Vol. 13, No. 2 (2025), pp.48–59

CHITKARA UNIVERSITY

Journal of Pharmaceutical Technology Research and Management

HPTLC Driven Analytical Approaches for Antidiabetic Pharmaceuticals: A Detailed Review

Atul A. Shirkhedkar[1*], Amod S. Patil[1], Inderbir Singh[2], Gurbinder Kaur[3], and Tejas Raju Jorvekar[1]

[1]*R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur (Dhule), Maharashtra, India.*

[2]*Chitkara College of Pharmacy, Chitkara University, Rajpura, Punjab, India.*

[3] *TIET-VT, Center of Excellence in Emerging Materials (CEEMS), Thapar Institute of Engineering and Technology, Patiala, Punjab, India.*

[*]shirkhedkar@gmail.com(Corresponding Author)

REVIEW ARTICLE **Open Access**

ARTICLE INFORMATION

Received: January 19, 2026
Accepted: February 13, 2026
Published Online: May 15, 2026

Keywords:
HPTLC, Antidiabetic drugs, Analytical methods, Validation

ABSTRACT

Background: Diabetes mellitus is a chronic disease affecting a large number of populations across the world, and it also requires management using anti-diabetic drugs. Various drugs such as metformin, vildagliptin, saxagliptin, sitagliptin, rosiglitazone, glipizide, and their combinations are commonly used for the treatment. Numerous pharmaceutical formulations are available in the market in combination forms as well. In order to ensure the quality, appropriate dosage, and regulatory compliance of these formulations, accurate and reliable analytical methods are essential.

Purpose: This review will provide comprehensive insight into HPTLC-based analytical approaches used for qualitative and quantitative analysis of anti-diabetic drugs in various pharmaceutical formulations.

Methods: This review critically summarizes the literature reporting HPTLC methods developed for the analysis of anti-diabetic drugs. Key aspects such as HPTLC method development, mobile phase and stationary phase selection, detection methods, and optimization strategies are discussed. Method validation parameters such as robustness, sensitivity, reproducibility, and accuracy are mentioned.

Results: The study demonstrated that the HPTLC method is a reliable and efficient analytical technique for the evaluation of anti-diabetic drugs due to its low cost, high reproducibility, minimal solvent consumption, and ability to analyze multiple samples simultaneously. For routine quality control and research applications, validated HPTLC methods have shown satisfactory performance.

Conclusion: HPTLC represents itself as a valuable tool for the qualitative as well as quantitative assessment of anti-diabetic formulations. This detailed review provides guidance for researchers as well as analysts in selecting and optimizing appropriate HPTLC protocols for research and quality control purposes. It also highlights the potential for further advancements in HPTLC-based drug analysis.

DOI: 10.15415/jptrm.2025.132003

1. Introduction

Diabetes mellitus (DM) is a metabolic condition characterized either by inadequate insulin production, impaired insulin action, or both. This insulin deficiency leads to prolonged hyperglycaemia and disturbs the normal metabolism of carbohydrates, lipids, and proteins (Poznyak *et al.*, 2020). The disease progression can result in tissue and blood vessel damage, resulting in severe complications such as retinopathy, neuropathy, nephropathy, cardiovascular disorders, and foot ulcers (Rossi *et al.*, 2019). Hence, diabetes represents a heterogeneous group of disorders.

Diabetes mellitus (DM) is classified into several types such as type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary forms caused by conditions such as endocrinopathies or steroid use. Among these, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) are the most prevalent forms. These two major forms dominate the majority of diabetes cases across the globe.

The prevalence of type 1 DM results from insufficient insulin secretion and mostly impacts children and adolescents, whereas type 2 DM is caused by insulin resistance and/or impaired secretion, usually occurring in middle-aged and

older adults. As type 1 DM and type 2 DM show differences in their pathophysiology, this leads to various causes, clinical features, and management strategies for each treatment (Sapra *et al.*, 2024; Kumar & Clark, 2002; Puavilai, 1999).

Conventionally, therapeutic strategies for hyperglycaemia mainly depend on the use of several drug classes. These include sulfonylureas, which stimulate insulin release; biguanides, which reduce liver glucose production; PPAR-gamma agonists, which enhance sensitivity; and α-glucosidase inhibitors, which slow down glucose absorption in the gut (Chaudhury *et al.*, 2017). These antidiabetic agents are used alone or in dual or triple combinations with other hypoglycaemic agents (Feingold *et al.*, 2024).

Maintaining proper blood glucose levels is crucial to reduce the chances of developing diabetes complications, including kidney failure, retinopathy, neuropathy, and cardiovascular disorders. Pharmaceutical analysis is a fundamental area of pharmaceutical sciences that is concerned with the identification of validated methods to determine quality parameters (e.g., purity profile) and safety parameters of active pharmaceutical constituents and finished dosage forms (Attimarad *et al.*, 2020). It constitutes methods for detecting compounds, revealing their strength, and evaluating their overall quality.

Pharmaceutical analysis is also crucial in the development of therapeutic processes. It is used in the analysis of bulk drugs, intermediates, formulations, impurities, as well as degradation products. Analytical techniques are used to determine drug stability, the presence of harmful contaminants, and drug concentration in pharmaceutical preparations. They are also obligatory in pharmacokinetic experimentation, which tracks drugs and their metabolites in biological fluids (Patel & Pandya, 2018).

HPLC, GC, and HPTLC chromatographic methods are highly investigated in regulatory laboratories to determine pharmaceutical raw materials as well as biological samples. Such methods are invaluable in all phases of the drug development process, starting with initial research and concluding with quality control assessment in routine operations. Several types of analytical procedures have been developed to quantify drug concentration in both pharmaceutical preparations and biological fluids to guarantee efficacy, safety, and compliance with regulatory procedures. It also involves procedures for separating, identifying, and quantifying the constituents of each sample.

Antidiabetic drugs in bulk and pharmaceutical formulations have employed various analytical procedures such as spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), ultra-performance liquid chromatography (UPLC), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis (CE), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-electrospray ionization/mass spectrometry (LC-ESI/MS) (Shaikh & Singla, 2018). These techniques are used not only for quality control of pharmaceutical matrices but also in diagnostic and clinical investigations to monitor drug levels, study pharmacokinetics, and detect impurities or degradation products.

