World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Review Article
ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 5
Page: 1028-1037

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METFORMIN BY RP-HPLC

Ravi Prakash*[1], Nitin Sharma[2], Saurabh Sharma[3], Dinesh Upadhyay[4], Subhranshu Panda[5]

[1,3]B Pharma Scholar, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan.
[2]Associate Professor, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan.
[4,5]Professor, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan.

Article Received: 11 April 2026 || Article Revised: 02 May 2026 || Article Accepted: 22 May 2026

***Corresponding Author: Ravi Prakash**
B Pharma Scholar, School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan.
DOI: https://doi.org/10.5281/zenodo.20396327

How to cite this Article: Ravi Prakash, Nitin Sharma, Saurabh Sharma, Dinesh Upadhyay, Subhranshu Panda (2026) ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF METFORMIN BY RP-HPLC. World Journal of Pharmaceutical Science and Research, 5(5), 1028-1037.

ABSTRACT

Metformin hydrochloride, a widely used biguanide antihyperglycemic agent, remains the first-line therapy for the management of type 2 diabetes mellitus due to its efficacy, safety, and cost-effectiveness. The present study focuses on the development and validation of a robust, precise, and reliable reversed-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative estimation of metformin in bulk drug and pharmaceutical dosage forms. The method was developed using a C18 column with an optimized mobile phase consisting of a suitable buffer and organic solvent system, delivered at a controlled flow rate. Detection was carried out using UV spectroscopy at an optimized wavelength in the low UV range. The developed method was systematically optimized by evaluating critical parameters such as mobile phase composition, pH, flow rate, and column conditions to achieve acceptable retention time, peak symmetry, and resolution. The method was validated in accordance with ICH guidelines for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability. The results demonstrated excellent linearity with a high correlation coefficient, satisfactory recovery within acceptable limits, and low %RSD values indicating good precision and reproducibility. The method also proved to be stability-indicating, effectively separating metformin from its degradation products under various stress conditions. Its applicability was confirmed for routine quality control analysis of bulk drug and tablet formulations, as well as for bioanalytical and stability studies. Overall, the developed RP-HPLC method is simple, rapid, accurate, and cost-effective, making it suitable for routine pharmaceutical analysis and regulatory compliance.

KEYWORDS: Metformin Hydrochloride, RP-HPLC, Method Validation, ICH Guidelines.

INTRODUCTION

Introduction Block

Metformin is a biguanide-class oral antihyperglycemic agent and the first-line pharmacological therapy for type 2 diabetes mellitus (T2DM) worldwide, owing to its efficacy, safety profile, and low cost. After oral administration, it is absorbed from the small intestine, distributed mainly in the liver and kidneys, and excreted largely unchanged by the renal route, with a plasma half-life of about 2–3 h but a prolonged glucose-lowering effect that allows once- to twice-daily dosing. Metformin lowers blood glucose primarily by reducing hepatic gluconeogenesis and enhancing peripheral insulin sensitivity, without causing hypoglycemia or weight gain, which distinguishes it from many other antidiabetic agents.[1,2,3,4]

Clinically, metformin is indicated not only for glycemic control in T2DM but also for prediabetes prevention, gestational diabetes, and polycystic ovary syndrome (PCOS), where it improves insulin resistance, restores ovulation, and helps regulate menstrual cycles. Emerging evidence further supports potential cardioprotective, nephroprotective, anticancer, and anti-aging effects, driving interest in repurposing metformin for a range of metabolic and chronic-disease states. This broad therapeutic spectrum underscores the need for robust, reliable, and precise analytical methods to ensure consistent drug strength, purity, and bioavailability across different formulations and manufacturing batches.[1,3,4]

Analytical method development is a critical step in pharmaceutical research and quality control, as it defines the procedures used to identify, quantify, and monitor the active pharmaceutical ingredient (API) and its impurities in drug substances, dosage forms, and biological matrices. A well-developed and validated method must be accurate, precise, specific, linear, robust, and reproducible, and it must comply with regulatory guidelines (e.g., ICH, FDA, EMA) to support process development, stability studies, pharmacokinetic investigations, and routine quality-assurance testing. In the context of metformin and similar small-molecule drugs, analytical methods underpin batch-release specifications, impurity profiling, degradation-studies, and bioavailability and bioequivalence assessments, all of which are essential for patient safety and regulatory approval.[4]

