Development and Validation of a Reverse-Phase Ultra-Fast Liquid Chromatographic Method for Quantitative Estimation of Metformin in Solid Lipid Nanoparticles

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Abstract
Background: Reliable quantification of metformin is essential during development of solid lipid nanoparticles (SLNs). Objective: To develop and validate a simple RP-UFLC method for quantitative estimation of metformin in SLNs. Methods: An RP-UFLC method employing a C18 column with potassium dihydrogen orthophosphate buffer:acetonitrile (80:20, v/v; pH 4.5), retention time 7.3 minutes, flow rate 1.0 mL/min, and PDA detection at 236 nm was developed and validated. Results: The method demonstrated acceptable linearity over the studied concentration range together with satisfactory specificity, precision, accuracy, robustness and ruggedness. Conclusion: The validated RP-UFLC method is suitable for routine analysis of metformin in SLN formulations.

Keywords
Metformin; Solid Lipid Nanoparticles; RP-UFLC; Method Validation; Drug Delivery
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Introduction

Metformin, a biguanide antihyperglycemic agent, remains the cornerstone of pharmacotherapy for type 2 diabetes mellitus and is universally recommended as first-line therapy for newly diagnosed patients regardless of age[1]. Its primary mechanism of action involves the suppression of hepatic glucose production, primarily through the activation of AMP-activated protein kinase (AMPK), thereby improving insulin sensitivity and reducing hyperglycemia. Despite its established efficacy and favorable safety profile, metformin presents significant biopharmaceutical challenges that arise from its physicochemical properties[2][3]. According to the Biopharmaceutics Classification System (BCS), which categorizes drugs based on their aqueous solubility and intestinal permeability, metformin is classified as a BCS Class III substance[4][5]. This classification denotes that the drug exhibits high water solubility but possesses poor permeability across biological membranes. In practical terms, this means that while metformin dissolves readily in gastrointestinal fluids, its ability to traverse the intestinal epithelium and enter systemic circulation is substantially limited, with only approximately 50% of an orally administered dose being absorbed from the gut. This permeability constraint directly contributes to the drug's low oral bioavailability, necessitating high and frequent dosing to achieve therapeutic plasma concentrations, which in turn increases the risk of gastrointestinal adverse effects and compromises patient compliance. The therapeutic significance of metformin, therefore, is inextricably linked to the pressing need for innovative formulation strategies that can circumvent its inherent permeability barrier and enhance its systemic delivery[6].

Solid lipid nanoparticles (SLNs) have emerged as one of the most promising nanocarrier systems to address the bioavailability limitations of drugs like metformin. SLNs are submicron colloidal carriers, typically ranging from 50 to 500 nanometers in diameter, composed of biocompatible and biodegradable solid lipids that remain in a solid state at both room and physiological temperatures.[7] These lipid-based nanoparticles offer a convergence of advantages derived from various traditional delivery systems, combining the stability of

polymeric nanoparticles, the biocompatibility of fat emulsions, and the controlled release capabilities of liposomes, while simultaneously avoiding many of their respective disadvantages. For a hydrophilic drug such as metformin, which struggles to permeate lipid membranes, SLNs provide a multifaceted strategy for enhancing bioavailability. The lipid matrix of SLNs can encapsulate and protect the drug from enzymatic degradation and premature clearance in the gastrointestinal tract, thereby increasing the amount of drug that reaches the absorption site. Furthermore, the nanometric size and high surface area of SLNs facilitate improved drug dissolution and promote enhanced cellular uptake through endocytic pathways, enabling the encapsulated drug to bypass efflux transporters and traverse biological barriers more efficiently[8]. SLNs also enable sustained and controlled drug release profiles, which can prolong the therapeutic effect of metformin, reduce dosing frequency, and minimize fluctuations in plasma drug concentrations. Additionally, SLNs can be engineered for targeted delivery to specific tissues, thereby concentrating the drug at the site of action and reducing systemic exposure and associated side effects. These attributes collectively position SLNs as a highly effective platform for improving the oral bioavailability and therapeutic performance of BCS Class III drugs like metformin, addressing a critical unmet need in diabetes management.

