Journal of Young Pharmacists, 2026; 18(1):64-69.
https://www.jyoungpharm.org

Review Article

A Review on Analytical Strategies for the Concurrent Estimation of Metformin and Dapagliflozin

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ABSTRACT

The rising clinical usage of fixed-dose combination drugs including Metformin and Sodium-Glucose Cotransporter-2 (SGLT2) inhibitors, in particular Dapagliflozin, has necessitated the development of reliable, sensitive, and robust analytical methodologies for their simultaneous assessment. This review comprehensively summarizes and critically evaluates reported analytical techniques for the simultaneous determination of metformin and dapagliflozin in bulk drugs, pharmaceutical dosage forms, and biological matrices. Various chromatographic approaches, including RP-HPLC, UPLC, LC–MS/MS, and green micellar liquid chromatography, are discussed with respect to chromatographic conditions, detection techniques, sample preparation strategies, validation parameters, and application scopes. Advanced LC-MS/MS approaches, including as polarity switching, multiple reaction monitoring, and stable isotope-labelled internal standards, are emphasized for their higher sensitivity and selectivity in pharmacokinetic and bioequivalence studies. Stability-indicating RP-HPLC techniques capable of separating pharmaceuticals from their degradation products under controlled degradation conditions are also highlighted for quality control applications. Furthermore, eco-friendly analytical techniques and greenness assessment tools such as Analytical Eco-scale, AGREE, and MoGAPI are addressed. Overall, the reviewed methodologies are demonstrated to be suitable for routine analysis, stability studies, and clinical investigations while according to ICH standards. This work presents an integrated analytical perspective to assist researcher & analysts in selecting appropriate methodologies and driving future development of analytical methods for combination dosage forms involving more than two antidiabetic drugs.

Keywords: Metformin, Dapagliflozin, LC–MS/MS, MLC, RP-HPLC.

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Received: 15-12-2025;
Revised: 05-01-2026;
Accepted: 23-02-2026.

INTRODUCTION

Insulin resistance and compromised glucose homeostasis are hallmarks of Type 2 Diabetes Mellitus (T2DM), a chronic metabolic disease. Millions of people worldwide suffer from diabetes mellitus, a serious global health issue (Jabbour and Goldstein, 2008). Complexity of Diabetes management involves a multifaceted approach, including lifestyle modifications, medication, and monitoring (Lambrinou *et al*., 2019). Mixed therapy, which requires two medications to manage blood glucose levels, improves medication compliance for diabetic patients by lessening the pill burden. Metformin, a biguanide antihyperglycemic drug, reduces hepatic glucose synthesis and increases insulin sensitivity, whereas Dapagliflozin, a Sodium-Glucose co-Transporter 2 (SGLT2) inhibitor, lowers plasma glucose by increasing urine glucose excretion. Due to their complimentary modes of action and potential to improve patient adherence, fixed-dose combinations of metformin and dapagliflozin are being used more frequently in clinical practice. The Xigduo and Xigduo XR fixed-dose combination of metformin and dapagliflozin was first approved by FDA in 2014 (*Xigduo XR(Dapagliflozin and Metformin HCl) Extended-Release Tablets*). Metformin and Dapagliflozin require reliable and confirmed analytical methodologies for their simultaneous determination. Although numerous chromatographic and hyphenated techniques have been described, the existing literature is fragmented, with no critical and systematic comparison of analytical methodologies across bulk drugs, pharmaceutical-dosage forms, and biological matrices. Furthermore, less emphasis has been placed on stability-indicating capabilities, sophisticated LC-MS/MS techniques, and the use of green analytical chemistry principles through standardized assessment tools. However, simultaneous estimation is analytically demanding because Metformin is highly polar and poorly retained on reverse-phase columns, whereas Dapagliflozin is lipophilic with strong UV absorbance and prolonged retention behaviour (Shah *et al*., 2019; Marie *et al*., 2025). As a result, the purpose of this study is to combine

DOI: 10.5530/jyp.20250384

and critically assess previously reported analytical approaches, identify existing gaps, and provide a systematic framework for future analytical method development in antidiabetic combination drugs.