Though advanced techniques such as LC-MS and UPLC provide superior selectivity and sensitivity, their routine application is usually hindered by extremely high cost, the need for skilled professionals to operate the instruments, and extensive sample preparation methods. Similarly, reverse-phase HPLC, despite being the most widely used technique, involves large quantities of organic solvent consumption and long analysis times, which limit its application in cost-sensitive laboratories. Moreover, commonly used solvents such as acetonitrile, methanol, and tetrahydrofuran are neurotoxic, teratogenic, and environmentally hazardous (Conelly, 2017).

Conversely, HPTLC requires considerably less solvent, making it a greener and safer alternative. In addition, HPTLC enables the analysis of multiple samples, requires less technical expertise, and generates minimal waste. HPTLC has gained attention due to its simplicity, cost-effectiveness, reproducibility, and ability to analyse multiple samples simultaneously. This makes it highly useful for the routine analysis of antidiabetic drugs (Prajapati *et al.*, 2023).

Additionally, HPTLC presents several advantages over other analytical approaches, including HPLC, spectrophotometry, and titrations. The method allows easy separation even for coloured compounds, and several samples can be investigated simultaneously on a single plate, making it faster, efficient, and economical. Two-dimensional separations are easy to carry out, and sensitive colour reagents can be used for precise identification of separated spots or bands. HPTLC supports various evaluation methods that facilitate the identification of compounds with diverse colours or light absorption properties.

In addition, TLC plates are single-use, eliminating the need for regeneration or cleaning. Since development and detection are performed separately, the plates can be stored and analysed later to obtain analytical data (Kalász & Báthori, 2001; Sethi, 1996).

The present review highlights the use of HPTLC techniques for the analysis and evaluation of antidiabetic pharmaceuticals.

2. Various Types, Therapeutic Uses, and Mechanisms of Action of Antidiabetic Drugs

Antidiabetic drugs are categorized based on their chemical structure, mechanism of action, and their role in regulating blood glucose levels in individuals with

diabetes. These drugs can broadly be classified into two main categories, i.e., oral hypoglycaemic agents and injectable insulin preparations. Each class comprises many subclasses that act on various pathways involved in glucose regulation, particularly in type 2 diabetes mellitus (T2DM) (Inzucchi *et al.*, 2014; Harris & McCarty; Ismail-Beigi, 2012).

The main drugs used to control hyperglycaemia work through different mechanisms. Sulfonylureas enhance insulin release by stimulating the pancreatic β-cells. Biguanides help lower blood sugar by decreasing glucose production in the liver and improving the body's response to insulin. PPAR-gamma agonists improve insulin action in peripheral tissues, which supports better glucose uptake. In addition, α-glucosidase inhibitors slow the absorption of carbohydrates in the intestine, helping to reduce the rise in blood glucose after meals. The detailed classification is shown in Figure 1.

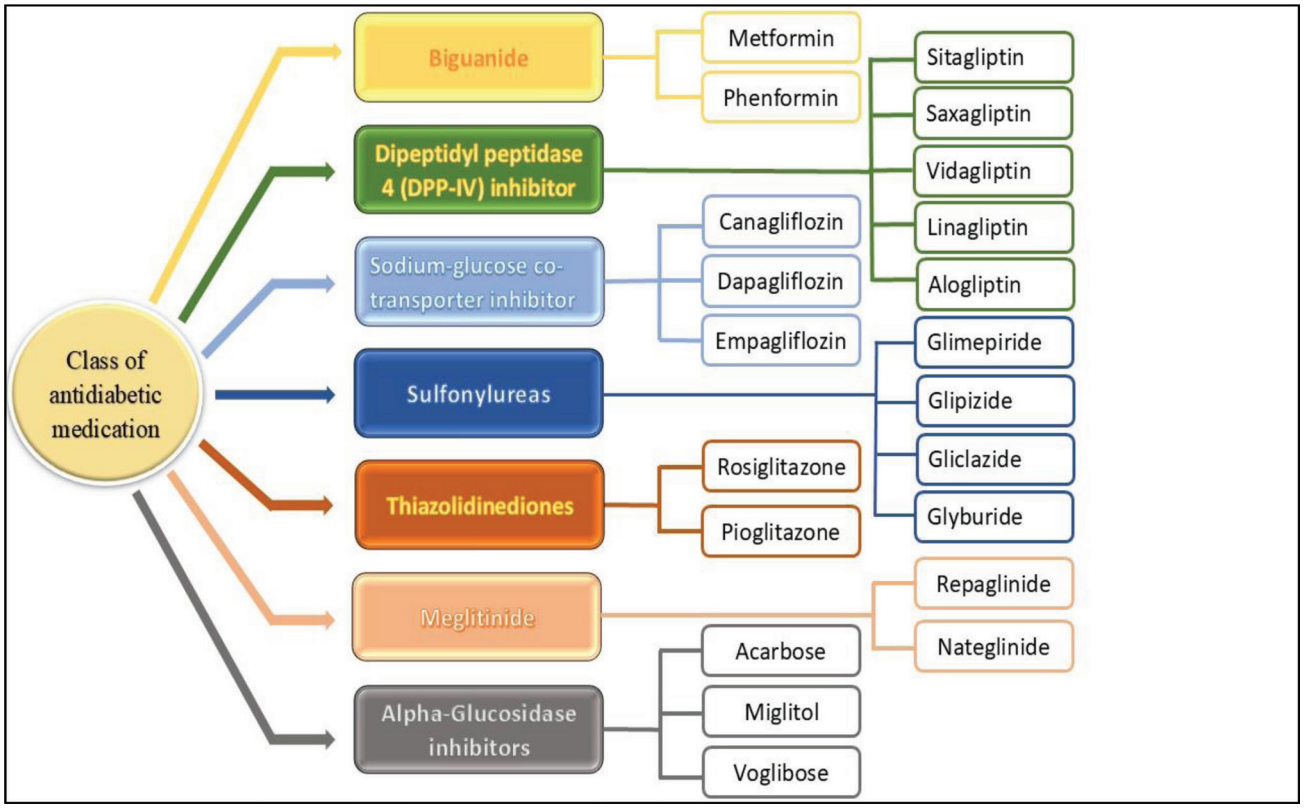

Figure 1: Categories of Medications Used in Diabetes Management

3. High-Performance Thin-Layer Chromatography Technique (HPTLC)

HPTLC is a more advanced version of traditional TLC. It uses high-quality adsorbent layers of silica gel with a uniform particle size, i.e., around 5 µm compared to 12 µm in conventional TLC. HPTLC also employs specialized instruments assisted with automatic developing chambers for better precision (Poole, 2003).

