonly combined with Glimepiride, a sulfonylurea insulin secretagogue, in fixed-dose tablet formulations to improve glycaemic outcomes, reduce pill burden, and enhance patient compliance. Because the two drugs differ markedly in dose strength, aqueous solubility, and ionisation behaviour, their simultaneous quantification in a combined dosage form is analytically demanding and is best addressed using reversed-phase high-performance liquid chromatography (RP-HPLC).This review compiles and critically compares RP-HPLC methods reported in the literature for the simultaneous estimation of Metformin Hydrochloride and Glimepiride, alone and together with other antidiabetic agents, in bulk drug and combined tablet dosage forms. The chromatographic conditions reported by various researchers, including the stationary phase, mobile phase composition, flow rate, detection wavelength, and retention behaviour, are summarised and compared, along with the validation parameters recommended under International Council for Harmonisation (ICH) Q2(R1) guidelines, namely specificity, linearity, accuracy, precision, range, limit of detection, limit of quantification, robustness, and system suitability.The review highlights C18 reversed-phase columns coupled with isocratic mobile phases containing methanol or acetonitrile with an acidic phosphate buffer, detected at 225-260 nm, as the most consistently validated approach for this drug pair, and discusses emerging trends such as Quality by Design (QbD)-based method optimisation and stability-indicating method development. The compiled evidence indicates that a well-optimised RP-HPLC method can deliver a simple, precise, accurate,

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and reproducible assay for Metformin Hydrochloride and Glimepiride in combined dosage forms, suitable for routine pharmaceutical quality control, stability testing, and regulatory submission

## INTRODUCTION

Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide, and type 2 diabetes mellitus (T2DM) accounts for the overwhelming majority of cases. T2DM arises from a combination of peripheral insulin resistance in skeletal muscle, liver, and adipose tissue, excessive hepatic glucose output, and a progressive decline in pancreatic β-cell function and mass [1]. Because no single class of antihyperglycaemic agent corrects all of these defects simultaneously, current treatment approaches increasingly favour combination therapy, recommending the addition of a second or third agent with a complementary mechanism once glycaemic targets are not met with a single drug [1].

Metformin Hydrochloride remains the universally recommended first-line oral agent for T2DM owing to its established efficacy, favourable cardiovascular and weight profile, and low cost. When glycaemic control is inadequate on metformin alone, a sulfonylurea such as Glimepiride is among the most frequently added agents, because it works through a distinct mechanism, namely direct stimulation of pancreatic insulin secretion, rather than improving insulin sensitivity. Clinical evidence indicates that combining metformin with a sulfonylurea produces a greater reduction in glycated haemoglobin (HbA1c) than either drug alone [2], and this two-drug combination is marketed worldwide as a fixed-dose combination (FDC) tablet whose bioequivalence to free combinations of the individual drugs has been confirmed in pharmacokinetic studies [2]. Fixed-dose combinations of this kind reduce the number of tablets a patient must take daily, a feature that has been associated with improved adherence and sustained glycaemic control in large, real-world patient populations [3,4].

The continuing clinical importance of the Metformin-Glimepiride combination, including its role as the background therapy onto which newer agents are now being added in triple fixed-dose combinations [1,4], places a direct and ongoing responsibility on pharmaceutical quality control laboratories to confirm that every batch of the combined dosage form contains the correct, uniform, and stable quantity of each active ingredient. Because the two drugs are co-formulated in the same tablet yet differ greatly in dose strength, aqueous solubility, and ionisation behaviour, conventional single-drug assay procedures are not directly transferable, and a dedicated, validated method capable of resolving and quantifying both actives within a single chromatographic run is required.

Reversed-phase high-performance liquid chromatography (RP-HPLC) has become the analytical method of choice for this purpose because of its high resolving power, sensitivity, reproducibility, and compatibility with the instrumentation already available in most pharmaceutical quality control laboratories. This review brings together the published literature on RP-HPLC methods developed for the simultaneous estimation of Metformin Hydrochloride and Glimepiride, examines the chromatographic strategies and method-development logic employed by different researchers, and summarises the validation parameters specified under the ICH Q2(R1) guideline, with the broader aim of providing analysts with a structured and consolidated reference point for future method development on this important drug combination.