DRUG PROFILE

Metformin

Metformin hydrochloride is a strong base due to its nitrogen-rich biguanide structure, with a pKa of around 12.4, resulting in full protonation under most analytical circumstances. The extensive ionization results in great aqueous solubility and a low partition coefficient (log P = −1.43), indicating strong hydrophilicity and little lipophilicity (Da Trindade *et al*., 2018). As a result, metformin has low retention on typical reversed-phase chromatographic columns. The drug is chemically stable and does not degrade easily, making it ideal for routine quantitative analysis and stability-indicating research (Kharabe *et al*., 2024). These physicochemical properties classify metformin as a BCS Class III compound (Crison *et al*., 2012) and have a significant impact on analytical method development, frequently necessitating ion-pair chromatography, stringent buffer pH control, or Hydrophilic Interaction Liquid Chromatography (HILIC) to achieve adequate retention and peak symmetry (Antonopoulos *et al*., 2018). Structure of Metformin in Figure 1.

Dapagliflozin

Dapagliflozin has a C-aryl glucoside structure, where a glucose-derived tetrahydropyran ring is linked via a Carbon–Carbon (C–C) bond to a substituted aromatic ring, conferring high metabolic stability against enzymatic hydrolysis compared with O-glucosides. This structural feature contributes to its oral bioavailability and pharmacological efficacy as a selective SGLT-2 inhibitor, as C-aryl glucosides resist intestinal β-glucosidase degradation, improving stability and duration of action *in vivo*. At physiological pH, dapagliflozin remains largely unionized due to its high pKa (≈ 12.57), and it exhibits moderate lipophilicity (log P ≈ 2.7), (Saiyed *et al*., 2024). Which enhances membrane permeability and retention on reverse-phase chromatographic systems, while influencing formulation and analytical method design. Dapagliflozin is poorly soluble in water but shows appreciable solubility in organic solvents such as methanol and acetonitrile, important considerations in both formulation development and analytical method optimization (Kang & Kim, 2023). Structure of Dapagliflozin in Figure 2.

Table 1 summarize the physicochemical properties and mode of action of the above mentioned drugs (Metformin), (Dapagliflozin) (AJ Scheen *et al*., 1996) (S Kasichayanula *et al*., 2014).

Literature study on Analytical Methods for Metformin and Dapagliflozin

Priyanka A Shah *et al*., developed a validated LC-MS/MS method for estimating metformin and dapagliflozin in human plasma utilizing ion-pair solid-phase extraction. Chromatographic separation was accomplished on a reversed-phase ACE 5CN column using acetonitrile-ammonium acetate (pH 4.5) as the mobile phase, and detection was performed in MRM mode with polarity switching. The approach demonstrated linearity for metformin concentrations ranging from 1.0 to 2000 ng/mL and 0.1 to 200 ng/mL for dapagliflozin, with low detection and quantification limits. No interference from normal, haemolyzed, or lipemic plasma was detected. The experiment was effectively used to evaluate the influence of food on the pharmacokinetics of both medications in healthy volunteers (Shah *et al*., 2019).

Aya A. Marie reported the development of a green Micellar Liquid Chromatography (MLC) technique for simultaneously determining dapagliflozin and metformin HCl in pure and tablet dosage forms. Separation was accomplished on a BDS Thermo-Hypersil C8 column with a hybrid micellar mobile phase at pH 3.3 and PDA detection at 223 nm under optimized chromatographic conditions. The approach demonstrated strong linearity across concentration ranges of 0.2-7 µg/mL for dapagliflozin and 50-700 µg/mL for metformin, with high accuracy and adequate recovery. It was validated using ICH specifications and successfully applied to the analysis of Dexigloflozin plus® 5/500 tablets. Greenness assessment with analytical eco-scale, Complex MoGAPI, and AGREE tools established the method's benefit to the environment (Marie *et al*., 2025).

M N Abou-Omar *et al*., Reported A rapid and sensitive UPLC–ESI–MS/MS method was developed for the simultaneous quantification of metformin and empagliflozin in human plasma using stable isotope-labeled internal standards. Sample preparation involved acetonitrile protein precipitation with a freezing step to reduce matrix effects, followed by chromatographic

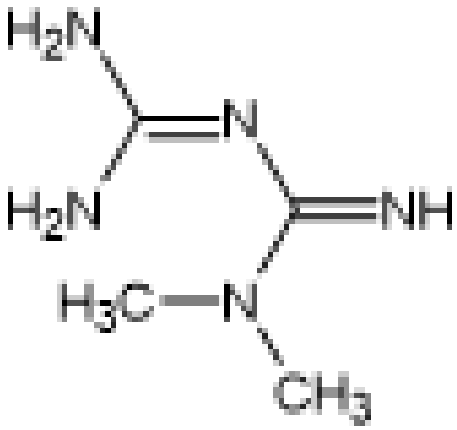

Figure 1: Metformin.