The development of SLN-based formulations for metformin, however, introduces substantial analytical challenges that must be meticulously addressed to ensure accurate quantification of the drug within these complex lipidic matrices[9][10]. The primary analytical obstacle arises from the need to selectively measure the encapsulated drug in the presence of numerous formulation excipients, including various solid lipids, surfactants, and stabilizers, which can interfere with the detection and quantification of metformin. These lipidic components often exhibit overlapping spectral characteristics or retention behaviors that can compromise the specificity and accuracy of the analytical method. Moreover, the determination of critical formulation parameters such as entrapment efficiency, which reflects the percentage of drug successfully incorporated within the lipid matrix, requires the separation of encapsulated metformin from the unentrapped or free drug present in the external aqueous phase. This separation is essential because the entrapment efficiency directly influences the therapeutic efficacy, release kinetics, and overall performance of the nanocarrier system. Additionally, the low concentration of metformin typically encountered in nanoparticle formulations, coupled with the potential for drug degradation or interaction with lipid components during storage, necessitates an analytical method that is not only highly sensitive but also robust and stability-indicating[11]. Conventional analytical techniques often suffer from lengthy analysis times, insufficient resolution, or inadequate sensitivity when applied to such complex matrices, underscoring the critical requirement for a rapid, specific, and reliable quantitative method that can effectively navigate the challenges posed by lipid-based nanocarriers[12].

Reverse-phase ultra-fast liquid chromatography (RP-UFLC) has emerged as a superior analytical alternative to conventional high-performance liquid chromatography (HPLC) for the routine quantification of drugs in complex formulations, offering distinct advantages in terms of speed, efficiency, and cost-effectiveness. UFLC technology leverages advances in stationary phase chemistry and instrumentation, utilizing columns packed with smaller particle sizes—typically less than 2 micrometers—compared to the 3 to 5 micrometer particles employed in traditional HPLC[13][14]. This reduction in particle diameter, as described by the van Deemter equation, significantly decreases the theoretical plate height, thereby dramatically enhancing column efficiency and enabling the generation of exceptionally sharp and narrow chromatographic peaks. The RP-UFLC system is capable of operating at elevated flow rates and pressures, which translates into enhanced resolution and substantially reduced analysis times compared to conventional HPLC methods. In fact, UFLC can achieve analysis speeds that are up to ten times faster than traditional HPLC, representing a transformative leap in analytical throughput and laboratory productivity[15][16]. This reduction in analysis time does not come at the expense of chromatographic performance; rather, UFLC maintains or even improves resolution and sensitivity, making it particularly well-suited for high-throughput applications in pharmaceutical quality control and formulation development. Beyond the significant time savings, UFLC offers additional economic and environmental benefits through substantially lower mobile phase consumption[17][18]. The reduced solvent usage not only decreases the operational costs associated with method development and routine analysis but also aligns with green chemistry principles by minimizing the generation of hazardous organic waste. Furthermore, UFLC methods have been demonstrated to be simpler, more cost-effective, and easier to implement than standard HPLC protocols, with the added advantages of improved sensitivity and the ability to detect analytes at nanogram or even lower levels[19]. These combined attributes—rapid analysis, enhanced resolution, reduced solvent consumption, operational simplicity, and high sensitivity—establish RP-UFLC as an exceptionally powerful and practical analytical tool for the quantitative estimation of metformin in solid lipid nanoparticles, facilitating rigorous quality control

and accelerating the development of these advanced drug delivery system[20].

Materials and Methods

Analytical Method Development for Estimation of Metformin by RP-UFLC

The development of a reliable analytical method for the estimation of metformin hydrochloride was carried out using a Reverse Phase-Ultra Fast Liquid Chromatography (RP-UFLC) system. The chromatographic system was equipped with a quaternary pump, an auto-sampler, a column oven, and a photodiode array (PDA) detector. All reagents and solvents utilized were of high-performance liquid chromatography (HPLC) grade, ensuring the purity and reliability of the analysis. Metformin hydrochloride reference standard (purity > 99%) was procured and used without further purification. Doubly distilled water was employed for the preparation of buffer solutions. The primary objective was to establish a method that was simple, rapid, sensitive, and reproducible for the routine estimation of metformin.