HPTLC involves a defined process for method development, optimization, documentation, and application of validated protocols. The technique is commonly employed for both qualitative and quantitative analysis of compounds in mixtures. In its quantitative analysis, the implementation of standardized protocols and accurate procedures confirms accurate and reproducible measurement of compounds in pharmaceutical and biological samples (Nyiredy, 2002).

The principle of HPTLC is based on adsorption, similar to conventional TLC. In this technique, the mobile phase moves the sample components along the stationary phase by capillary action. The separation occurs according to the principle that the compounds interact with the stationary phase to different extents. Components that have low affinity with the stationary phase travel faster with the mobile phase, while more affine compounds slow down, enabling good separation according to adsorption (Inflibnet, 2025).

Modern HPTLC systems have been developed with instrumentation that uses advanced components to improve analytical accuracy and reproducibility. The sample applicator offers precision and uniformity in sample spotting

using computerized procedures (Spangenberg, 2011). Separations are performed under controlled humidity and saturation conditions in plate development chambers (Waksmundzka-Hajnos *et al.*, 2008). Qualitative and quantitative investigations are supported by high-resolution documentation systems with appropriate light sources. In addition, densitometers allow accurate quantification of compounds by absorption or fluorescence measurements (Görög, 2011).

4. Approaches for Optimisation Analysis of HPTLC

The optimization of various variables is essential when developing an HPTLC procedure to obtain accurate and reliable results. The stationary phase is important, especially silica gel plates, which can generally be more selective to certain compounds. The choice of stationary phase has a profound effect on the resolution and selectivity of HPTLC analysis. Although silica gel 60 F254 is usually used because it has high adsorption capacity and is suitable for UV detection, variations in particle size, surface thickness, and layer modification may change the sharpness of bands, migration characteristics, and separation efficiency.

Highly polar compounds have greater interaction with silica surfaces, which may result in broader bands or a lower migration rate unless the mobile phase composition is well tuned. In multicomponent formulations, stationary phase properties are important for attaining sufficient selectivity among structurally similar drugs. Thus, effective selection and optimization of the stationary phase are necessary to achieve efficient separation and reproducible analytical performance (Shewiyo *et al.*, 2012).

Mobile phase optimization is a stepwise process that starts with pre-screening of appropriate solvent systems, followed by modification of the solvent system to achieve optimum separation (Gope *et al.*, 2024). Other important parameters include chamber saturation, development distance, and humidity control, which have a major impact on the quality and resolution of separations.

In recent applications, HPTLC methods have increasingly been employed in the analysis of anti-diabetic drugs in both single and multi-component formulations. Current methodological trends show enhanced separation efficiency through optimized solvent systems, improved plate quality, and accurate control of development conditions, resulting in sharper bands and improved repeatability. Computer-aided method development has made this process quicker and has also enhanced the accuracy of quantitative analysis. Such procedures facilitate the application of HPTLC methods in compliance with regulatory validation requirements, emphasizing robustness, accuracy, and reproducibility (McCalley, 2003).

Other factors include the choice of detection techniques and post-chromatographic derivatization techniques, which have become more widely used in recent years to improve sensitivity and selectivity, especially for compounds with low natural chromophoric ability. Optimization of the methodology ensures high-quality qualitative and quantitative analysis, minimal solvent usage, and good reproducibility, and is therefore an important step in HPTLC-based pharmaceutical analysis.

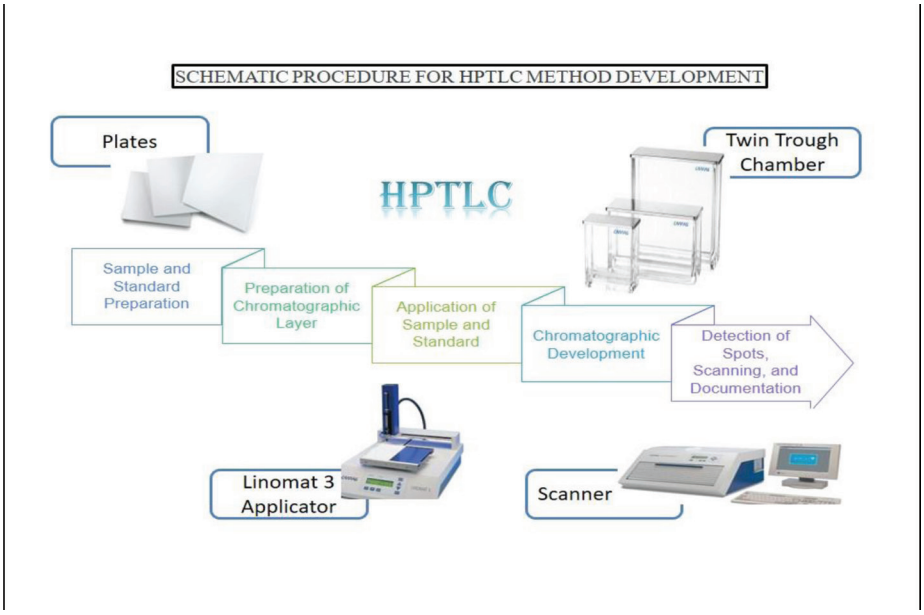

Figure 2: Stepwise Procedure for HPTLC Method Development

The stepwise procedure for HPTLC method development is detailed and presented in Figure 2. Additionally, HPTLC acts as an effective stability-indicating analytical technique for antidiabetic pharmaceuticals. The method provides clear separation of active compounds from potential degradation products formed under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic exposure. Owing to its high resolving capacity and visual band detection, it facilitates monitoring of degradation behaviour along with assessment of formulation integrity during storage.

Further, its ability to analyse multiple samples simultaneously also aids in comparative stability evaluation in routine quality control. Thus, HPTLC provides a cost-effective and reliable approach for preliminary stability screening and stability studies of antidiabetic drug formulations.