Among modern separation techniques, reversed-phase high-performance liquid chromatography (RP-HPLC) has become the most widely used method in pharmaceutical analysis due to its versatility, reliability, and capacity to resolve complex mixtures. RP-HPLC separates compounds based on their hydrophobic interactions with a non-polar stationary phase (typically C18-based silica) and a polar mobile phase (often water-acetonitrile or water-methanol mixtures), enabling the quantification of metformin, its related substances, and degradation products with high sensitivity and resolution. Its simplicity, adaptability over a wide polarity range, and compatibility with UV, PDA, and MS detection make RP-HPLC an ideal platform for method development, validation, and routine analysis of metformin-containing formulations, as well as for investigating its stability and forced-degradation behavior under various stress conditions.[5,6]

Drug Profile of Metformin

Metformin, most commonly used as metformin hydrochloride (HCl), is a biguanide-class oral antihyperglycemic agent and the first-line pharmacological therapy for type 2 diabetes mellitus (T2DM). It lowers blood glucose by reducing hepatic gluconeogenesis and improving insulin sensitivity in peripheral tissues without causing significant hypoglycemia, making it a preferred agent in metabolic and insulin-resistance-related disorders.[1,7]

Chemical Structure

Metformin consists of a biguanide backbone with two guanidine-like moieties linked by a central carbon, and in the pharmacopoeial active form it is employed as its hydrochloride salt (metformin HCl). The parent biguanide core is highly polar and nitrogen-rich, responsible for its strong cationic character at physiological pH.[8]

Chemical Name and Molecular Formula

- Chemical name (IUPAC-style): 1,1-Dimethylbiguanide hydrochloride (also named *N, N-dimethylimidodicarbonimidic diamide monohydrochloride* in drug-dictionary entries).
- Molecular formula of metformin (free base): $C_4H_{11}N_5$ (MW ≈ 129.17 g/mol).
- Molecular formula of metformin hydrochloride: $C_4H_{11}N_5 \cdot HCl$ (MW ≈ 165.63 g/mol).[8]

Physicochemical Properties

- Metformin is a small, hydrophilic base that exists predominantly as a positively charged cation (>99.9%) at physiological pH, which limits passive diffusion through lipid membranes.[8,9]
- It is freely soluble in water, poorly soluble in organic solvents, and highly stable in solid-dosage forms; its hydrochloride salt appears as a white to almost white crystalline powder with a melting point around 219–223 °C.[8]
- The molecule lacks strong chromophores but can be readily detected by UV at low-wavelength range (or more robustly by MS or conductivity-based detectors), which is exploited in RP-HPLC and other analytical methods.[4,10]

Mechanism of Action

- Primary metabolic action: Metformin reduces hepatic glucose production by inhibiting gluconeogenesis and opposing the action of glucagon-mediated signaling, thereby lowering fasting and postprandial plasma glucose.[1,7,11]
- Mitochondrial and AMPK-linked effects: It inhibits mitochondrial complex I and possibly mitochondrial glycerophosphate dehydrogenase, increasing the cellular AMP: ATP ratio; this activates AMP-activated protein kinase (AMPK), which enhances insulin sensitivity, promotes fatty-acid oxidation, and suppresses anabolic pathways.
- Gut-centric actions: Recent evidence highlights a role of metformin in the gut, including modulation of microbiota, bile-acid metabolism, and intestinal glucose absorption, contributing to its overall glucose-lowering and metabolic-benefit profile.[8,11]

Pharmacokinetic Profile

- Absorption: Metformin is absorbed chiefly from the small intestine; its oral bioavailability is about 40–60%, with absorption showing saturability at higher doses, suggesting carrier-mediated uptake via organic cation transporters (OCTs).[9,10]
- Distribution and protein binding: It is rapidly distributed to tissues (especially liver and kidney), is not significantly protein-bound, and does not undergo hepatic metabolism; no major metabolites have been identified.[9,10]
- Elimination: Metformin is excreted unchanged by the kidneys, with a plasma elimination half-life of approximately 4–6 hours in subjects with normal renal function. In renal impairment, both half-life and exposure increase, necessitating dose reduction or contraindication to avoid lactic acidosis.[9,10]