Table 1: **Physiochemical Properties of Metformin & Dapagliflozin.**

Parameters	Description	
Drug Name	**Metformin**	**Dapagliflozin**
CAS Number	1115-70-4	461432-26-8
Category	Antidiabetic Agent	Antidiabetic Agent, SGLT2 inhibitor
Chemical Formula	$C_4H_{11}N_5$	$C_{21}H_{25}ClO_6$
Molecular Weight	129.16 g/mol	408.9g/mol
Physical State and Appearance	White to faint Yellow Solid	White / white crystalline powder
Melting point	74-78°C	76–78°C
Solubility	Ethanol, Methanol, Dimethyl formamide	Sparingly soluble in water; freely soluble in ethyl alcohol and methyl alcohol.
Mode of Action	It reduces blood glucose substantially by decreasing hepatic gluconeogenesis through AMPK activation. It enhances peripheral insulin sensitivity, lowers the absorption of glucose in the gut, and inhibits mitochondrial complex I.	Acts by selectively blocking SGLT2 in the proximal tubules of kidney, thereby diminishing glucose reuptake, promoting its urinary excretion, and lowering circulating glucose concentrations.

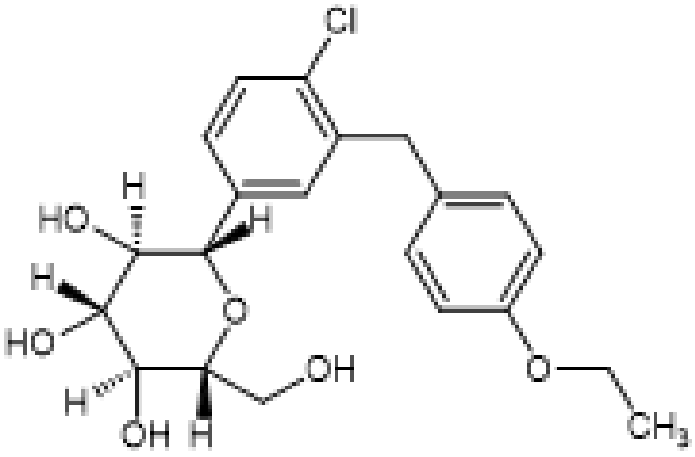

Figure 2: Dapagliflozin.

separation on a BEH C18 column under isocratic conditions. Quantification was performed using multiple reaction monitoring in positive ion mode, demonstrating good linearity, accuracy, and sensitivity with a total run time of less than 3 minutes. The method was successfully applied to pharmacokinetic studies and therapeutic drug monitoring of fixed-dose combinations with high reproducibility and robustness (Abou-Omar *et al.*, 2021).

Sonia T. Hassib described a simple and accurate liquid chromatographic method for determining canagliflozin, dapagliflozin propandiol monohydrate, or empagliflozin in the presence of metformin's major degradation product, cyanoguanidine. The method used isocratic elution on a Prontosil (Lichrosorb 100-5-NH_2) column, with a NaH_2PO_4 buffer-acetonitrile mobile phase and UV detection at 225 nm. The method was validated in accordance with ICH Low limits of detection and quantification validated the method's sensitivity. The developed assay was found to be suitable for regular quality control studies (Hassib *et al.*, 2019).

Ragini Ghotale reported a simple, precise, and validated RP-HPLC method for the simultaneous estimation of metformin hydrochloride and dapagliflozin in solid dosage forms. Chromatographic separation was achieved on an Agilent TC-C18 column using a mobile phase of 0.02% triethylamine and acetonitrile (50:50 v/v), with PDA detection at 236 nm. The method showed good linearity, accuracy, and precision, with retention times of 2.6 min for metformin and 5.6 min for dapagliflozin and high percentage recoveries. Validation results confirmed that the method complies with ICH Q2R2 guidelines and is suitable for routine analysis (Ghotale *et al.*, 2025).