5. HPTLC-Based Method Validation for Quality Assessment of Pharmaceuticals

Analytical method validation includes several important criteria for assuring the precision of the results. Recent research trends have shown growing interest in the application of HPTLC for the analysis of antidiabetic pharmaceuticals, especially for multidrug formulations and assessment of stability studies. Methodological advancements emphasize improved solvent optimization, enhanced densitometric detection, and validation in accordance with regulatory guidelines. These developments indicate that HPTLC can be considered an evolving technique, which serves beyond a conventional screening tool and is taken as a reliable quantitative and stability-indicating analytical approach. The increasing trend of eco-friendly solvent systems and advanced plate technology further supports its relevance in modern pharmaceutical quality control practices (Nishad *et al.*, 2023).

Accuracy refers to the similarity of a measured value to the true value. Precision defines how consistent repeated measurements of a homogeneous sample are when analyzed under the same conditions. Repeatability reflects the closeness of agreement of results obtained by the same analyst on the same instrument under similar conditions. Conversely, reproducibility refers to variation observed when the same procedure is performed over time or by different analysts and instruments. Sensitivity refers to the capability of the method to respond to slight variations in the concentration of a drug or to detect its presence in a mixture. Specificity is the ability of the method to accurately measure the target analyte without interference from other constituents in the sample.

Linearity refers to the ability of the method to generate test results that are proportional to analyte concentration within a specified range, whereas the range defines the lowest and highest analyte levels at which the method demonstrates acceptable accuracy, precision, and linearity. Robustness assesses the reliability of the method when small, intentional variations in analytical conditions are introduced, ensuring consistent performance. To ensure that the entire analytical system, including the instrument, reagents, and method parameters, is functioning properly before sample analysis, system suitability testing is performed.

In addition, the Limit of Quantification (LOQ) and Limit of Detection (LOD) should be determined to assess the sensitivity of the method. The LOQ is the lowest concentration of an analyte that can be quantitatively determined with acceptable accuracy and precision. The LOD, on the other hand, represents the lowest concentration of an analyte that can be detected but not necessarily quantified reliably.

Table 1 provides a summary of validation parameters and shows that HPTLC procedures established to detect single antidiabetic agents in bulk and pharmaceutical preparations exhibit acceptable linearity and sensitivity over microgram to nanogram concentrations. For example, metformin shows linearity between 200–1000 ng/mL, with LOD and LOQ values of 95 and 200 ng/mL, respectively, indicating sufficient sensitivity for routine tablet analysis.

By comparison, validation data presented in Table 2 for combined formulations demonstrate variability in the sensitivity of co-formulated drugs. For instance, when repaglinide is used in combination with metformin, metformin shows a much higher LOD (17 ng/band) and LOQ (51 ng/band) compared to repaglinide (98 ng/band) under the same chromatographic conditions. This indicates that analytical sensitivity in HPTLC depends on molecular structure, chromophoric properties, and detection response, rather than solely on mobile phase composition.

Overall, comparative analysis between the two tables demonstrates that HPTLC procedures consistently achieve acceptable linearity ($r^2 = 0.99$) and quantification limits suitable for pharmaceutical quality control. However, variability in sensitivity for individual and combination drugs highlights the need for specific method optimization for each analyte and formulation.

Details of the analytical profiling of antidiabetic agents using HPTLC in bulk samples and pharmaceutical preparations are presented in Table 1, while HPTLC methods for the analysis of antidiabetic drugs in combined formulations and bulk materials are summarized in Table 2.

Table 1: Analytical Profiling of Antidiabetic Agents Using HPTLC in Bulk Samples and Pharmaceutical Preparations

Sr. No.	Drug	Sample	Description (Stationary Phase, Mobile Phase)	Rf	Linearity, r^2, LOD, LOQ	Detection Wavelength (nm)	Ref. No.
1	Metformin	Bulk material and Tablets	Silica gel 60 F254; Ammonium sulphate (0.5%): 2-propanol: methanol (8.0:1.6:1.6)	0.50 ± 0.03	Linearity: 200–1000 ng/mL (r^2 = 0.9991); LOD: 95 ng/mL; LOQ: 200 ng/mL	238	(Havele & Dhaneshwar, 2010)
2	Sitagliptin	Bulk material and Tablets	Silica gel 60 F254; Acetone: methanol: toluene: formic acid (4:3:2:1)	0.63 ± 0.02	Linearity: 200–500 ng/band (r^2 = 0.99); LOD: 27 ng/band; LOQ: 87 ng/band	220	(Sandeep Pawar *et al.*, 2015)
3	Vildagliptin	Bulk material and Tablets	TLC aluminium plate 60 F254; Toluene: methanol: n-butanol (4.5:4:1:0.5)	0.75	Linearity: 10–30 ng/band (r^2 = 0.993); LOD: 0.09 ng/band; LOQ: 0.9 ng/band	233	(Badke *et al.*, 2022)
4	Linagliptin	Bulk material and Tablets	Silica gel 60 F254; Acetone: methanol: chloroform: formic acid (3:1:5:1)	0.72	Linearity: 100–600 ng/spot (r^2 = 0.999); LOD: 5.19 ng/spot; LOQ: 15.74 ng/spot	230	(Srivani *et al.*, 2016)
5	Alogliptin	Bulk material and Tablets	Silica gel aluminium plate 60 F254; Acetonitrile: 1% ammonium acetate in methanol (4.5:5.5)	0.37	Linearity: 100–5000 ng/band (r^2 = 0.999); LOD: 2.356 ng/band; LOQ: 7.14 ng/band	277	(Sharma & Parle, 2015)
6	Saxagliptin	Bulk material and Tablets	Silica gel aluminium plate 60 F254; Methanol: chloroform (6:4)	0.50 ± 0.02	Linearity: 400–1200 ng/band (r^2 = 0.99); LOD: 7.96 ng/band; LOQ: 26.54 ng/band	222	(Srividya *et al.*, 2015)
7	Canagliflozin	Bulk material and Tablets	Silica gel aluminium plate 60 F254; Toluene: ethyl acetate: methanol (2:2:1)	0.50 ± 0.03	Linearity: 10–500 ng/spot (r^2 = 0.9988); LOD: 0.39 ng/spot; LOQ: 1.19 ng/spot	290	(Kaur *et al.*, 2016)
8	Dapagliflozin	Bulk material and Tablets	Silica gel aluminium plate 60 F254; Chloroform: methanol (9:1)	0.21 ± 0.004	Linearity: 400–1200 ng/band (r^2 = 0.999); LOD: 1.2083 ng/band; LOQ: 3.6616 ng/band	223	(Suma *et al.*, 2019)
9	Empagliflozin	Bulk material and Tablets	Silica gel 60 GF254; Ethyl acetate: chloroform: acetonitrile (55:25:20)	0.53	Linearity: 0.2–1.2 µg/spot (r^2 = 0.99); LOD: 0.061 µg/spot; LOQ: 0.177 µg/spot	254	(El-Desouky *et al.*, 2021)
10	Glimepiride	Bulk material and Tablets	Silica gel 60 F254; 0.5% ammonium sulfate: methanol (7.5:2.5)	0.73	Linearity: 600–2100 ng/band (r^2 = 0.99); LOD: 0.05 ng/band; LOQ: 0.16 ng/band	228	(Patel *et al.*, 2015)
11	Glipizide	Bulk material and Tablets	Silica gel 60 F254; Water: methanol: 0.5% ammonium sulfate (6:3:1.5)	0.85 ± 0.01	Linearity: 50–250 ng/band (r^2 = 0.993); LOD: 9.57 ng/band; LOQ: 29.01 ng/band	236	(Modi & Patel, 2012)
12	Gliclazide	Bulk material and Tablets	Silica gel 60 F254; Toluene: acetonitrile: ethanol: 0.25% ammonium sulphate (4:4:4:3)	0.69	Linearity: 200–1000 ng/spot (r^2 = 0.99); LOD: 86.14 ng/spot; LOQ: 261.03 ng/spot	228	(Patil *et al.*, 2014)
13	Glibenclamide	Bulk material and Tablets	Silica gel 60 F254; Toluene: ethyl acetate: methanol (8:0.5:1)	0.45 ± 0.07	Linearity: 40–200 ng/spot (r^2 = 0.9994); LOD: 6 ng/spot; LOQ: 20 ng/spot	229	(Havele & Dhaneshwar, 2010)