- Therapeutic-plasma-concentration range: Steady-state plasma concentrations commonly fall in the order of 0.5–2 mg/L, though routine monitoring is rarely needed except in suspected toxicity or lactic acidosis.[9,10]

Principle of RP-HPLC

RP-HPLC separates compounds based on differences in their hydrophobicity between a non-polar (hydrophobic) stationary phase and a polar mobile phase (usually water-methanol or water-acetonitrile mixtures). The analyte molecules partition between the hydrophobic stationary phase and the aqueous-organic mobile phase; more hydrophobic components are retained longer on the column, emerging as later peaks in the chromatogram, whereas polar/hydrophilic components elute earlier. This partition-based mechanism allows efficient resolution of complex mixtures, including drug substances, excipients, and degradation products.[12,13]

Instrumentation

A typical RP-HPLC system consists of:

- Solvent reservoirs and degassing unit for mobile-phase storage and bubble-free delivery.
- High-pressure pumps that deliver a constant, precise flow of the mobile phase through the column.
- Injector (manual or autosampler) to introduce a fixed volume of sample onto the column [14].
- Analytical column packed with a reversed-phase packing material (most commonly C18-silica).
- Detector (e.g., UV-Vis, PDA, fluorescence, or MS) that measures the eluted components and generates a chromatogram.
- Data-acquisition system (computer and software) to record and quantify peak areas and retention times.[15]

Selection of Column

The choice of column is critical for resolution, speed, and robustness. In RP-HPLC, C18-bonded silica is the most widely used stationary phase for polar and semi-polar pharmaceuticals, including metformin and many related drugs. Other variants (C8, C4, phenyl, or polar-embedded phases) are selected when the analyte is very hydrophobic, very hydrophilic, or prone to undesirable interactions. Column dimensions (length, internal diameter, and particle size) are chosen to balance resolution, run time, and backpressure; shorter columns with small particles are often preferred for fast, high-throughput analyses.[16]

Mobile Phase Selection

In RP-HPLC, mobile phases are typically binary or ternary mixtures of water (or buffer) and an organic modifier such as methanol or acetonitrile.

- Water-organic ratio controls retention: increasing organic content shortens retention time and speeds up the run; gradients of increasing organic modifier are used to resolve complex mixtures.
- Buffers and pH modifiers (e.g., phosphate, acetate, formate, or low-TFA) adjust ionization and improve peak shape for ionizable analytes.
- Low UV-absorbance solvents (especially acetonitrile at low wavelengths) and low-viscosity mixtures are preferred for sensitivity and system stability.[17]

Detection Methods

- UV-Visible detection is the most common method in RP-HPLC for pharmaceuticals; a fixed or multi-wavelength (PDA) detector records absorbance and produces quantifiable peaks based on Beer–Lambert law.

- Fluorescence detection offers higher sensitivity for compounds that are inherently fluorescent or can be derivatized.
- Mass-spectrometric detection (LC-MS) provides structural information, identification of unknowns, and superior selectivity for complex biological or degradation-product matrices.[14]

Advantages of RP-HPLC

- Versatility: RP-HPLC can separate a wide range of analytes, from highly polar to moderately hydrophobic, including drugs, metabolites, and peptides.
- High resolution and repeatability: Modern C18 columns and well-optimized mobile-phase gradients provide sharp, reproducible peaks suitable for assay and impurity profiling.
- Compatibility with aqueous samples and LC-MS: RP-HPLC readily accepts biological fluids and dosage-form extracts, and it is the preferred mode for LC-MS-based applications in pharmacokinetics and bioanalytical work.
- Regulatory-friendly: RP-HPLC methods are easily validated (accuracy, precision, linearity, robustness, specificity) and widely accepted by ICH/FDA/EMA for impurity control, content uniformity, and stability-indicating analyses of pharmaceuticals.[14]