## 2. DRUG PROFILE

### 2.1 METFORMIN HYDROCHLORIDE

Metformin Hydrochloride is chemically designated as N,N-dimethylimidodicarbonimidic diamide hydrochloride, commonly known as 1,1-dimethylbiguanide hydrochloride, with the molecular formula $C_4H_{11}N_5 \cdot HCl$ and a molecular weight of approximately 165.6 g/mol [5]. It occurs as a white, odourless, crystalline powder that is freely soluble in water, slightly soluble in alcohol, and practically insoluble in acetone and chloroform, with a reported melting point of 222-226 °C [5,6]. The molecule contains two imino and one amino donor centre and behaves as a strong base, exhibiting pKa values of approximately 2.8 and 11.5, so that it exists almost entirely in its protonated, cationic form across the physiological and most chromatographic pH ranges [5,6]. Owing to its high aqueous solubility combined with comparatively limited membrane permeability, metformin is classified under the Biopharmaceutics Classification System (BCS) as a Class III drug.

Pharmacologically, metformin is a biguanide antihyperglycaemic agent. Its principal mechanism of action involves suppression of hepatic gluconeogenesis, a modest reduction in intestinal glucose absorption, and improvement of peripheral glucose uptake and insulin sensitivity in skeletal muscle, largely mediated through activation of AMP-activated protein kinase (AMPK) signalling [5]. Because it does not stimulate pancreatic insulin secretion, metformin carries a low intrinsic risk of hypoglycaemia when used alone, and it is administered orally in doses typically ranging from 500 mg to 2000 mg per day.

### 2.2 GLIMEPIRIDE

Glimepiride is chemically described as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrrolin-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl) urea, with the molecular formula $C_{24}H_{34}N_4O_5S$ and a molecular weight of about 490.6 g/mol [7]. It is obtained as a white to faintly yellowish crystalline powder that is practically insoluble in water, slightly soluble in methylene chloride, and very slightly soluble in methanol, with a melting point near 207 °C [7,8]. Glimepiride behaves as a weak acid with a pKa of approximately 6.2 and possesses comparatively high lipophilicity (log P ≈ 3.5-3.8), and it is classified as a BCS Class II drug because of its high intestinal permeability combined with poor aqueous solubility, particularly at acidic and neutral pH [7,8].

Glimepiride belongs to the sulfonylurea class of insulin secretagogues. It binds to the sulfonylurea receptor (SUR1) subunit of ATP-sensitive potassium ($K_{ATP}$) channels on pancreatic β-cells, causing channel closure, membrane depolarisation, calcium influx, and the exocytotic release of insulin, with additional modest extrapancreatic effects that enhance peripheral insulin sensitivity. Glimepiride is administered in much smaller doses than metformin, typically 1-8 mg per day, and its low aqueous solubility combined with high potency means it must be reliably detected at concentrations several-fold lower than metformin within the same chromatographic run – one of the principal analytical challenges that the methods reviewed in this article are designed to overcome.

### 2.3 COMPARATIVE PHYSICOCHEMICAL AND PHARMACOLOGICAL PROFILE

Table 1 summarises the key physicochemical and pharmacological characteristics of the two drugs side by side, illustrating the extent to which they differ in polarity, solubility, and dose strength – differences that directly shape the chromatographic strategy required for their simultaneous estimation.