Samkit Shah reported the development and validation of a simple, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of dapagliflozin, linagliptin, and metformin hydrochloride in fixed-dose combination tablets. Chromatographic separation was achieved using a Phenomenex Luna C18 column with a mobile phase of acetonitrile and phosphate buffer (pH 6.8) in a 40:60 v/v ratio, a flow rate of 0.8 mL/min, and UV detection at 230 nm. The method showed excellent linearity for metformin hydrochloride (20–140 µg/mL), linagliptin (0.2–1.4 µg/mL), and dapagliflozin (0.6–2.8 µg/mL), with correlation coefficients greater than 0.995. Validation in accordance with ICH Q2 (R2) guidelines confirmed the method's accuracy, precision, robustness, specificity, and sensitivity, with %RSD values below 2%. Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic

conditions demonstrated effective separation of the drugs from their degradation products, confirming the stability-indicating nature of the method. Application to commercial tablets yielded assay results close to 100%, supporting its suitability for routine quality control and stability testing of antidiabetic combination products (Shah & Kotadiya, 2025).

Krishna Rao Vankalapati reported the development and validation of a simple, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of dapagliflozin, linagliptin, and metformin hydrochloride in fixed-dose combination tablets. Chromatographic separation was achieved using a Phenomenex Luna C18 column with a mobile phase of acetonitrile and phosphate buffer (pH 6.8) in a 40:60 v/v ratio, at a flow rate of 0.8 mL/min and UV detection at 230 nm. The method demonstrated excellent linearity over the concentration ranges of 20–140 µg/mL for metformin hydrochloride, 0.2–1.4 µg/mL for linagliptin, and 0.6–2.8 µg/mL for dapagliflozin, with correlation coefficients exceeding 0.995. Validation performed according to ICH Q2 (R2) guidelines confirmed the method's accuracy, precision (%RSD < 2%), robustness, specificity, and sensitivity. Forced degradation studies under various stress conditions confirmed the stability-indicating capability of the method (Vankalapati *et al*., 2022).

Waqar Siddique detailed the creation and verification of a quick and dependable RP-HPLC technique for the simultaneous measurement of metformin and dapagliflozin in bulk medications and pharmaceutical formulations. The approach was verified following the guidelines of the International Conference on Harmonization (ICH) and the United States Pharmacopeia (USP). Chromatographic separation was completed in under 4 minutes utilizing a mobile phase made of phosphate buffer (pH 6.8) and acetonitrile in a 45:55 v/v proportion, at a flow rate of 1.0 mL/min. The technique showed strong precision, with recovery percentages for metformin and dapagliflozin between around 98.8% and 101.5% at concentration levels of 70, 100, and 130 µg/mL. Examination of both bulk and tablet formulations revealed no interference from excipients, validating the specificity of the method. These findings suggest that the proposed RP-HPLC technique is appropriate for regular quality control assessment of metformin and dapagliflozin combination products (Siddique

Table 2: Summary of the selected studies.

Matrix	Technique	Key Findings	References
Human plasma	LC-MS/MS	Highly sensitive and selective method using ion-pair SPE and MRM with polarity switching; excellent linearity (metformin: 1-2000 ng/mL, dapagliflozin: 0.1-200 ng/mL); successfully applied to pharmacokinetic and food-effect studies; no matrix interference observed.	(Shah *et al*., 2019)
Tablet dosage form	Green Micellar Liquid Chromatography (MLC)	Environment-friendly stability-indicating method with strong linearity and accuracy; validated as per ICH guidelines; successfully applied to commercial with favourable greenness assessment.	(Marie *et al*., 2025)
Human plasma	UPLC–ESI–MS/MS	Rapid (≤3 min), sensitive, and reproducible method for simultaneous estimation of metformin and empagliflozin using isotope-labeled internal standards; suitable for pharmacokinetic and therapeutic monitoring studies.	(Abou-Omar *et al*., 2021)
Bulk & dosage form with degradation products	LC-UV	Selective determination of SGLT2 inhibitors in the presence of metformin degradation product (cyanoguanidine); low LOD and LOQ; suitable for quality control studies.	(Hassib *et al*., 2019)
Solid dosage form	RP-HPLC	Simple and precise method with good linearity and high recovery; validated according to ICH Q2 (R2) guidelines; suitable for routine analysis of fixed-dose combinations.	(Ghotale *et al*., 2025)
Tablet Dosage Form (FDC)	Stability-indicating RP-HPLC	Effective separation of metformin, linagliptin, and dapagliflozin with excellent linearity; forced degradation confirmed stability-indicating capability; applicable for quality control and stability testing.	(S. Shah & Kotadiya, 2025)
Bulk drug and tablet dosage form	Stability-indicating RP-HPLC	Robust and precise method, validated as per ICH Q2 (R2); effective resolution of analytes and degradation products; suitable for stability studies.	(Vankalapati *et al*., 2022)
Bulk & tablet dosage forms	RP-HPLC	Accurate and precise method with recoveries close to 100%; rapid analysis and good specificity; appropriate for routine quality control.	(Siddique *et al*., 2025)

et al., 2025). Important information from each article was summarized in Table 2.