Analytical Method Development for Metformin

Selection of Solvent and Reagents

Metformin hydrochloride (M-HCl) is a highly polar, water-soluble base, so the solvent system is typically based on a polar aqueous buffer and a water-miscible organic modifier for RP-HPLC. Common reagents include phosphate or acetate buffer (e.g., 0.02–0.05 M) and methanol or acetonitrile as the organic component, together with high-purity water (HPLC-grade) and degassed solvents to minimize baseline noise and column pressure. Additives such as dilute acids (e.g., phosphoric or acetic acid) are often used to adjust pH and suppress ionization, improving peak shape and retention reproducibility.[18,19,20,21]

Preparation of Standard and Sample Solutions

A standard stock solution of metformin hydrochloride is prepared by accurately weighing an appropriate quantity of pure drug reference standard and dissolving it in the initial mobile-phase composition or a mixture of water and a small proportion of organic modifier, followed by dilution to obtain the working standard concentration (e.g., 100–500 µg/mL). For tablet dosage forms, a representative number of tablets are finely powdered, and an aliquot equivalent to one tablet is extracted with the same solvent under sonication or shaking, filtered, and diluted to the desired assay or impurity-concentration range. The final drug concentration in the sample should fall within the linear range of the method, with a clear separation of active ingredient from excipient peaks and any degradation products.[18,21]

Optimization of Chromatographic Conditions

Optimization involves systematic variation of critical method parameters (CMPs) such as buffer pH, organic:aqueous ratio, flow rate, column temperature, and injection volume, typically guided by a quality-by-design (QbD) or design-of-experiments (DoE) approach. For metformin, a common optimized mobile-phase system is a buffer–organic blend (e.g., 0.02 M acetate buffer pH 3.0:methanol, 70:30 v/v) delivered at a flow rate of about 1.0 mL/min on a C18 column at around 25–35 °C. Method robustness is evaluated by slightly varying each parameter around the optimized value and monitoring retention time, tailing factor, and resolution, ensuring that the method remains suitable for routine analysis.[18,19]

Selection of Wavelength

Metformin is a low-chromophore compound with characteristic UV absorption at relatively short wavelengths; the optimal detection wavelength is usually selected in the range of 215–240 nm, with many validated methods using 233–238 nm for sufficient sensitivity and selectivity. UV-scanning of the standard solution across the spectrum helps identify the wavelength with maximum absorbance and acceptable baseline stability, and PDA detection can be used to confirm spectral purity and absence of co-eluting peaks.

Flow Rate Optimization

Flow rate directly affects retention time, backpressure, and peak resolution. In metformin-RP-HPLC methods, flow rates in the range of 0.8–1.2 mL/min are commonly used, with 1.0 mL/min being a typical compromise between adequate resolution and acceptable run time. Higher flow rates shorten the analysis time but may reduce resolution and increase column pressure, whereas lower flow rates improve resolution at the cost of longer chromatographic runs. Design-of-experiments can be employed to study the effect of flow rate on critical quality attributes (retention time, peak area, and peak symmetry) and to select a robust operating window.[28]

pH Optimization

Since metformin is an ionizable base, pH of the aqueous buffer strongly influences its degree of ionization, retention, and peak shape. In many developed methods, a pH between 3.0 and 4.5 (commonly acetate or phosphate buffer at pH ~3.0–3.5) is used to partially suppress ionization, yielding well-retained, symmetrical peaks without excessive interaction with residual silanols on the C18 column. A systematic DoE-based pH study (e.g., pH 3.0–5.0) allows identification of the pH value that optimizes retention, symmetry factor, and resolution while maintaining method robustness.[21]

Retention Time and Peak Resolution

The retention time of metformin in optimized RP-HPLC methods typically falls in the range of 3–8 minutes, depending on the column, mobile-phase composition, and flow rate. For a stability-indicating method, resolution between metformin and its degradation products or impurities is critical; acceptable resolution values are generally ≥1.5, especially for potential forced-degradation impurities. Parameters such as theoretical plates, tailing factor, and peak symmetry are monitored to confirm that the method offers sharp, well-resolved, and reproducible peaks suitable for accurate quantification and impurity profiling in pharmaceutical dosage forms and biological matrices.[21]

Method Validation According to ICH Guidelines Specificity

Specificity demonstrates the method's ability to accurately measure the analyte (metformin) in the presence of potential interferences such as excipients, degradation products, and impurities. This is typically assessed by chromatographic separation and peak purity in stressed samples (acid, base, oxidative, thermal, and photolytic conditions), placebo, and blank solutions, with acceptable resolution between the drug peak and closely eluting species. For metformin-RP-HPLC methods validated under ICH Q2(R1), baseline separation from major degradation products and absence of peak distortion in excipient-containing solutions are considered key indicators of specificity.