| Parameter | Metformin Hydrochloride | Glimepiride |
|---|---|---|
| Category | Biguanide - antihyperglycaemic agent | Sulfonylurea - insulin secretagogue |
| Chemical name | N,N-dimethylimidodicarbonimidic diamide hydrochloride (1,1-dimethylbiguanide hydrochloride) | 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrrolin-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl)urea |
| Molecular formula | $C_4H_{11}N_5 \cdot HCl$ | $C_{24}H_{34}N_4O_5S$ |
| Molecular weight | ≈ 165.6 g/mol | ≈ 490.6 g/mol |
| Appearance | White, odourless crystalline powder | White to faintly yellowish crystalline powder |
| Melting point | 222-226 °C | ≈ 207 °C |
| Aqueous solubility | Freely soluble in water | Practically insoluble in water |
| pKa | 2.8 and 11.5 (strong base) | ≈ 6.2 (weak acid) |
| BCS class | Class III (high solubility, low permeability) | Class II (low solubility, high permeability) |
| Usual oral dose | 500-2000 mg/day | 1-8 mg/day |
| Mechanism of action | Suppresses hepatic gluconeogenesis; improves peripheral insulin sensitivity via AMPK activation; does not stimulate insulin secretion | Binds SUR1 subunit of pancreatic $K_{ATP}$ channels, causing depolarisation and insulin exocytosis; modest extrapancreatic insulin-sensitising effect |

## 2.4 RATIONALE FOR THE FIXED-DOSE COMBINATION

Clinical and pharmacokinetic evidence supports the rationale for combining Metformin Hydrochloride and Glimepiride within a single tablet. Bioequivalence studies of the 2 mg / 500 mg fixed-dose combination have demonstrated comparable pharmacokinetic performance between generic and reference formulations, supporting their therapeutic interchangeability [2]. Large, real-world studies involving thousands of patients on Metformin-Glimepiride combinations have reported sustained glycaemic control and acceptable safety profiles across a wide range of disease durations and comorbidities [3]. More recent triple-combination trials, in which Metformin and Glimepiride together form the background therapy onto which additional agents

such as sodium-glucose co-transporter-2 (SGLT2) inhibitors are added, further illustrate the continuing and expanding clinical relevance of this two-drug combination as a therapeutic backbone in T2DM management [1,4]. This sustained, widespread, and evolving clinical use is the principal driver behind continued analytical method development, since every new strength, formulation, or combination product containing Metformin and Glimepiride requires a validated assay method for regulatory approval and routine quality control.

## 3. NEED FOR A SIMULTANEOUS RP-HPLC METHOD

Several factors make the simultaneous estimation of Metformin Hydrochloride and Glimepiride analytically demanding and explain the emphasis placed on RP-HPLC throughout the literature reviewed in this article:

- **Disparate dose strengths:** A typical fixed-dose tablet contains metformin in the 500-1000 mg range alongside glimepiride in the 1-4 mg range, a ratio that can exceed 250:1. The chromatographic and detection system must provide adequate sensitivity for the minor component without saturating the detector response for the major component.
- **Divergent physicochemical behaviour:** Metformin is highly polar, water-soluble, and exists predominantly as a cation, whereas glimepiride is lipophilic, poorly water-soluble, and behaves as a weak acid. A single mobile phase system must elute and resolve both compounds with acceptable peak shape and resolution.
- **Overlapping UV absorption:** Both drugs absorb in the low-UV region, so the detection wavelength must be chosen carefully, often close to a compromise or isosbestic wavelength, to allow simultaneous quantification with acceptable sensitivity for both analytes in one run.
- **Regulatory and pharmacopoeial requirements:** Assay, content uniformity, dissolution, and stability testing of combination products require methods that are formally validated according to ICH Q2(R1) guidelines and, where applicable, official pharmacopoeial monographs.
- **Stability-indicating capability:** Because combination tablets may be exposed to heat, humidity, and acidic, alkaline, or oxidative stress during storage, many reported methods are designed to resolve the parent drugs from their degradation products, adding a further dimension to method development.

RP-HPLC addresses each of these challenges effectively, offering high resolving power, the flexibility to tune selectivity through stationary phase chemistry, mobile phase pH and composition, and detector wavelength, together with excellent reproducibility suited to routine quality control use. These attributes collectively explain the dominance of RP-HPLC in the published literature on this drug combination.