14	Rosiglitazone	Bulk material and Tablets	Silica gel 60 F254; Methanol: toluene: ethyl acetate (1:8:1)	0.39 ± 0.03	Linearity: 100–1500 ng/spot (r^2 = 0.9989); LOD: 35 ng/spot; LOQ: 90 ng/spot	228	(Dhole *et al.*, 2013)
15	Pioglitazone	Bulk material and Tablets	Silica gel 60 F254; Benzene: ethyl acetate: diethyl ether (6:3:1)	0.50 ± 0.03	Linearity: 600–3600 ng/mL (r^2 = 0.9984); LOD: 57.22 ng/mL; LOQ: 190.73 ng/mL	254	(Akabari *et al.*, 2025)
16	Repaglinide	Bulk material and Tablets	Silica gel 60 RP-18 F254; Chloroform: methanol: ammonia (4.5:0.8:0.05)	0.55 ± 0.03	Linearity: 600–1600 ng/spot (r^2 = 0.998); LOD: 22.64 ng/spot; LOQ: 68.84 ng/spot	288	(Jain *et al.*, 2013)
17	Nateglinide	Bulk material and Tablets	Silica gel 60 F254; Chloroform: ethyl acetate: acetic acid (4:6:0.1)	0.8	Linearity: 200–2400 ng/band (r^2 = 0.996); LOD: 0.020 ng/band; LOQ: 0.060 ng/band	216	(Thomas *et al.*, 2011)
18	Voglibose	Bulk material and Tablets	Silica gel 60 F254; Acetonitrile: methanol: ammonia (15:4:0.1)	0.66 ± 0.03	Linearity: 100–450 ng/spot (r^2 = 0.99); LOD: 40 ng/spot; LOQ: 100 ng/spot	284	(Rao & Konda, 2020)

Table 2: HPTLC Methods for the Analysis of Antidiabetic Drug in Combined Formulations and Bulk Material

Sr. No.	**Drug Combination**	**Sample**	**Description**	**Rf**	**Linearity, r^2, LOD, LOQ**	**Detection Wavelength (nm)**	**Ref. No.**
1	Metformin + Repaglinide	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Methanol: 0.25% ammonium sulphate (2.5:7.5)	0.34 & 0.60	Linearity: 500–2500 ng/band (r^2 = 0.9999) & 100–500 ng/band (r^2 = 0.995); LOD: 98 ng/band & 17 ng/band; LOQ: 296 ng/band & 51 ng/band	236	(Ahir *et al.*, 2013)
2	Sitagliptin + Simvastatin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Chloroform: methanol (8:2)	0.13 & 0.75	Linearity: 2000–7000 ng/spot (r^2 = 0.9983) & 250–750 ng/spot (r^2 = 0.9974); LOD: 150 ng/spot & 50 ng/spot; LOQ: 2000 ng/spot & 660 ng/spot	217	(Vinit *et al.*, 2014)
3	Vildagliptin + Metformin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: 1% ammonium acetate in methanol: toluene (10:0.5)	0.55 & 0.44	Linearity: 500–2000 ng/spot (r^2 = 0.991) & 1000–5000 ng/spot (r^2 = 0.999); LOD: 34.60 ng/spot & 17.22 ng/spot; LOQ: 104.85 ng/spot & 52.20 ng/spot	214	(Shirode *et al.*, 2014)
4	Linagliptin + Metformin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Acetone: methanol: toluene: formic acid (4:3:2:1)	0.82 & 0.61	Linearity: 20–100 ng/spot (r^2 = 0.9993) & 400–2000 ng/spot (r^2 = 0.9991); LOD: 10 ng/spot & 20 ng/spot; LOQ: 20 ng/spot & 100 ng/spot	259	(Chandrabatla Varaprasad *et al.*, 2015)
5	Alogliptin + Metformin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Acetonitrile: 1% ammonium acetate: methanol (4.5:5.5)	0.34 & 0.24	Linearity: 100–2500 ng/spot & 100–2500 ng/spot	235	(Rana & Sharma, 2021)
6	Dapagliflozin + Linagliptin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Toluene: chloroform: methanol: triethylamine (7:2:1:0.2)	0.23 & 0.40	Linearity: 200–1200 ng/band (r^2 = 0.9991); LOD: 25.80 ng/band & 13.09 ng/band; LOQ: 72.22 ng/band & 42.14 ng/band	224	(Shukla *et al.*, 2024)