Linearity and Range

Linearity is evaluated by plotting peak area (or height) versus concentration over a defined concentration range (e.g., 50–150% of the target assay concentration for metformin) using a series of standard solutions prepared by serial dilution. A correlation coefficient (r) of ≥0.999 and a low residual sum of squares or percent relative standard error usually indicate adequate linearity. The range is the interval between the upper and lower concentration limits where the method demonstrates acceptable linearity, accuracy, and precision; for metformin-assay-type methods, this is typically aligned with the label claim and expected assay variations.

Accuracy

Accuracy reflects the closeness of the measured value to the true or reference value, usually estimated by % recovery in spiked samples at different concentration levels (e.g., 80%, 100%, 120%). For metformin-tablet or solution methods, recoveries in the range of 98–102% with low % RSD are generally considered acceptable, confirming that the extraction and chromatographic system do not significantly over- or under-estimate the drug content.

Precision

Precision describes the degree of agreement among repeated measurements under the same or varying conditions and is evaluated as:

- Repeatability (intra-day precision): multiple injections of the same sample on the same day ($n \geq 6$),
- Intermediate precision (inter-day precision): analysis on different days, by different analysts, or with different instruments.

For metformin-RP-HPLC methods compliant with ICH, the % RSD for assay values is typically ≤2%, and for impurity methods it is often ≤10–15% depending on the concentration level.

Limit of Detection (LOD)

LOD is the lowest concentration at which the analyte can be reliably detected but not necessarily quantified. It is commonly estimated using the signal-to-noise (S/N) ratio (LOD ≈ S/N = 3) or the standard-deviation-of-the-intercept method from the calibration curve. For metformin-assay methods, LOD is usually reported in µg/mL or ng/mL and is used to confirm the method’s ability to detect trace impurities or degradation products.

Limit of Quantification (LOQ)

LOQ is the lowest concentration at which the analyte can be quantitatively determined with acceptable accuracy and precision. LOQ is generally defined as S/N ≈ 10 or by a fixed coefficient of variation (e.g., 10–20% RSD) at low-level standards. In ICH-compliant metformin methods, LOQ is reported along with the expected % RSD and bias, ensuring that low-level impurities or metabolites can be reliably quantified.

Robustness

Robustness evaluates the method’s resilience to small, deliberate variations in critical parameters, such as mobile-phase composition, pH, flow rate, column temperature, and wavelength. For metformin-RP-HPLC, robustness is demonstrated if retention time, tailing factor, resolution, and % assay remain within pre-defined limits when one parameter is varied at a time. A robustness study may be designed using a DoE approach to systematically assess these variables around the optimized conditions.

Ruggedness

Ruggedness assesses the method's inter-laboratory or inter-operator reproducibility under normal usage conditions. It is tested by performing the analysis on different days, by different analysts, and/or on different instruments or columns, and by comparing mean assay values and % RSD. Ruggedness ensures that the method remains reliable when transferred to other laboratories or scaled to routine quality-control use.

System Suitability Parameters

System suitability tests are performed before routine analysis to confirm that the chromatographic system is performing adequately. For metformin-RP-HPLC, these parameters typically include:

- Retention time (within a defined range),
- Theoretical plates (≥2000 for metformin in many published methods),
- Tailing factor (≤2.0),
- Resolution (≥1.5 for critical pairs such as metformin and its nearest degradant),
- Repeatability (% RSD of replicate injections, usually ≤1–2%).

