A Comprehensive Review on Analytical methods reported for the Quantitative Estimation of Glimepiride, Dapagliflozin and Metformin Hydrochloride in tablet dosage forms

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***Abstract*—The development of accurate and reliable analytical methods is essential for the quantitative estimation of pharmaceutical compounds in combined dosage forms. Glimepiride, Dapagliflozin, and Metformin Hydrochloride are widely used antidiabetic agents belonging to different pharmacological classes—DPP-4 inhibitor, SGLT2 inhibitor, and biguanide, respectively. Their combination therapy offers synergistic control over blood glucose levels in patients with type 2 diabetes mellitus. This review provides a comprehensive overview of various UV-spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods developed and validated for the simultaneous determination of these three drugs in bulk and tablet dosage forms. Emphasis is placed on method development parameters such as selection of detection wavelength, mobile phase composition, stationary phase, flow rate, retention time, linearity range, and validation as per ICH guidelines. The comparative assessment highlights that RP-HPLC methods demonstrate superior sensitivity, selectivity, and precision compared to UV methods, although UV-spectrophotometry remains advantageous for routine, cost-effective analysis in resource-limited laboratories. The review concludes that optimized RP-HPLC methods are more suitable for the simultaneous estimation of Glimepiride, Dapagliflozin, and Metformin Hydrochloride in fixed-dose formulations for routine quality control applications.**

***Keywords*— Glimepiride, Dapagliflozin, Metformin Hydrochloride, UV spectrophotometry, RP-HPLC**

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. It represents one of the most prevalent non-communicable diseases worldwide and remains a significant public health concern due to its increasing incidence, morbidity, and mortality. The two major forms—Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM)—differ in their pathophysiology, with T1DM involving autoimmune destruction of pancreatic β-cells and absolute insulin deficiency, while T2DM involves insulin resistance, impaired insulin secretion, and progressive β-cell dysfunction. Among these, T2DM accounts for approximately 90–95% of all diagnosed cases globally.

Chronic hyperglycaemia in diabetes is associated with long-term complications affecting vital organs, including the eyes, kidneys, heart, and peripheral nerves. Microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular complications (cardiovascular, cerebrovascular, and peripheral vascular diseases) significantly reduce quality of life and increase mortality. Because of these complications, effective glycaemic control is essential in preventing disease progression and improving patient outcomes.

The global burden of diabetes continues to rise due to sedentary lifestyles, urbanization, obesity, and aging populations. According to the International Diabetes Federation (IDF), over 500 million adults worldwide are currently living with diabetes, and this number is expected to increase sharply in the coming decades. This growing prevalence highlights the urgent need for effective therapeutic strategies and reliable analytical techniques for drug analysis and quality control.[1]

MOA of Glimepiride, Dapagliflozin and Metformin hydrochloride

Glimepiride acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors.Among secondary sulfonylureas, Glimepiride has lesser risk of hypoglycemia.[2]

Dapagliflozin is an SGLT2 inhibitor that lowers blood glucose by acting on the kidneys. It selectively blocks the sodium-glucose co-transporter-2 (SGLT2) in the proximal renal tubules, which is responsible for reabsorbing most filtered glucose. Inhibition of SGLT2 promotes urinary glucose excretion, reducing plasma glucose levels independently of insulin. It also leads to mild osmotic diuresis and natriuresis, contributing to weight loss and blood-pressure reduction.[3]

Metformin HCl (a biguanide) primarily decreases hepatic glucose production by inhibiting gluconeogenesis. It activates AMP-activated protein kinase (AMPK), which suppresses hepatic glucose output and increases insulin sensitivity in peripheral tissues. Metformin also enhances glucose uptake in skeletal muscle and reduces intestinal glucose absorption. It does not cause hypoglycaemia and is weight-neutral.[4]

Together, these medications target different pathways—increase glucose level (Glimepiride), renal glucose loss (dapagliflozin), and reduced hepatic glucose output + improved insulin sensitivity (metformin)—providing complementary control of Type 2 diabetes.