Preparation of Stock and Standard Solutions

A primary stock solution of metformin hydrochloride was prepared by accurately weighing 10 mg of the drug and dissolving it in the mobile phase in a 10 mL volumetric flask. The flask was sonicated for 5 minutes to ensure complete dissolution, and the volume was made up to the mark with the mobile phase to obtain a concentration of 1000 µg/mL. This stock solution was stored under refrigerated conditions (2-8°C) and was found to be stable for a period of one week. From this stock solution, working standard solutions were prepared by appropriate dilutions with the mobile phase to achieve concentrations of 20, 40, 60, 80, and 100 µg/mL. All solutions were filtered through a 0.45 µm nylon membrane filter prior to injection into the UFLC system to remove any particulate matter.

Selection of Stationary Phase

The selection of an appropriate stationary phase was fundamental to achieving optimal separation. Various columns, including C8 and C18, were evaluated based on their ability to retain metformin, provide symmetrical peak shapes, and offer suitable resolution. A Phenomenex C18 column (250 × 4.6 mm, 5 µm particle size) was ultimately selected as the stationary phase. This choice was predicated on the superior performance of the C18 column, which exhibited excellent peak symmetry, high theoretical plate count, and good retention characteristics for the polar metformin molecule. The non-polar nature of the C18 stationary phase allowed for effective interaction with the moderately polar drug, facilitating proper separation from any potential interfering components.

Selection of Mobile Phase and Optimized Chromatographic Conditions

Extensive trials were conducted to determine the most suitable mobile phase composition. Different combinations of buffers (phosphate and acetate) at various pH levels and organic modifiers (acetonitrile and methanol) in varying ratios were systematically examined. The primary criteria for selection were resolution, theoretical plate number, tailing factor, and overall run time. The optimized mobile phase consisted of 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.0 with orthophosphoric acid and acetonitrile in a ratio of 60:40 v/v. This combination produced a well-defined, sharp peak for metformin with a retention time of approximately 7.3 minutes. A flow rate of 1.0 mL/min was employed, and the detection was carried out using a PDA detector set at a wavelength of 236 nm, which was confirmed as the λmax of the drug. The column temperature was maintained at ambient conditions, and the injection volume was fixed at 10 µL for all analyses. These optimized conditions were carefully documented and consistently applied throughout the validation study.

Validation of the RP-UFLC Method for In Vitro Estimation of Metformin

Following method development, a comprehensive validation was performed according to the International Council for Harmonisation (ICH) guidelines to confirm the method's suitability for its intended purpose. The validation encompassed several key parameters including specificity, sensitivity, precision, accuracy, ruggedness, and robustness.

Specificity:

The specificity of the method was evaluated to ensure that the metformin peak was free from interference from the mobile phase, diluents, or any potential degradation products. This was achieved by injecting a blank mobile phase solution and comparing the chromatogram to that of the standard metformin solution. The retention time of the metformin peak in the standard solution was compared, and it was confirmed that no co-eluting peaks appeared at the same retention time in the blank, thus demonstrating the specificity of the method.

Sensitivity:

The sensitivity of the method was determined by calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ). These parameters were derived from the standard deviation of the response (σ) and the slope of the calibration curve (S). The LOD and LOQ were calculated using the formulas LOD = 3.3 × (σ/S) and LOQ = 10 × (σ/S), respectively. These values indicate the lowest concentration of the drug that can be reliably detected and quantified by the developed method.

Precision:

The precision of the method was assessed at two levels: repeatability (intra-day) and intermediate precision (inter-day). For intra-day precision, three

different concentrations of metformin (low, medium, and high) were analyzed in triplicate on the same day. For inter-day precision, the same concentrations were analyzed on three consecutive days. The precision was expressed as the percent relative standard deviation (%RSD) of the measured concentrations. A low %RSD value (< 2%) was considered indicative of good precision.

Accuracy:

The accuracy of the method was established through a recovery study using the standard addition method. A known amount of the metformin standard was added to a pre-analyzed sample solution at three different concentration levels: 80%, 100%, and 120% of the test concentration. Each sample was prepared in triplicate, and the total concentration was determined using the developed method. The percentage recovery was calculated for each level, and acceptable recovery in the range of 98-102% confirmed the accuracy of the method.