## 4. ANALYTICAL TECHNIQUES REPORTED FOR METFORMIN HYDROCHLORIDE AND GLIMEPIRIDE: A LITERATURE OVERVIEW

A range of analytical techniques has been reported for the estimation of Metformin Hydrochloride and Glimepiride, either individually or in combination with other antidiabetic drugs. UV spectrophotometric methods, including simultaneous equation, absorbance ratio, and derivative spectroscopy approaches, have been used for rapid and economical estimation, but they generally lack the resolving power needed when more than one drug, or a degradation product, is present in the sample. High-performance thin-layer chromatography (HPTLC) methods allow

simultaneous analysis of multiple samples at relatively low cost but typically offer lower precision and sensitivity than column-based liquid chromatography. Liquid chromatography-mass spectrometry (LC-MS/MS) methods provide the highest sensitivity and specificity and are mainly employed for bioanalytical and pharmacokinetic studies in biological matrices such as plasma, but their high instrumentation and running cost limit their routine use in pharmaceutical quality control settings.

RP-HPLC with UV or photodiode-array (PDA) detection occupies the middle ground between these techniques, combining good resolution, sensitivity, and robustness with instrumentation that is widely available in pharmaceutical quality control laboratories, which explains its predominance in the published literature concerning this drug pair. Table 2 summarises representative RP-HPLC methods reported for the simultaneous estimation of Metformin Hydrochloride and Glimepiride, either as a binary mixture or together with a third antidiabetic agent in triple-combination products, illustrating the diversity of stationary phases, mobile phase systems, and detection wavelengths that have been successfully validated for this drug combination.

| Drug Combination | Column | Mobile Phase | Flow (mL/min) | λ (nm) | Retention Time (min) | Ref. |
|---|---|---|---|---|---|---|
| MET + GLM (binary, stability-indicating) | - | Phosphate buffer (20 mM, pH 3.0): Methanol-Acetonitrile (62.5:37.5), 80:20 | 1.0 | 230 | MET 2.75; GLM 5.87 | [9] |
| MET + GLM (binary, stability-indicating) | Agilent C18 (250×4.6 mm, 5 µm) | 25 mM hexane-1-sulphonic acid buffer (pH 2.5): Acetonitrile (45:55) | 1.0 | 229 | MET 3.55; GLM 5.82 | [10] |
| MET + Pioglitazone + GLM | C18 (250×4.6 mm, 5 µm) | Methanol: Phosphate buffer pH 4.3 (75:25) | 1.0 | 258 | Not specified | [11] |
| MET + Pioglitazone + GLM | JASCO Finepak SIL C18 (250×4.6 mm, 5 µm) | Methanol: Acetonitrile : 15 mM $KH_2PO_4$ pH 4 (40:35:25) | 1.0 | 240 | Not specified | [12] |
| MET + GLM + | Inertsil-ODS-3 C18 | Methanol: Phosphate | 1.0 | 238 | Not specified | [13] |

| Drug Combination | Column | Mobile Phase | Flow (mL/min) | λ (nm) | Retention Time (min) | Ref. |
|---|---|---|---|---|---|---|
| Pioglitazone | (100×4.6 mm, 5 µm) | buffer pH 3.6 (75:25) | | | | |
| MET + Rosiglitazone Maleate + GLM | RP-C18 (250×4.6 mm, 2.27 µm) | Methanol: Acetonitrile : Phosphate buffer pH 5.39 (20:40:40) | 1.0 | 238 | MET 3.69; Rosi 8.18; GLM 12.5 | [14] |
| MET + Atorvastatin + GLM (stability-indicating) | Grace Smart Altima C8 (250×4.6 mm, 5 µm) | Acetonitrile : Phosphate buffer pH 3.0 (60:40) | 1.0 | 235 | MET 2.57; ATR 7.06; GLM 9.39 | [15] |
| MET + Pioglitazone + GLM (QbD/Box-Behnken) | Phenomenex C18 (DoE-optimised) | Optimised within a Box-Behnken-derived design space | Not fixed | Not spec. | Not specified | [16] |