Acceptable system-suitability results are required for each analytical run, as per ICH and pharmacopoeial expectations.[23,24]

Applications of RP-HPLC Method for Metformin

RP-HPLC has become a versatile and widely applied technique for the analysis of metformin across multiple domains, including bulk-drug characterization, pharmaceutical-dosage-form assay, bioanalytical pharmacokinetic and pharmacodynamic studies, and stability-indicating investigations. Its selectivity, linearity, precision, and robustness make it particularly suitable for both quality control and research-oriented applications.[25,26]

Analysis in Bulk Drug

RP-HPLC is routinely used for assaying metformin hydrochloride in bulk drug substances, providing a rapid, accurate, and precise measurement of drug strength and purity. Validated methods on C18 columns with simple aqueous-organic mobile phases (e.g., buffer-acetonitrile or buffer-methanol) allow estimation of metformin concentration over a defined linearity range (often 50–150% of test concentration), with recoveries close to 100% and low % RSD, confirming the method's suitability for raw-material evaluation and release testing of the active ingredient.[26]

Analysis in Pharmaceutical Dosage Forms

RP-HPLC is extensively employed to determine metformin content in tablets and fixed-dose combination products, often in isocratic or gradient modes on C18-type columns. Methods have been validated for content-uniformity, assay, and impurity profiling in monotherapy metformin tablets and combination products (e.g., metformin + glimepiride, pioglitazone, dapagliflozin, or DPP-4 inhibitors), demonstrating high accuracy (98–102%), excellent precision (% RSD < 2%), and reliable separation of the drug from excipients and degradation products. These methods are integral to routine quality-control laboratories for batch-release testing and method-transfer studies.[27]

Bioanalytical Applications

In bioanalytical settings, RP-HPLC–UV methods have been developed and validated for metformin in human plasma and other biological matrices to support pharmacokinetic and drug-interaction studies. These methods typically involve liquid–liquid or solid-phase extraction of plasma samples, followed by injection onto an RP-HPLC column with a low-wavelength UV detector; they demonstrate wide linearity ranges (e.g., 100–500 µg/mL in plasma for metformin alone or in combination with companion drugs) and adequate sensitivity and selectivity for clinical pharmacokinetics, dose-proportionality, and drug-interaction assessments.[28]

Stability Studies

RP-HPLC plays a critical role in stability-indicating studies of metformin, both in bulk and in dosage forms. By applying stressed conditions (acid, base, oxidative, thermal, and photolytic), RP-HPLC can monitor degradation of metformin and resolve major degradation products, ensuring that the peak area of the parent drug changes in a predictable and quantifiable manner. Such methods are validated for specificity, linearity, and robustness and are used to establish shelf-life, storage conditions, and packaging suitability for metformin-containing products, thereby supporting regulatory filing and post-approval stability commitments.[29]

ACKNOWLEDGMENT

The authors express their sincere gratitude to the School of Pharmaceutical Sciences, Jaipur National University, Jaipur, for providing the necessary facilities and support to carry out this research work. The authors also acknowledge the valuable guidance of faculty members and technical staff for their assistance throughout the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

REFERENCES

1. Corcoran, C., & Jacobs, T. F., Metformin, 2018.
2. Huang, R., Dalton, J., Eichbaum, Y., Heard, J., Ezeonu, T., Fras, S., ... & Schroeder, G. D., The Impact of Metformin on Postoperative Opioid Consumption and Patient Reported Outcomes after Lumbar Fusion. *Spine*, 2025; 10-1097.
3. Drzewoski, J., & Hanefeld, M., The current and potential therapeutic use of metformin—the good old drug. *Pharmaceuticals*, 2021; *14*(2): 122.
4. Du, Y., Zhu, Y. J., Zhou, Y. X., Ding, J., & Liu, J. Y., Metformin in therapeutic applications in human diseases: its mechanism of action and clinical study. *Molecular Biomedicine*, 2022; *3*(1): 41.
5. Kumar, S. D., & Kumar, D. H., Importance of RP-HPLC in analytical method development: a review. *International journal of pharmaceutical sciences and research*, 2012; *3*(12): 4626.
6. Aguilar, M. I., Reversed-phase high-performance liquid chromatography. In *HPLC of peptides and proteins: Methods and protocols*, 2004; (pp. 9-22). Totowa, NJ: Springer New York.
7. Pernicova, I., & Korbonits, M., Metformin—mode of action and clinical implications for diabetes and cancer. *Nature Reviews Endocrinology*, 2014; *10*(3): 143-156.
8. Klepser, T. B., & Kelly, M. W., Metformin hydrochloride: an antihyperglycemic agent. *American journal of health-system pharmacy*, 1997; *54*(8): 893-903.
9. Graham, G. G., Punt, J., Arora, M., Day, R. O., Doogue, M. P., Duong, J., ... & Williams, K. M., Clinical pharmacokinetics of metformin. *Clinical pharmacokinetics*, 2011; *50*(2): 81-98.
10. Scheen, A. J., Clinical pharmacokinetics of metformin. *Clinical pharmacokinetics*, 1996; *30*(5): 359-371.
11. Rena, G., Hardie, D. G., & Pearson, E. R., The mechanisms of action of metformin. *Diabetologia*, 2017; *60*(9): 1577-1585.