7	Metformin + Glibenclamide	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Methanol: water: 0.4% sodium sulphate (7:5:11)	0.27 & 0.80	Linearity: 250–1750 ng/spot for both drugs; LOD: 1.2412 µg & 0.9944 µg; LOQ: 3.7613 µg & 3.0133 µg	232	(Malgundkar & Mulla, 2014)
8	Nateglinide + Metformin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Chloroform: ethyl acetate: acetic acid (4:6:0.1)	0.996 & 0.995	Linearity: 200–2400 ng/band & 500–3000 ng/band; LOD: 0.020 & 0.060; LOQ: 0.022 & 0.066	216	(Thomas *et al.*, 2011)
9	Sitagliptin + Metformin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Water: methanol: ammonium sulphate (4.5:4.5:1.5)	0.68 & 0.59	Linearity: 3000–24000 ng/spot & 50–400 ng/spot; LOD: 1.10 ng/spot & 0.28 ng/spot; LOQ: 3.32 ng/spot & 0.80 ng/spot	254	(Ravishankar *et al.*, 2013)
10	Metformin + Glipizide	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Water: methanol: 0.5% ammonium sulphate (6:3:1.5)	0.22 ± 0.01 & 0.85 ± 0.01	Linearity: r^2 = 0.9962 & 0.9930; LOD: 991.30 ng/band & 9.57 ng/band; LOQ: 3003.95 ng/band & 29.01 ng/band	236	(Modi & Patel, 2012)
11	Canagliflozin + Metformin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: Methanol: toluene: ethyl acetate: ammonia (2:4:4:0.1)	0.50 & 0.15	Linearity: 50–300 ng/band & 0.5–3.0 µg/band	254	(Vichare *et al.*, 2022)
12	Metformin + Empagliflozin	Bulk material and tablets	Stationary phase: Silica gel 60 F254; Mobile phase: 2% ammonium acetate: isopropyl alcohol: triethylamine (4:6:0.1)	0.82 ± 0.02 & 0.50 ± 0.02	Linearity: 5000–30000 ng/band & 125–750 ng/band; LOD: 705.21 ng/band & 24.65 ng/band; LOQ: 2136.99 ng/band & 74.70 ng/band	242	(Munde *et al.*, 2020)

6. Conclusion

The article is a review article containing a compilation of HPTLC analytical methods that are employed in the determination of antidiabetic drugs. These techniques include both bulk material and pharmaceutical dosage form analyses. Antidiabetic medications, applied separately or in combination, are significant in the management and treatment of various kinds of diabetes. It is important that such drugs are properly analysed to ensure their quality and efficiency as therapeutic agents.

HPTLC is an easy, sensitive, inexpensive, and environmentally friendly method, which is why it is a good alternative to traditional analytical methods such as HPLC; however, systematic evaluation using validated metrics of greenness needs to be carried out to ensure proper validation. It includes information concerning mobile phase compositions, detection wavelengths, validation parameters, and methodological simplicity.

Eighteen single drugs and twelve combination preparations were evaluated based on reported HPTLC methods and current literature, showing their appropriateness in terms of analytical evaluation. The protocols are suitable for the study of individual component formulations, and HPTLC with densitometric detection is still widely used for routine analysis of pharmaceutical compounds.

As new oral antidiabetic agents continue to be developed and introduced to the market each year, the availability of robust analytical strategies is crucial. By compiling and comparing existing HPTLC methods, this review provides a useful reference for researchers and analysts. It supports method development, quality control, and the evaluation of both established and newly emerging antidiabetic compounds.

Acknowledgement

The authors express their sincere gratitude to S. J. Surana, Principal, R. C. Patel Institute of Pharmaceutical Education

and Research, Shirpur, Dist. Dhule (MS), and Inderbir Singh, Chitkara College of Pharmacy, Chitkara University, for providing the necessary facilities and support to carry out this work.

Authorship Contribution

Tejas Raju: Conceptualization, investigation, data curation, and preparation of the original draft.
Gurbinder Kaur: Conceptualization, investigation, data curation, and preparation of the original draft.
Amod S. Patil: Organization, presentation, and critical comparison of the formulation strategy.
Atul A. Shirkhedkar: Supervision, validation, and critical review of the manuscript.
Inderbir Singh: Supervision, validation, and critical review of the manuscript.

Ethical Approval

The authors declare that no ethical approval was required for this study.

Funding

The authors declare that no funding or grants were received for this study.

Declarations

The authors confirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. The final version of the manuscript has been approved by all authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this manuscript.

Data Availability Statement

Data sharing is not applicable to this article, as no new data were generated or analyzed.

References

Ahir, K. B., Patelia, E. M., & Shah, A. (2013). Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical formulation by HPTLC-densitometry method. *Journal of Chromatography & Separation Techniques, 4*(1), 166. https://doi.org/10.4172/2157-7064.1000166

Akabari, A. H., Gajiwala, H., Patel, S. K., Surati, J., Solanki, D., Shah, K. V., Patel, T. J., & Patel, S. P. (2025). Stability-indicating TLC-densitometric and HPLC methods for simultaneous determination of teneligliptin and pioglitazone in pharmaceutical dosage forms with eco-friendly assessment. *Journal of Chromatographic Science, 63*(2), bmae038. https://doi.org/10.1093/chromsci/bmae038

Attimarad, M., Elgorashe, R. E., Subramaniam, R., Islam, M. M., Venugopala, K. N., Nagaraja, S., & Balgoname, A. A. (2020). Development and validation of rapid RP-HPLC and green second-derivative UV spectroscopic methods for simultaneous quantification of metformin and remogliflozin in formulation using experimental design. *Separations, 7*(4), 59. https://doi.org/10.3390/separations7040059

Badke, S. V., Kakad, K. S., & Malode, S. S. (2022). High-performance thin-layer chromatography (HPTLC) method development and validation for determination of remogliflozin etabonate and vildagliptin in bulk and its tablet formulation. *International Journal of Applied Pharmaceutics, 14*, 147–153. https://doi.org/10.22159/ijap.2022.v14ti.42

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N. S., Montales, M. T., Kuriakose, K., & Sasapu, A. (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Frontiers in Endocrinology, 8*, 6. https://doi.org/10.3389/fendo.2017.00006

Chavan, V., Ghante, M., & Sawant, S. (2014). Development and validation of RP-HPLC method for simultaneous estimation of sitagliptin phosphate and simvastatin in bulk and dosage form. *Journal of Applied Pharmacy, 6*, 327–338.