Importance of Oral Antidiabetic Agents

Oral antidiabetic agents (OADAs) are essential in the management of T2DM, particularly in cases where lifestyle modification alone is insufficient to maintain glycaemic targets. These agents lower blood glucose levels through various mechanisms, such as enhancing insulin secretion, reducing hepatic glucose output, improving insulin sensitivity, or increasing urinary glucose excretion.

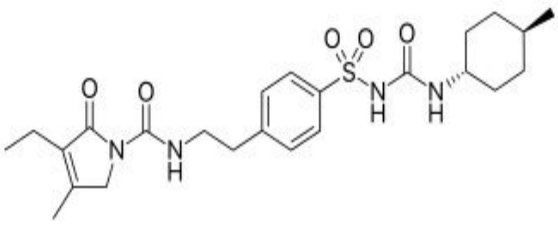
Several classes of OADAs are currently used in clinical practice, including biguanides, sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium–glucose co-transporter 2 (SGLT2) inhibitors, α-glucosidase inhibitors, and meglitinides. Among these, metformin, a biguanide, remains the first-line therapy due to its efficacy, safety profile, and cardio-protective benefits. Newer agents such as linagliptin (a DPP-4 inhibitor) and dapagliflozin (an SGLT2 inhibitor) offer advantages including weight neutrality, low risk of hypoglycaemia, and additional cardiovascular or renal benefits.

Despite the availability of multiple drug classes, monotherapy often becomes insufficient due to the progressive nature of T2DM. As a result, combination therapies are increasingly required to achieve and sustain optimal glycaemic control.[5]

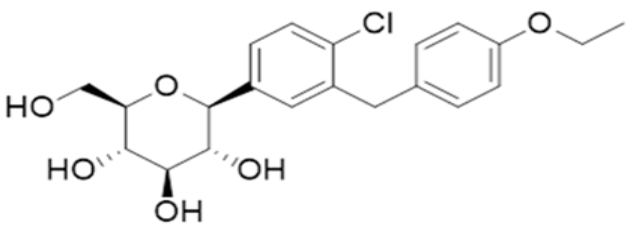
Need for Combination Therapies (Glimepiride + Dapagliflozin + Metformin)

Combination therapy has become a cornerstone in T2DM management due to complementary mechanisms of action and improved therapeutic efficacy. The combination of glimepiride, dapagliflozin, and metformin represents a rational and synergistic approach to glycaemic control.

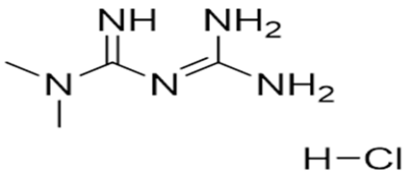
Glimepiride



Dapagliflozin



Metformin Hydrochloride



Glimepiride
Inhibits SUR1 on K+ channels in beta cells.
Prevent causes depolarization & increases insulin secretion.
Decreases glucogen levels.
Lower blood glucose via increased glucose uptake by tissues.[6]

Dapagliflozin
SGLT2 inhibitor that increases urinary glucose excretion
Reduces plasma glucose, body weight, and blood pressure
Offers cardiovascular and renal protective properties [7]

Metformin HCL
Biguanide decreases hepatic glucose production (gluconeogenesis)
Enhances insulin sensitivity
Improves peripheral glucose uptake [8]

UV-Visible Spectroscopy (UV)
UV-Visible spectroscopy is an analytical technique that measures the absorption of ultraviolet or visible light by molecules. Absorption occurs due to electronic transitions, and the degree of absorbance is related to concentration via Beer-Lambert's law. UV spectroscopy is widely used for qualitative and quantitative analysis in pharmaceuticals, biochemistry, and environmental studies. Timeously, resulting in superior glycaemia control compared with monotherapy. Combination formulations also improve patient compliance by reducing pill burden.

