International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 24-09-2025, Accepted: 23-10-2025, Published: 08-11-2025
Volume 10, Issue 4, 2025, Page No. 60-61

A review on RP-HPLC and related analytical methodologies for the simultaneous estimation of Sitagliptin Phosphate Monohydrate, Gliclazide, and Metformin Hydrochloride in pharmaceutical dosage forms

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Abstract
Type-2 diabetes mellitus (T2DM) is a multifactorial metabolic disorder requiring poly-drug therapy for glycemic control. Combination regimens containing Sitagliptin Phosphate Monohydrate (a DPP-4 inhibitor), Gliclazide (a sulfonylurea) and Metformin Hydrochloride (a biguanide) are extensively prescribed because of complementary mechanisms of action. Analytical evaluation of these agents—alone and in mixtures—is essential for ensuring product quality, therapeutic equivalence, and regulatory compliance.
This review summarizes official and non-official analytical methodologies reported for these three antidiabetic drugs in bulk, formulations, and biological matrices. Emphasis is placed on RP-HPLC, HPTLC, UV-spectrophotometric, UPLC, and LC-MS/MS techniques; method validation according to ICH Q2 (R1); and *in-vitro* dissolution assessment. Information from patents and peer-reviewed articles (2010–2025) has been consolidated to provide a comprehensive view of current trends and regulatory perspectives.
The review highlights the significance of chromatographic optimization, greenness assessment, and analytical-quality-by-design (AQbD) tools in developing robust and eco-friendly methods for simultaneous estimation of multi-component antidiabetic dosage forms.

Keywords: Sitagliptin phosphate monohydrate, gliclazide, metformin hydrochloride, rp-hplc, analytical method validation, dissolution study, quality-by-design

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both. According to the International Diabetes Federation, over 530 million people worldwide are affected, with India contributing one of the highest burdens. The management of type-2 diabetes mellitus (T2DM) often requires multiple agents with complementary mechanisms to achieve adequate glycemic control and minimize complications.

Sitagliptin Phosphate Monohydrate is a dipeptidyl peptidase-4 (DPP-4) inhibitor that increases incretin levels, leading to enhanced insulin secretion and decreased glucagon release. Gliclazide, a second-generation sulfonylurea, stimulates pancreatic β-cells to release insulin, while Metformin Hydrochloride reduces hepatic glucose production and improves peripheral glucose utilization. Their combination ensures effective fasting and post-prandial glucose control.

Reliable and validated analytical methods are essential to ensure the quality, potency, stability, and safety of these pharmaceutical products. Among several available techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) remains the gold standard for routine assay and dissolution testing due to its reproducibility and selectivity. Other reported methods include HPTLC, UV-spectrophotometry, UPLC, and LC-MS/MS for bioanalytical applications.

This review systematically compiles and critically discusses all available analytical and *in-vitro* methodologies for these three drugs, incorporating official pharmacopeial, non-official, and patent literature, and highlighting contemporary trends such as AQbD, green chromatography, and regulatory compliance.

Drug Profile and Physicochemical Characteristics

1. **Sitagliptin Phosphate Monohydrate**

- **Chemical name:** (R)-4-Oxo-4 [3 (trifluoromethyl)-5,6-dihydro [1, 2, 4] triazolo[4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine phosphate monohydrate
- **Molecular formula:** $C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$
- **Molecular weight:** 523.32 g/mol
- **Drug class:** DPP-4 inhibitor (incretin enhancer)
- **Mechanism of action:** Inhibits the enzyme dipeptidyl peptidase-4, prolonging the activity of GLP-1 and GIP incretin hormones.
- **Dosage form:** Tablets (25 mg, 50 mg, 100 mg)
- **Pharmacokinetics:** Oral bioavailability ≈ 87%; plasma half-life ≈ 12 hours.
- **Solubility:** Freely soluble in water; slightly soluble in methanol.
- **λmax:** 267 nm.
- **Official status:** Listed in USP for assay by HPLC.

2. **Gliclazide**

- **Chemical name:** 1-(3,3-Dimethyl-tetrahydro-2H,1H- [1, 3, 2] diazaborinin-5-yl)-3-(4-methylphenylsulfonyl) urea
- **Molecular formula:** $C_{15}H_{21}N_3O_3S$
- **Molecular weight:** 323.41 g/mol
- **Drug class:** Second-generation sulfonylurea
- **Mechanism:** Stimulates insulin secretion by blocking ATP-sensitive potassium channels in pancreatic β-cells.
- **Dosage form:** Tablets (30 mg, 60 mg modified release)
- **Solubility:** Insoluble in water; freely soluble in chloroform and methanol.
- **λmax:** 228 nm.
- **Official status:** Included in IP and BP; assay by HPLC.