Connelly, J. (2017). ICH Q3C impurities: Guideline for residual solvents. In *ICH quality guidelines: An implementation guide* (pp. 199–232). Wiley.

Dhole, S. M., Khedekar, P. B., & Amnerkar, N. D. (2013). Development and validation of HPTLC densitometry method for simultaneous estimation of rosiglitazone and glimepiride in fixed tablet dosage form. *Journal of the Chilean Chemical Society, 58*(2), 1663–1666. https://doi.org/10.4067/S0717-97072013000200004

El-Desouky, E. A., Abdel-Raoof, A. M., Abdel-Fattah, A., Abdel-Zaher, A., Osman, A. O., Abdel-Monem, A. H., & Morshedy, S. (2021). Determination of linagliptin and empagliflozin by UPLC and HPTLC techniques

aided by lean six sigma approach. *Biomedical Chromatography, 35*(7), e5102. https://doi.org/10.1002/bmc.5102

Feingold, K. R. (2024). Cholesterol lowering drugs. In K. R. Feingold, B. Anawalt, & A. Boyce (Eds.), *Endotext*. https://www.ncbi.nlm.nih.gov/books/NBK395573

Gope, E. R., Begum, S. M., Anisetti, P. P., Kasa, G. G., Eedarada, V. G., Nalli, J., & Thummidi, R. S. (2024). A review of principles, applications, and recent developments in HPTLC and HPLC. *Journal of Pharma Insights and Research, 2*(6), 56–64. https://doi.org/10.69613/315vge42

Görög, S. (2011). Advances in the analysis of steroid hormone drugs in pharmaceuticals and environmental samples (2004–2010). *Journal of Pharmaceutical and Biomedical Analysis, 55*(4), 728–743. https://doi.org/10.1016/j.jpba.2010.11.011

Harris, K. B., & McCarty, D. J. (2015). Efficacy and tolerability of glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes mellitus. *Therapeutic Advances in Endocrinology and Metabolism, 6*(1), 3–18. https://doi.org/10.1177/2042018814558242

Havele, S. S., & Dhaneshwar, S. R. (2010). Determination of glibenclamide in tablets by densitometric HPTLC. *Der Pharmacia Lettre, 2*(4), 440–446.

Havele, S., & Dhaneshwar, S. (2010). Estimation of metformin in bulk drug and in formulation by HPTLC. *Journal of Nanomedicine & Nanotechnology, 1*(102), 1–3. https://doi.org/10.4172/2157-7439.1000102

Inflibnet. (n.d.). High performance thin layer chromatography (HPTLC) – Analytical chemistry. Retrieved January 7, 2025, from https://ebooks.inflibnet.ac.in/esp02/chapter/high-performance-thin-layer-chromatography-hptlc/

Inzucchi, S. E., Lipska, K. J., Mayo, H., Bailey, C. J., & McGuire, D. K. (2014). Metformin in patients with type 2 diabetes and kidney disease: A systematic review. *JAMA, 312*(24), 2668–2675. https://doi.org/10.1001/jama.2014.15298

Ismail-Beigi, F. (2012). Pathogenesis and glycemic management of type 2 diabetes mellitus: A physiological approach. *Archives of Iranian Medicine, 15*(4), 239–246.

Jain, P. S., Patel, M. K., & Surana, S. J. (2013). Development and validation of stability-indicating high-performance thin-layer chromatography method for estimation of repaglinide in bulk and in pharmaceutical formulation. *Acta Chromatographica, 25*(3), 531–544. https://doi.org/10.1556/achrom.25.2013.3.9

Kalász, H., & Báthori, M. (2001). Pharmaceutical applications of TLC. *LC GC Europe, 14*(5), 311–321.

Kaur, I., Wakode, S., & Singh, H. P. (2016). Development and validation of a stability-indicating high-performance thin layer chromatography (HPTLC) method for estimation of canagliflozin in bulk and pharmaceutical dosage form. *Journal of Applied Pharmaceutical Science, 6*(5), 51–57. https://doi.org/10.7324/JAPS.2016.60508

Kumar, P. J., & Clark, M. (2002). *Textbook of clinical medicine*. Saunders.

McCalley, D. V. (2003). Selection of suitable stationary phases and optimum conditions for their application in the separation of basic compounds by reversed-phase HPLC. *Journal of Separation Science, 26*(3–4), 187–200. https://doi.org/10.1002/jssc.200390026

Modi, D. K., & Patel, B. H. (2012). Simultaneous determination of metformin hydrochloride and glipizide in tablet formulation by HPTLC. *Journal of Liquid Chromatography & Related Technologies, 35*(1), 28–39. https://doi.org/10.1080/10826076.2011.593227

Munde, M. K., Kulkarni, N. S., Sen, A. K., & Sen, D. B. (2020). A novel validated stability indicating analytical method for simultaneous quantification of metformin hydrochloride and empagliflozin in bulk and marketed formulation by HPTLC using box–Wilson experimental design approach. *BMC Chemistry, 17*(1), 110. https://doi.org/10.1186/s13065-023-00969

Nyiredy, S. (2002). Planar chromatographic method development using the PRISMA optimization system and flow charts. *Journal of Chromatographic Science, 40*(10), 553–563. https://doi.org/10.1093/chromsci/40.10.553

Patel, K. K., Karkhanis, V. V., & Gajjar, S. S. (2015). Development and validation of stability indicating HPTLC method for estimation of glimepiride and metformin hydrochloride. *International Journal of Pharmaceutical Sciences and Research, 6*(3), 1222–1228. https://doi.org/10.13040/IJPSR.0975-8232.6(3).1222-29

Patel, P. D., & Pandya, S. S. (2018). A review on analytical methods for determination of oral anti-diabetic drugs like biguanides, gliptins and gliflozins in bulk and pharmaceutical dosage forms. *World Journal of Pharmaceutical Sciences, 6*(1), 29–39.