Working Principle of UV–Visible Spectroscopy (UV):
UV spectroscopy is an analytical technique based on the absorption of ultraviolet or visible light by molecules containing chromophores ($\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ transitions). When a sample is exposed to UV light of a specific wavelength, electrons in these chromophores absorb energy and transition to higher electronic states. The instrument measures the decrease in light intensity (absorbance), which is directly proportional to the concentration of the absorbing species according to the Beer–Lambert law ($A = \varepsilon bc$). UV detectors are widely used for quantitative analysis of compounds that absorb in the 190–800 nm range. The method is simple, rapid, and commonly integrated with liquid chromatography for detection of analytes.[9]

Reverse-Phase HPLC (RP-HPLC)
RP-HPLC is a type of HPLC where the stationary phase is non-polar (e.g., C18), and the mobile phase is relatively polar. Analytes are separated primarily based on hydrophobic interactions, with more non-polar compounds retained longer. RP-HPLC is extensively used for the analysis of drugs, peptides, and biomolecules due to its versatility and reproducibility.

Working Principle of Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC):
RP-HPLC is a chromatographic separation technique in which the stationary phase is non-polar (e.g., C18-bonded silica) and the mobile phase is polar (typically water mixed with methanol or acetonitrile). Separation is achieved based on differences in hydrophobic interactions: non-polar analytes are retained longer on the stationary phase, while more polar analytes elute earlier. By altering the mobile-phase composition (isocratic or gradient elution), the retention and resolution of compounds can be precisely controlled. Detection is often performed using a UV detector, since many organic compounds absorb UV light. RP-HPLC provides high sensitivity, excellent resolution, and reproducible quantification for complex mixtures in pharmaceuticals, environmental samples, and biological matrices.[10]

Table 1 Analytical method For Estimation of Glimepiride

Sr No	Drug Name	Analytical Method	Description	Ref no
01	Glimepiride	UV	Solvent: Methanol/Ethanol Detection of wavelength:230 nm Linearity(µg/ml):2-20(µg/ml) r2:0.999	11
02	Glimepiride	UV	Solvent: phosphate buffer Detection of wavelength:235 nm Linearity(µg/ml):2-20 (µg/ml)	12

			r2: 0.999	
03	Glimepiride	RP-HPLC	Column: C18(250×4.6mm,5μm) Mobile Phase: Acetonitrile; Phophate buffer 60:40 v/v (pH - 3.0) Detection of wavelength: 230 nm Flowrate: 1.0 mL/min	13
04	Glimepiride	RP-HPLC	Column:C18 Silica Mobile Phase; Acetonitrile; Phophate buffer 60:40 v/v (pH - 3.0) Detection of wavelength:230nm Flowrate:0.8 mL/min	14
05	Glimepiride	RP-HPLC	Column: C18(150×4.6mm,3.5µm) Mobile Phase: 0.02 M potassium dihydrogen phosphate (60;40 v/v) (HPLC grade) Detection of wavelength:232 nm Flowrate:1.0 mL/min	15
06	Glimepiride	RP-HPLC	Column: C18(150×4.6mm,5µm) Mobile Phase: Acetronitrile : 0.02 M buffer, (pH - 3.0) Detection of wavelength:230 nm Flowrate: 1 mL/min	16
07	Glimepiride and Dapagliflozin	UV	Solvent: Water Detection of wavelength(nm): 230(Glimepiride), 235(Dapagliflozin) Linearity: 2-20(Glimepiride) 5-10 (dapagliflozin)	17
08	Glimepiride and Dapagliflozin	UV	Solvent: methanol Detection of wavelength: 234 nm(Glimepiride),223 nm(dapagliflozin) Linearity:1-20(Glimepiride),5-10(Dapagliflozin)	18
09	Glimepiride and Dapagliflozin	RP-HPLC	Column: Thermo Scientific synchronic C18,)5µm Mobile phase: Phosphate buffer: acetonitrile Detection of wavelength:230 nm Flow rate: 1.0 mL/min	19
10	Glimepiride and Dapagliflozin	RP-HPLC	Column: Qualisil 5 model (C18(250×4.6mm,5µm) Mobile phase:0.1% O-phosphoric acid pH adjusts to 4 with TEA and Acetonitrile (45:55%) (v/v) Detection of wavelength:293 nm Flow rate: 1.0 mL/min	20
11	Glimepiride and Dapagliflozin	RP-HPLC	Column: Hypersil C18((250×4.6mm,5µm) Mobile phase: acetonitrile: water r^2:0.995(Dapagliflozin),0.999(linagliptin) Flow rate:1 mL/min	21
12	Glimepiride and Dapagliflozin	RP-HPLC	Column:C18 column (250×4.6mm,5µm) Mobile phase: Acetonitrile: phosphate buffer (0.01 M KH2PO4, pH 6.5) (47:57) (v/v) Detection of wavelength:225 nm Flow rate: 1mL/min	22