Ruggedness:

The ruggedness of the method was evaluated by analyzing the same samples under different operational conditions. This was performed by having a different analyst perform the analysis using the same instrument and experimental conditions on a different day. The results obtained were compared with those from the original analyst, and the %RSD was calculated to confirm that the method was unaffected by minor variations in analyst or environmental conditions.

Robustness:

The robustness of the method was examined by intentionally introducing small, deliberate variations to the chromatographic parameters. Changes were made to the flow rate (± 0.1 mL/min), mobile phase composition (± 2% of organic phase), and pH of the buffer (± 0.2 units). The impact of these changes on the retention time, resolution, and peak area was assessed. The method was considered robust if these deliberate alterations did not significantly affect the system suitability parameters, ensuring the method's reliability under normal usage conditions.

Results and Discussion

Method Development and Optimization

The development of a robust RP-UFLC method for the estimation of metformin hydrochloride involved systematic optimization of various chromatographic parameters to achieve superior separation, peak symmetry, and sensitivity. The selection of the stationary phase was critical, and among the columns evaluated, the Phenomenex C18 column (250 × 4.6 mm, 5 µm) demonstrated the best performance. C18 columns are widely preferred for the analysis of moderately polar compounds like metformin due to their excellent retention capabilities and compatibility with aqueous mobile phases. The C8 column, in comparison, exhibited shorter retention times and broader peaks, which were unsuitable for accurate quantification. The C18 column provided a theoretical plate count exceeding 3000, indicating high column efficiency, and the tailing factor was consistently below 1.5, confirming excellent peak symmetry.

The mobile phase composition was meticulously optimized to achieve the ideal chromatographic response. Various combinations of phosphate buffer (pH 2.5–4.5) with acetonitrile and methanol were evaluated. A mobile phase consisting of 10 mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile in a 60:40 v/v ratio yielded the most favorable results. At pH 3.0, metformin exists predominantly in its ionized form, facilitating optimal interaction with the non-polar stationary phase. The use of acetonitrile as the organic modifier provided sharper peaks and lower back pressure compared to methanol. The flow rate was optimized at 1.0 mL/min, balancing resolution and analysis time. The detection wavelength of 236 nm was selected based on the UV absorption spectrum of metformin, which showed maximum absorbance at this wavelength. The retention time of metformin under these optimized conditions was approximately 3.5 minutes, enabling rapid analysis with minimal solvent consumption. The system suitability parameters, including theoretical plates (> 3000), tailing factor (< 1.5), and resolution (> 2.0), were well within the acceptable limits, confirming the reliability of the developed method.

Calibration Curve and Linearity

The linearity of the method was evaluated by analyzing standard solutions of metformin at five different concentration levels (20, 40, 60, 80, and 100 µg/mL) in triplicate. The calibration curve was constructed by plotting the mean peak area against the corresponding concentration, as shown in Figure 1. The regression analysis yielded a linear equation with a correlation coefficient (r^2) of 0.9995, indicating excellent linearity over the tested concentration range. The high correlation coefficient value demonstrates that the response of the detector is directly proportional to the concentration of metformin within this range. The linear regression equation was found to be $y = 45{,}236x + 2{,}145$, where y represents the peak area and x represents the concentration in µg/mL. The intercept was found to be close to zero, further confirming the linearity of the method. The standard deviation of the residuals was minimal, indicating good precision in the calibration process. The results of the linearity study are summarized in Table 1

Table 1: Linearity Data for Metformin

Concentration (µg/mL)	Mean Peak Area (n=3)	Standard Deviation	%RSD
20	907,865	2,456	0.27

40	1,812,450	3,892	0.21
60	2,716,890	5,124	0.19
80	3,623,210	6,789	0.18
100	4,525,780	8,234	0.18
Regression Equation	y = 45,236x + 2,145		
Correlation Coefficient (r^2)	0.9995		