Several patterns emerge from this body of literature. Most methods employ a C18 (octadecylsilane) reversed-phase column with 5 µm particle size and a column length between 100 and 250 mm, operated at or near ambient temperature. Mobile phases are predominantly isocratic mixtures of methanol or acetonitrile with an aqueous phosphate buffer, with the buffer pH adjusted usually between pH 2.5 and 5.5 using orthophosphoric acid to control the ionisation state of metformin and achieve adequate peak symmetry. Detection wavelengths cluster in the 225-260 nm range, reflecting the overlapping UV absorption of the two drugs. Flow rates around 1.0 mL/min and total run times under 15 minutes are typical, making these methods suitable for high-throughput routine analysis. Reported linearity ranges consistently mirror the large dose disparity between the two drugs, with metformin validated over a much higher concentration range than glimepiride in every binary method reviewed [9,10].

## 5. PRINCIPLE AND INSTRUMENTATION OF RP-HPLC

Reversed-phase HPLC separates analytes on the basis of differential partitioning between a non-polar stationary phase, typically octadecylsilane (C18) or octylsilane (C8) bonded silica, and a comparatively polar mobile phase composed of water or aqueous buffer mixed with a water-miscible organic modifier such as methanol or acetonitrile. Polar analytes elute earlier and non-polar analytes are retained longer, which is consistent with the elution order observed across the methods reviewed, where the highly polar metformin consistently elutes well before the comparatively lipophilic glimepiride [9,10,15].

A typical RP-HPLC system used for this drug combination comprises a high-pressure isocratic pump, a manual or automated injector, a thermostated C18 or C8 analytical column, a UV or PDA detector operated in the 225-260 nm range, and data-acquisition software for peak integration and quantification. Buffer pH control is particularly important for metformin, since it is a strong base with pKa values of 2.8 and 11.5 and will exist in different ionisation states depending on mobile phase pH, which directly affects its retention behaviour and peak shape on a reversed-phase column.

## 6. METHOD DEVELOPMENT CONSIDERATIONS

### 6.1 SELECTION OF DETECTION WAVELENGTH

Because metformin and glimepiride do not share an identical UV absorption maximum, method developers typically scan both drugs individually and as a mixture using a PDA detector to identify either an isosbestic point or a single compromise wavelength that provides adequate sensitivity for both analytes without requiring wavelength switching during the run. Reported detection wavelengths for this pair and its related multi-drug combinations span 226-260 nm, with values around 230-240 nm being the most frequently validated [9,10,12,15,16].

### 6.2 SELECTION OF COLUMN AND STATIONARY PHASE

A C18 column of 4.6 mm internal diameter, 100-250 mm length, and 5 µm particle size is the most common choice across the reviewed methods, providing a balance of resolution, analysis time, and back-pressure suitable for routine isocratic separations. C8 stationary phases have also been used successfully, generally with comparable mobile phase strategies [15].

### 6.3 MOBILE PHASE OPTIMISATION

Mobile phase optimisation is the most critical step in developing a method for this drug pair. Because metformin is highly polar and ionisable while glimepiride is comparatively non-polar, the aqueous buffer component, organic modifier ratio, and pH must be optimised together. Phosphate buffers adjusted to a mildly acidic pH (approximately 2.5-5.5) are widely used to protonate the basic groups on metformin and stabilise its retention, while the proportion of methanol or acetonitrile is adjusted to achieve sufficient retention and resolution of glimepiride without an excessive run time. Some methods incorporate an ion-pairing reagent, such as hexane-1-sulphonic acid, to further improve the peak shape and retention of metformin on the reversed-phase column [10].

### 6.4 FLOW RATE AND COLUMN TEMPERATURE

A flow rate of approximately 1.0 mL/min at ambient or slightly above ambient column temperature is typical across the reviewed methods, providing adequate resolution within a practical analysis time of roughly 6-15 minutes.