CONCLUSION

A critical assessment of the published analytical procedures for the simultaneous determination of metformin with Dapagliflozin reveals substantial progress in sensitivity, selectivity, speed, and applicability across in the field of pharmaceutical and bioanalytical domains. However, each analytical approach has specific advantages and drawbacks that affect its applicability for routine quality control, stability testing, and pharmacokinetic investigations.

Among the reviewed, the LC-MS/MS and UPLC-MS/MS techniques were the most advanced and sensitive. These methods provide excellent selectivity, very low limits of detection and quantification, broad linear dynamic ranges, and minimal matrix interference, making them highly suitable for bioanalytical applications such as pharmacokinetic, bioequivalence, food-effect, and therapeutic drug monitoring studies. The use of isotopically labelled internal standards, polarity switching, and optimal sample preparation techniques (Ion-pair SPE or protein precipitation with freezing) improves precision and reproducibility. Despite these benefits, LC-MS/MS procedures necessitate expensive instrumentation, high operational expenses, and expert people, thereby limiting their routine use in resource-constrained quality control laboratories.

Conventional RP-HPLC and stability-indicating HPLC techniques with UV/PDA detection are still very useful for routine pharmaceutical analysis. These procedures are simple, cost-effective, reliable, and ICH-compliant, with appropriate linearity, precision, accuracy, and specificity for bulk pharmaceuticals and fixed-dose combination tablets. Stability-indicating capabilities under a variety of stress settings increase their usefulness for regulatory submissions and shelf-life assessments. However, their relatively greater detection limits and susceptibility to matrix interferences limit their application in biological matrices.

Green Micellar Liquid Chromatography (MLC) is a significant improvement in sustainable analytical chemistry. By lowering organic solvent usage and adding greenness assessment techniques (Eco-scale, MoGAPI, and AGREE), this method meets recent regulatory and ecological requirements. While ideal for dosage form analysis with high accuracy and precision, its applicability to biological samples is limited due to sensitivity limitations.

Overall, no specific analytical method can be considered universally ideal for all applications. LC-MS/MS methods are more suited for plasma and pharmacokinetic study, but RP-HPLC and stability-indicating HPLC methods are more feasible for pharmaceutical formulation quality control and stability testing. Emerging green analytical techniques offer potential alternatives, but further validation is required for bioanalytical applications. Future research ought to hinge on creating cost-effective, environmentally friendly, and highly sensitive hybrid methods that bridge the gap between routine pharmaceutical analysis and advanced bioanalytical applications, fulfilling both regulatory and sustainability standards.

ACKNOWLEDGEMENT

We are thankful to SBV University, Puducherry & East West College of Pharmacy, Bangalore, for providing guidance and support for this review work.

ABBREVIATIONS

T2DM: Type 2 Diabetes Mellitus; **SGLT2:** Sodium-Glucose Co-Transporter 2; **FDA:** Food and Drug Administration; **XR:** Extended Release; **LC-MS/MS:** Liquid Chromatography–Tandem Mass Spectrometry; **UPLC:** Ultra-Performance Liquid Chromatography; **ESI:** Electrospray Ionization; **MRM:** Multiple Reaction Monitoring; **RP-HPLC:** Reverse-Phase High-Performance Liquid Chromatography; **HPLC:** High-Performance Liquid Chromatography; **UV:** Ultraviolet; **PDA:** Photodiode Array Detector; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **USP:** United States Pharmacopeia; **SPE:** Solid-Phase Extraction; **MLC:** Micellar Liquid Chromatography; **HILIC:** Hydrophilic Interaction Liquid Chromatography; **BCS:** Biopharmaceutics Classification System; **MoGAPI:** Metric of Greenness Assessment of Analytical Procedures Index; **AGREE:** Analytical GREEnness Metric Approach; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard Deviation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENT OF AI'S AND QUILLBOT USE

OpenAI's ChatGPT and QuillBot assisted with structural editing and linguistic improvement for this manuscript. The authors have verified the scientific material. No AI-generated figures were used.

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Cite this article: Vaithiyanathan VS, Ramesh DR. A Review on Analytical Strategies for the Concurrent Estimation of Metformin and Dapagliflozin. J Young Pharm. 2026;18(1):64-9.