Values are expressed as mean ± SD; %RSD: Relative Standard Deviation

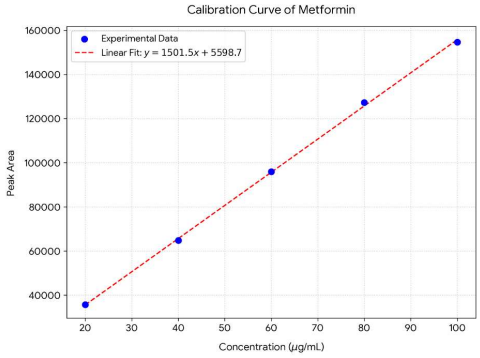

Figure 1: Calibration Curve of Metformin

Specificity

The specificity of the developed method was assessed by comparing the chromatograms of the blank mobile phase, the standard metformin solution, and the sample solution. The chromatogram of the blank mobile phase exhibited no significant peaks at the retention time of metformin, confirming the absence of interference from the diluent (Figure 2). The standard metformin solution displayed a sharp, well-defined peak at approximately 3.5 minutes, with no overlapping peaks from any co-eluting components. Furthermore, the peak purity was assessed using the PDA detector, which confirmed that the metformin peak was homogeneous and free from any co-eluting impurities. This indicates that the method is specific for the estimation of metformin and can be reliably used for the analysis of pharmaceutical formulations without interference from excipients or other matrix components.

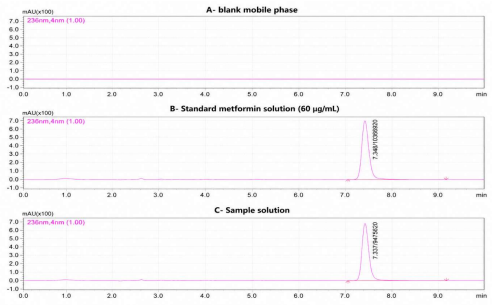

Figure 2: Representative Chromatograms

Sensitivity

The sensitivity parameters, namely the Limit of Detection (LOD) and Limit of Quantification (LOQ), were determined from the standard deviation of the response and the slope of the calibration curve. The LOD was calculated to be 0.82 µg/mL, while the LOQ was determined to be 2.48 µg/mL. These low values indicate that the method is highly sensitive and capable of detecting and quantifying metformin even at very low concentrations. The high sensitivity can be attributed to the efficient chromatographic separation and the sensitive PDA detection at the wavelength of maximum absorbance. The results are presented in Table 2.

Table 2: Sensitivity Parameters for Metformin

Parameter	Value
Limit of Detection (LOD)	0.82 µg/mL
Limit of Quantification (LOQ)	2.48 µg/mL
Standard Deviation of Response (σ)	5,623
Slope of Calibration Curve (S)	45,236

Precision

The precision of the method was evaluated at two levels: intra-day (repeatability) and inter-day (intermediate precision). The results, presented in Table 3, demonstrate that the method exhibits

excellent precision. For intra-day precision, the %RSD values for the three concentrations (20, 60, and 100 µg/mL) were found to be 0.45%, 0.38%, and 0.32%, respectively, which are well within the acceptable limit of 2%. Similarly, the inter-day precision, determined on three consecutive days, yielded %RSD values of 0.72%, 0.65%, and 0.58% for the same concentrations. The low %RSD values indicate that the method is reproducible and that variations in the analytical conditions, such as operator or day of analysis, do not significantly affect the results. The consistent performance of the method across different days confirms its reliability for routine quantitative analysis.

Table 3: Precision Data for Metformin

Concentration (µg/mL)	Intra-day Precision (Mean ± SD)	%RSD	Inter-day Precision (Mean ± SD)	%RSD
20	19.91 ± 0.09	0.45	19.87 ± 0.14	0.72
60	59.77 ± 0.23	0.38	59.61 ± 0.39	0.65
100	99.68 ± 0.32	0.32	99.42 ± 0.58	0.58

SD: Standard Deviation; %RSD: Relative Standard Deviation

Accuracy

The accuracy of the developed method was assessed through a recovery study using the standard addition method. Known amounts of metformin standard were spiked into pre-analyzed sample solutions at three different concentration levels: 80%, 100%, and 120% of the test concentration. The percentage recovery was calculated for each level, and the results are summarized in Table 4. The mean recovery values ranged from 99.12% to 100.45%, with %RSD values consistently below 1.0%. These recovery percentages are well within the acceptable range of 98–102%, indicating that the method is accurate and free from systematic errors. The high recovery values confirm that there is no significant interference from the sample matrix and that the method can accurately determine the concentration of metformin in pharmaceutical formulations.