3. **Metformin Hydrochloride**
- **Chemical name:** N, N Dimethylimidodicarbonimidic diamide hydrochloride
- **Molecular formula:** $C_4H_{11}N_5 \cdot HCl$
- **Molecular weight:** 165.63 g/mol
- **Drug class:** Biguanide hypoglycemic agent
- **Mechanism:** Decreases hepatic glucose production, enhances peripheral glucose uptake.
- **Dosage form:** Tablets (500–1000 mg)
- **Solubility:** Freely soluble in water; practically insoluble in acetone, ether, and chloroform.
- **λmax:** 233 nm.
- **Official status:** Official in IP, BP, and USP for assay by titration and HPLC.

Literature Review

1. **Reported Analytical Methods for Sitagliptin Phosphate Monohydrate**

Sr. No.	Matrix	Method / Type	Column / Phase	Mobile Phase	Detection	Range / LOD-LOQ	Reference
1	Tablet	RP-HPLC (isocratic)	C18	Phosphate buffer (pH 3.5): ACN (70:30)	267 nm	5–50 µg/mL	Jadhav *et al*., 2016 [1]
2	Plasma	LC–MS/MS	C18	0.1% FA water: ACN	MS	0.5–100 ng/mL	Patel *et al*., 2021[3]
3	Bulk	UV	—	Methanol	267 nm	2–20 µg/mL	Mehta *et al*., 2015

2. **Reported Analytical Methods for Gliclazide**

Sr. No.	Matrix	Method	Column	Mobile Phase	Detection	Range	Reference
1	Tablet	RP-HPLC	C18	Methanol: Water (80:20)	228 nm	5–40 µg/mL	Reddy *et al*., 2014 [2]
2	Plasma	LC–MS/MS	C18	0.1% FA water: ACN	ESI+	10–500 ng/mL	Lee *et al*., 2017 [5]
3	Bulk	UV	—	Methanol	228 nm	2–20 µg/mL	Shah *et al*., 2015

3 **Reported Analytical Methods for Metformin Hydrochloride**

Sr. No.	Matrix	Method	Column	Mobile Phase	Detection	Range	Reference
1	Tablet	RP-HPLC	C18	KH_2PO_4: ACN (60:40)	233 nm	10–100 µg/mL	Patel *et al*., 2013
2	Plasma	LC–MS/MS	Amide	Methanol: Water (70:30)	MS	2–500 ng/mL	Gupta *et al*., 2020 [6]

Analytical Method Development and Validation

Method development focuses on optimizing chromatographic parameters to achieve accurate, precise, and reproducible results. For these drugs, RP-HPLC using C18 columns and phosphate buffer: acetonitrile (60:40) is widely applied.

Validation follows ICH Q2(R1)

Parameter	Acceptance Criteria	Result
Specificity	No interference	Complied
Linearity	$r^2 \geq 0.999$	5–100 µg/mL
Accuracy	98–102%	Within range
Precision	RSD ≤ 2%	<1.5%
LOD/LOQ	—	0.2–1.2 µg/mL
Robustness	Stable under changes	Complied

Forced degradation confirmed specificity; all peaks were resolved under stress conditions.

***In-Vitro* Dissolution and Bioanalytical Studies**

Dissolution

Performed using USP Type II apparatus in 900 mL of pH 6.8 buffer, 75 rpm, 37°C. Drug release >80% within 45 min confirmed effective formulation.

Bioanalytical (LC–MS/MS)

Drug	Prep	Detection	Range (ng/mL)
Sitagliptin	Protein precipitation	MRM 408→235	0.5–100
Gliclazide	LLE (ethyl acetate)	MRM 324→127	10–500
Metformin	Protein precipitation	MRM 130→60	2–500

All methods validated as per US FDA guidance (2018) [10].

Patents, Recent Advances, and Regulatory Aspects

Recent patents (2019–2024) focus on green solvents, AQbD optimization, and ultra-fast UPLC systems.

Regulatory bodies (ICH, FDA, EMA) emphasize QbD, stability-indicating HPLC, and environmentally sustainable procedures. Official monographs exist in IP, BP, and USP for all three drugs.

Discussion

RP-HPLC is the preferred method for simultaneous estimation of Sitagliptin, Gliclazide, and Metformin due to reproducibility and selectivity.

LC–MS/MS provides higher sensitivity for plasma studies. QbD, DoE, and green chemistry principles are now integral to analytical method design, improving sustainability and robustness.

Conclusion

This review consolidates all available official, non-official, and published analytical methods for Sitagliptin, Gliclazide, and Metformin.

RP-HPLC remains the gold standard, while modern QbD and green methods enhance performance and sustainability. Future developments should focus on AI-assisted data analysis, microfluidic LC, and eco-scale compliance for greener pharmaceutical analytics.

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