Table 2 Analytical method For Estimation of Dapagliflozin

Sr No	Drug Name	Analytical method	Description	Ref no
01	Dapagliflozin	UV	Solvent: Distilled water Detection of wavelength: 278 nm Linearity(µg/ml): 5-10 (µg/ml) r^2:0.9992	23
02	Dapagliflozin	UV	Solvent: Methanol: water (15:85) Detection of wavelength: 220 nm Linearity(µg/ml): 5-30(µg/ml) r2:0.999	24
03	Dapagliflozin	RP-HPLC	Column: pinecone C18 Mobile phase: Acetonitrile:0.1 triethylamine(pH-5.0) (50:50) (v/v) Detection of wavelength: 224 nm Flow rate:1 mL/min	25
04	Dapagliflozin	RP-HPLC	Column: Inspire (150×4.6mm,5µm) Mobile phase: methanol: water (80:20) (v/v) Detection of wavelength:235 nm Flow rate:1.0 mL/min	26
05	Dapagliflozin	RP-HPLC	Column: Waters C18(250×4.6mm,5µm) Mobile phase: Phosphate buffer: acetonitrile (40:60) (v/v) Detection of wavelength: 237 nm Flow rate:1.0 mL/min	27
06	Dapagliflozin	RP-HPLC	Column: Zorbax Eclipse Plus, Agilent Technology column (250×4.6mm,5µm) Mobile phase: Methanol: water Detection of wavelength:230 nm Flow rate: 1 mL/min	28
07	Dapagliflozin and metformin HCL	UV	Solvent: methanol Detection of wavelength:235 nm (Dapagliflozin), 272 nm (metformin HCL) Linearity(µg/ml):0.5-2.5 (µg/ml) (Dapagliflozin) 25-125 (µg/ml) (metformin HCL r^2:0.984(dapagliflozin), 0.982(Metformin HCL)	29
08	Dapagliflozin and metformin HCL	UV	Solvent: Distilled water Detection of wavelength: 275 nm (dapagliflozin) 245 nm (Metformin HCL) Linearity(µg/ml):2-10 (µg/ml) (Dapagliflozin), 20-100(µg/ml) (Metformin HCL) r^2:0.9984(Dapagliflozin) 0.9994(Metformin HCL)	30
09	Dapagliflozin and metformin HCL	RP-HPLC	Column: Phenomenex C18(250mm×4.6 mm, 5µm) Mobile phase: Water: methanol (50:50) (v/v) Detection of wavelength: 230 nm Flow rate: 1.0 mL/min	31
10	Dapagliflozin and metformin HCL	RP-HPLC	Column: Intersil ODS column (250×4.6mm, 5µm) Mobile phase: ACN: KH2PO4 pH- 4.5 (65:35%) (v/v) Detection of wavelength: 227 nm Flow rate: 1 mL/min	32

11	Dapagliflozin and metformin HCL	RP-HPLC	Column: Agilent 1260 infinity 2 with an Agilent 5 TC-C18(2) (250mm ×4.6mm) Mobile phase: 0.02% triethylamine: acetonitrile (v/v) Detection of wavelength: 236 nm Flow rate: 1 mL/min	33
12	Dapagliflozin and metformin HCl	RP-HPLC	Column: C18 analytical reverse phase column Mobile phase: Methanol: sodium acetate buffer (70:30) (v/v) Detection of wavelength: 240 nm Flow rate: 1.0 mL/min	34