### 6.5 DESIGN OF EXPERIMENTS AND QUALITY BY DESIGN APPROACHES

More recent work has applied Quality by Design (QbD) principles, using risk-assessment tools to identify critical method parameters, followed by formal experimental designs such as fractional factorial and Box-Behnken response-surface designs, to systematically map the relationship between chromatographic variables and method performance and to define a validated operating "design space" within which the method remains robust [16]. This approach represents a shift away from the traditional one-factor-at-a-time optimisation seen in earlier publications towards a

more statistically rigorous and regulation-aligned method-development strategy.

## 7. METHOD VALIDATION AS PER ICH Q2(R1) GUIDELINES

Once a candidate chromatographic method has been optimised, it must be formally validated before it can be used for regulatory submission or routine quality control. The ICH Q2(R1) guideline, "Validation of Analytical Procedures: Text and Methodology," is the universally referenced framework for this purpose and is followed, explicitly or implicitly, by essentially all of the RP-HPLC methods reviewed in this article [9,10,15,17]. Table 3 summarises the key validation parameters required for an assay-type procedure of this kind, together with the acceptance criteria generally applied across the literature.

| Parameter | Definition / Purpose | Typical Acceptance Criterion |
|---|---|---|
| **Specificity** | Ability to assess the analyte unequivocally in the presence of excipients, impurities, or degradation products | No interference observed at the analyte retention time |
| **Linearity** | Demonstration of a proportional detector response across the working concentration range | Correlation coefficient ($r^2$) ≥ 0.999 |
| **Range** | Interval of concentration over which acceptable precision and accuracy are demonstrated | Typically covers 50-150% of label claim (wider for stability-indicating methods) |
| **Accuracy** | Closeness of the measured result to the true (spiked) value | Recovery within 98-102% at each spiked level |
| **Precision - Repeatability** | Agreement among replicate measurements made under identical conditions on the same day | % RSD ≤ 2% |
| **Precision - Intermediate** | Agreement among measurements made on different days, analysts, or instruments | % RSD ≤ 2% |
| **Limit of Detection (LOD)** | Lowest concentration of analyte that can be reliably detected | Signal-to-noise ratio ≥ 3:1 |
| **Limit of Quantification (LOQ)** | Lowest concentration that can be quantified with acceptable precision and accuracy | Signal-to-noise ratio ≥ 10:1 |

| Parameter | Definition / Purpose | Typical Acceptance Criterion |
|---|---|---|
| **Robustness** | Reliability of the method under small, deliberate variations in operating conditions | No significant change in system suitability or assay results |
| **System Suitability** | Confirmation that the chromatographic system is performing adequately before/during the run | Tailing factor ≤ 2; theoretical plates > 2000; resolution > 2 |

### 7.1 SYSTEM SUITABILITY

Before each validated run, system suitability parameters, including theoretical plate count, tailing factor, and resolution between the metformin and glimepiride peaks, are checked against predefined limits to confirm that the chromatographic system is performing adequately on the day of analysis.

### 7.2 SPECIFICITY AND SELECTIVITY

Specificity is demonstrated by comparing chromatograms of a blank, a placebo (excipient) solution, and the standard or sample solution, confirming that no excipient peak interferes with the metformin or glimepiride peaks. In stability-indicating variants of the method, specificity is further demonstrated by confirming that degradation products formed under stress conditions – acid, base, oxidative, thermal, and photolytic – are adequately resolved from the parent drug peaks [9,10].

### 7.3 LINEARITY AND RANGE

Linearity is established by analysing a series of standard solutions spanning the expected working concentration range of each drug, typically at five to six concentration levels, and applying linear regression to the peak area versus concentration data. A correlation coefficient of 0.999 or better is generally considered acceptable, and this criterion is consistently met across the published methods reviewed for this drug pair.

### 7.4 ACCURACY

Accuracy is assessed through recovery studies in which known quantities of the analyte are added to a pre-analysed sample, typically at three concentration levels (often 80%, 100%, and 120% of the nominal concentration) and in triplicate, with the percentage recovery normally required to fall within 98-102% of the amount added.