12. LoBrutto, R., & Kazakevich, Y., Reversed-Phase HPLC. *HPLC for Pharmaceutical Scientists*, 2007; 139-239.
13. Kirkland, J. J., Development of some stationary phases for reversed-phase HPLC. *Journal of Chromatography A*, 2004; *1060*(1-2): 9-21.
14. Aguilar, M. I., Reversed-phase high-performance liquid chromatography. In *HPLC of peptides and proteins: Methods and protocols*, 2004; (pp. 9-22). Totowa, NJ: Springer New York.
15. Colin, H., & Guiochon, G., Introduction to reversed-phase high-performance liquid chromatography. *Journal of Chromatography A*, 1977; *141*(3): 289-312.
16. Rosés, M., Determination of the pH of binary mobile phases for reversed-phase liquid chromatography. *Journal of Chromatography A*, 2004; *1037*(1-2): 283-298.
17. Stepnowski, P., & Mrozik, W., Analysis of selected ionic liquid cations by ion exchange chromatography and reversed-phase high performance liquid chromatography. *Journal of separation science*, 2005; *28*(2): 149-154.
18. Sha'at, M., Spac, A. F., Stoleriu, I., Bujor, A., Cretan, M. S., Hartan, M., & Ochiuz, L., Implementation of QbD approach to the analytical method development and validation for the estimation of metformin hydrochloride in tablet dosage forms by HPLC. *Pharmaceutics*, 2022; *14*(6): 1187.
19. Bhor, R. J., Sable, K. S., Bhosale, M. S., & Dighe, S. B., Synthesis and Anti Convulsant Activity of "N'-{4-[2-(1h-Benzimidazol-2-Yl)-2-Oxoethyl] Phenyl}-2-Hydroxyacetohydrazide and Its Derivatives". *Adv. Pharmacol. Pharm*, 2023; *11*(1): 46-56.
20. Jones, M. D., & William, B., *A UPLC Method for Analysis of Metformin and Related Substances by Hydrophilic Interaction Chromatography (HILIC)*, 2013.
21. Sowjanya, P., RP-HPLC Method development of metformin in pharmaceutical dosage form. *J Pharm Anal*, 2012; *4*: 9-20.
22. Chhetri, H. P., Thapa, P., & Van Schepdael, A., Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. *Saudi Pharmaceutical Journal*, 2014; *22*(5): 483-487.
23. https://database.ich.org/sites/default/files/Q2(R1)%20Guideline.pdf
24. Swartz, M. E., & Krull, I. S., *Analytical method development and validation*. CRC press, 2018.
25. Rele, R. V., & Patil, S. P., Application of RP-HPLC technique for development of analytical method for validation of metformin hydrochloride from bulk drug and dosage form. *Asian Journal of Research in Chemistry*, 2021; *14*(4): 265-268.
26. HIREMATH, J. A., & KUMAR, H., A novel RP-HPLC method development and validation for the quantification of a potential anti-diabetic drug metformin hydrochloride in tablet dosage form. *Int J Curr Pharm Res*, 2022; *14*(5): 20-24.
27. Nirupa, G., & Tripathi, U. M., RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: Glimepiride, pioglitazone, and metformin and its pharmaceutical dosage forms. *Journal of chemistry*, 2013; *2013*(1): 726235.
28. Vetapalem, R., Yejella, R. P., & Atmakuri, L. R., Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of teneligliptin and metformin. *Turkish journal of pharmaceutical sciences*, 2020; *17*(2): 141.
29. ar, M., & Choudhury, P. K., HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. *Indian Journal of pharmaceutical sciences*, 2009; *71*(3): 318.