Pawar, S., Patel, J., Sharma, R., Khan, S., & Patel, R. (2022). Method development and validation for anti-diabetic drugs by RP-HPLC. *International Journal of Pharmaceutical Sciences & Medicine, 7*(10), 6–29. https://doi.org/10.47760/IJPSM.2022.V07I10.002

Poole, C. F. (2003). Thin-layer chromatography: Challenges and opportunities. *Journal of Chromatography A, 1000*(1–2), 963–984. https://doi.org/10.1016/S0021-9673(03)00435-7

Poznyak, A., Grechko, A. V., Poggio, P., Myasoedova, V. A., Alfieri, V., & Orekhov, A. N. (2020). The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *International Journal of Molecular Sciences, 21*(5), 1835. https://doi.org/10.3390/ijms21051835

Prajapati, P., Rana, B., Pulusu, V. S., & Mishra, A. (2024). Multipurpose RP-HPLC method for simultaneous estimation of fixed-dose combinations of anti-diabetic drugs: integrating green, economical, and robust approaches with design of experiments and white analytical chemistry. *Chemistry Africa*, *7*(3), 1385-1400. https://doi.org/10.1007/s42250-023-00835-9

Puavilai, G., Chanprasertyotin, S., & Sriphrapradaeng, A. (1999). Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. *Diabetes research and clinical practice, 44*(1), 21-26. https://doi.org/10.1016/S0168-8227(99)00008-X

Rana, K., & Sharma, P. (2021). Analytical method development and validation for the simultaneous estimation of metformin hydrochloride and alogliptin by RP-HPLC in bulk and tablet dosage forms. *Research Journal of Science and Technology, 13*(2), 111–118. https://doi.org/10.52711/2349-2988.2021.00018

Rao, N. M., & Konda, R. (2020). Development and validation of high-performance thin layer chromatographic method for the determination of voglibose in bulk and their formulations. *Letters in Applied NanoBioScience, 9*(2), 1074–1078. https://doi.org/10.33263/LIANBS92.10741078

Rossi, M. C., Nicolucci, A., Ozzello, A., Gentile, S., Aglialoro, A., Chiambretti, A., ... & Cucinotta, D. (2019). Impact of severe and symptomatic hypoglycemia on quality of life and fear of hypoglycemia in type 1 and type 2 diabetes. Results of the Hypos-1 observational study. *Nutrition, Metabolism and Cardiovascular Diseases, 29*(7), 736-743. https://doi.org/10.1016/j.numecd.2019.04.009

Sapra, A., & Bhandari, P. (2024). *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK551501

Sethi, P. D. (1996). *High-performance thin-layer chromatography: Quantitative analysis of pharmaceutical formulations*. CBS Publishers & Distributors, New Delhi.

Sharma, K., & Parle, A. (2015). Development and validation of HPTLC method for estimation of alogliptin benzoate. *International Bulletin of Drug Research, 5*(8), 81–89.

Shewiyo, D. H., Kaale, E. A. K. K., Risha, P. G., Dejaegher, B., Smeyers-Verbeke, J., & Vander Heyden, Y. (2012). HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. *Journal of pharmaceutical and biomedical analysis, 66*, 11-23. https://doi.org/10.1016/j.jpba.2012.03.034

Shirode, A. R., Maduskar, P. D., Deodhar, M. S., & Kadam, V. J. (2014). RP-HPLC and HPTLC Methods for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin from Bulk and Marketed Formulation: Development and Validation. *British Journal of Pharmaceutical Research, 4*(20), 2370–2386. https://doi.org/10.9734/BJPR/2014/12820

Shukla, A., Chhalotiya, U., Shah, D., Tandel, J., Kachhiya, H., & Parmar, M. (2024). *Development and validation of stability indicating HPTLC method for simultaneous estimation of dapagliflozin and linagliptin*. Discover Chemistry, *1*(1), Article 4. https://doi.org/10.1007/s44371-024-00002-0

Spangenberg, B., Poole, C. F., & Weins, C. (2011). *Quantitative thin-layer chromatography: A practical survey*. Springer. https://doi.org/10.1007/978-3-642-10729-0

Srivani, J., Umamahesh, B., & Veeresham, C. J. (2016). Development and validation of stability indicating HPTLC method for simultaneous determination of linagliptin and metformin. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(1), 112–115.

Srividya, S., Swetha, E., & Veeresham, C. (2015). *Quantitative analysis of saxagliptin by stability indicating HPTLC method in bulk and pharmaceutical dosage form*. *American Journal of Analytical Chemistry, 6*(10), 797–804. https://doi.org/10.4236/ajac.2015.610076

Suma, B. V., Deveswaran, R., & Premnath, S. H. (2019). *HPTLC method development of dapagliflozin*. *International Journal of Pharmacy and Pharmaceutical Sciences, 11*(8), 58–63. https://doi.org/10.22159/ijpps.2019v11i8.34339

Thomas, A. B., Patil, S. D., Nanda, R. K., Kothapalli, L. P., Bhosle, S. S., & Deshpande, A. D. (2011). *Stability-indicating HPTLC method for simultaneous*

determination of nateglinide and metformin hydrochloride in pharmaceutical dosage form. *Saudi Pharmaceutical Journal, 19*(4), 221–231. https://doi.org/10.1016/j.jsps.2011.06.005

Varaprasad, C., Asif, M., & Ramakrishna, K. (2015). RP-HPLC method for simultaneous estimation of metformin and linagliptin in tablet dosage form. *Rasayan Journal of Chemistry, 8*(4), 426–432.

Vichare, V. S., Choudhari, V. P., & Reddy, M. V. (2022). *Development of new validated HPTLC method for simultaneous estimation of canagliflozin and metformin in tablet formulation*. *Research Journal of Pharmacy and Technology, 15*(6), 2599–2604. https://doi.org/10.52711/0974-360X.2022.00434

Waksmundzka-Hajnos, M., Sherma, J., & Kowalska, T. (2008). *Thin-layer chromatography in phytochemistry*. CRC Press. https://doi.org/10.1201/9781420046786