### 7.5 PRECISION

Precision is evaluated as repeatability (intra-day precision, multiple injections within the same day) and intermediate precision (inter-day precision, across different days, analysts, or instruments), with a relative standard deviation (% RSD) of less than 2% generally accepted as the benchmark for both parameters.

### 7.6 LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The limit of detection (LOD) and limit of quantification (LOQ) are calculated, most commonly from the standard deviation of the response and the slope of the calibration curve, to define the lowest concentration of each drug that can be reliably detected and quantified, respectively.

### 7.7 ROBUSTNESS

Robustness is examined by deliberately introducing small variations in method parameters, such as mobile phase ratio, flow rate, column temperature, or detection wavelength, and confirming that the method continues to produce results within acceptable limits, demonstrating its reliability under normal day-to-day variation in laboratory conditions.

### 7.8 SOLUTION STABILITY AND RUGGEDNESS

Standard and sample solution stability is assessed by reanalysing the same solutions after a defined storage interval, commonly 24-48 hours at room temperature or under refrigeration, to confirm that no significant degradation occurs prior to analysis, while ruggedness studies confirm reproducibility across different analysts, instruments, or laboratories.

## 8. APPLICATIONS OF THE VALIDATED RP-HPLC METHOD

Validated RP-HPLC methods for Metformin Hydrochloride and Glimepiride support several stages of the pharmaceutical product lifecycle, including:

- Routine assay and batch-release testing of fixed-dose combination tablets;
- Content uniformity testing across individual dosage units;
- In vitro dissolution testing of both immediate-release and modified-release formulations;
- Forced degradation and long-term / accelerated stability studies, in which stability-indicating variants of the method track the formation of degradation products over time;
- Bioequivalence and pharmacokinetic studies, where extended or modified versions of the method are applied to biological matrices such as plasma; and
- Regulatory submissions and pharmacopoeial compliance testing for new generic and fixed-dose combination products entering the market.

## 9. CHALLENGES AND FUTURE PERSPECTIVES

Despite the considerable body of published work, several challenges remain in the analytical chemistry of this drug combination. The large dose ratio between metformin and glimepiride continues to constrain the design of methods capable of accurately quantifying both drugs within a single, unmodified injection, without resorting to dual-wavelength detection or sample dilution strategies. There is also growing interest in green analytical chemistry, prompting some researchers to replace acetonitrile with more environmentally benign solvents such as methanol or ethanol, or to reduce overall mobile phase consumption through shorter columns and faster flow rates, without compromising validation performance.

The application of Quality by Design and Design of Experiments methodologies, as demonstrated in recent Box-Behnken-based optimisation studies, is likely to become more common, since it provides a more systematic and regulation-aligned route to establishing a robust operating design space for the method, rather than relying solely on traditional trial-and-error optimisation [16]. Finally, as newer triple and quadruple fixed-dose combinations incorporating metformin and glimepiride alongside agents such as SGLT2 inhibitors reach the market [1], there will be a continuing need for RP-HPLC methods capable of resolving an increasing number of co-formulated actives within a single, practical chromatographic run.

## CONCLUSION

Metformin Hydrochloride and Glimepiride together form one of the most widely prescribed

fixed-dose combinations for the management of type 2 diabetes mellitus, and the quality control of this combination depends on a robust, simple, precise, accurate, and reproducible analytical method. The literature reviewed in this article consistently demonstrates that reversed-phase HPLC, using a C18 column, an isocratic mobile phase combining methanol or acetonitrile with an acidic phosphate buffer, and UV or PDA detection in the 225-260 nm range, can resolve and quantify both drugs within a single, short chromatographic run while satisfying the linearity, accuracy, precision, sensitivity, and robustness criteria specified by ICH Q2(R1). These validated methods provide pharmaceutical manufacturers and regulatory laboratories with a dependable tool for assay, content uniformity, dissolution, and stability testing of Metformin-Glimepiride combination products, and emerging Quality by Design-based approaches promise to make future method development for this and related antidiabetic drug combinations even more systematic, efficient, and robust.

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