Table 3 Analytical method for Estimation of Metformin Hydrochloride

Sr No	Drug name	Analytical method	Description	Ref no
01	Metformin HCl	UV	Solvent: Distilled water Detection of wavelength: 232 nm Linearity(µg/ml): 2-10 (µg/ml) r^2: 0.999	35
02	Metformin HCl	UV	Solvent: Methanol: ethanol: acetonitrile: phosphate buffer Detection of wavelength: 234 nm Linearity(µg/ml): 10-15(µg/ml) r^2: 0.9998	36
03	Metformin HCl	RP-HPLC	Column: C18 analytical reverse phase column Mobile phase: Methanol: water (30:70) (v/v) Detection of wavelength: 233 nm Flow rate: 0.5 mL/min	37
04	Metformin HCl & Glimepiride	UV	Solvent: Distilled water Detection of wavelength:294.4nm(Glimepiride) 230.4 nm (Metformin HCl) Linearity(µg/ml):10-14 (µg/ml) (Glimepiride) 2-4(µg/ml) Metformin HCL r^2: 0.999	38
05	Metformin HCl & Glimepiride	UV	Solvent: Methanol water Detection of wavelength: 295 nm(Glimepiride) 234 nm (Metformin HCl) Linearity(µg/ml): 10-18 (µg/ml) (Glimepiride)3-11(µg/ml) Metformin HCl,r^2:0.9987(Glimepiride) ,0.9947(Metformin HCL)	39
06	Metformin HCl	RP-HPLC	Column: Water' X-bridge C18 (150×4.6 mm, 5µ) Mobile phase: Acetonitrile:0.02 M phosphate buffer (pH-5.0) (35:65) (v/v) Detection of wavelength: 225 nm Flow rate: 1.0 mL/min	40
07	Metformin HCl	RP-HPLC	Column: C18 column(150×4.6mm, 4µm) Mobile phase: Acetonitrile: 0.0 1 M di-potassium hydrogen phosphate buffer (75:25) Detection of wavelength: 237 nm	41

			Flow rate: 0.1 mL/min	
08	Metformin HCl & Dapagliflozin	RP-HPLC	Column: Hypersil BDSC18 column (4.6×250 mm,5µm) Mobile phase: KH2Po4: acetonitrile (40:60) Detection of wavelength: 225 nm Flow rate: 1.0 mL/min	42
09	Metformin HCl	RP-HPLC	Column: Water's X-bridge C18(150×4.6mm,5µm) Mobile phase: Acetonitrile: phosphate buffer (35:65) Detection of wavelength: 225 nm Flow rate: 1.0 mL/min	43

II. CONCLUSION

The review emphasizes the need for accurate and reliable analytical methods to estimate Glimepiride, Dapagliflozin and Metformin HCl in combined dosage forms. UV spectroscopy is simple, fast and economical, but its selectivity is limited for multicomponent formulations. RP-HPLC methods, on the other hand, provide superior precision, sensitivity and specificity, with excellent validation results using optimized C18 columns and mobile phases. Overall, RP-HPLC is identified as the most suitable and reliable technique for routine quality-control and stability-indicating analysis of this triple-drug combination.

III. SUMMARY

This review focuses on UV and RP-HPLC analytical methods used for estimating Glimepiride, Dapagliflozin, and Metformin HCl in tablet dosage forms. These three drugs, which act through different mechanisms, are widely used in combination for better glycaemic control in Type 2 diabetes. The literature collected shows that UV spectroscopy is simple and economical, while RP-HPLC provides higher accuracy, sensitivity, and selectivity. Different columns, mobile phases, wavelengths, and validation parameters were compared to understand the most efficient analytical conditions for simultaneous estimation.

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