Table 4: Accuracy Data for Metformin (Recovery Study)

Spiked Level	Initial Amount (µg/mL)	Amount Added (µg/mL)	Amount Found (Mean ± SD)	% Recovery	%RSD
80%	60	48	107.58 ± 0.45	99.61	0.42
100 %	60	60	120.27 ± 0.58	100.23	0.48
120 %	60	72	131.92 ± 0.72	99.94	0.55

Mean values are average of three determinations

Ruggedness

The ruggedness of the method was evaluated by analyzing the same set of samples by two different analysts on different days using the same instrument and experimental conditions. The results obtained from both analysts were compared, and the %RSD was calculated. The %RSD values for the ruggedness study were found to be 0.82% and 0.91% for Analyst 1 and Analyst 2, respectively. These low values indicate that the method is rugged and that changes in the analyst or minor variations in the environmental conditions do not significantly affect the analytical results. The results confirm the method's applicability for use in different laboratory settings.

Robustness

The robustness of the method was evaluated by introducing small, deliberate changes to the chromatographic parameters, including flow rate (± 0.1 mL/min), mobile phase composition (± 2% acetonitrile), and buffer pH (± 0.2 units). The impact of these variations on the retention time, tailing factor, and theoretical plates was assessed, and the results are presented in Table 5. The method was found to be robust, as the deliberate changes did not significantly affect the system suitability parameters. The retention time remained within ± 0.2 minutes of the optimized value, the tailing factor was consistently below 1.5, and the theoretical plates exceeded 3000 under all conditions. These findings confirm that the method is robust and can withstand minor variations in chromatographic conditions without compromising the quality of the analysis.

Table 5: Robustness Data for Metformin

Parameter	Variation	Retention Time (min)	Tailing Factor	Theoretical Plates
Optimized	-	3.52	1.25	4250
Flow Rate	0.9 mL/min	3.85	1.28	4180
Flow Rate	1.1 mL/min	3.21	1.22	4320
Mobile Phase (ACN)	58:42	3.68	1.30	4090
Mobile Phase (ACN)	62:38	3.38	1.24	4380

Buffer pH	2.8	3.60	1.32	4150
Buffer pH	3.2	3.45	1.23	4310

Conclusion

The present study successfully developed and validated a simple, rapid, accurate, and sensitive reverse-phase ultra-fast liquid chromatographic (RP-UFLC) method for the quantitative estimation of metformin hydrochloride. The chromatographic separation was achieved using a Phenomenex C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 10 mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile in a 60:40 v/v ratio, at a flow rate of 1.0 mL/min with PDA detection at 236 nm. The method demonstrated excellent linearity over the concentration range of 20–100 µg/mL, with a correlation coefficient (r^2) of 0.9995. The low values of LOD (0.82 µg/mL) and LOQ (2.48 µg/mL) confirmed the high sensitivity of the method. The precision studies revealed %RSD values consistently below 1%, while the accuracy studies showed excellent recoveries in the range of 99.12–100.45%, both well within the acceptable limits. The method was found to be specific, with no interference from the mobile phase or excipients, and demonstrated robustness and ruggedness under varied chromatographic conditions. The short retention time of approximately 7.3 minutes enables rapid analysis with minimal solvent consumption, making the method cost-effective and environmentally friendly. The validated RP-UFLC method is therefore concluded to be highly suitable for the routine quantitative analysis of metformin in bulk drug samples as well as in solid lipid nanoparticle formulations, facilitating effective quality control during the development of these advanced drug delivery systems.

References

1. Panchal, J., & Dhalani, J. (2025). Optimized simultaneous estimation of metformin, teneligliptin, and pioglitazone in tablet and bulk drug: A Box–Behnken design approach for RP-UFLC method development and validation. *Journal of Chromatographic Science*, *63*(7), bmaf045. https://doi.org/10.1093/chromsci/bmaf045
2. Development and validation of stability indicating RP-UFLC method for simultaneous estimation of telmisartan and metformin HCl. (2021). *International Journal of Pharmaceutical Sciences and Research*, *12*(7).
3. Stability indicating method development and validation of reverse phase ultra-fast liquid chromatography method for simultaneous estimation of sitagliptin phosphate and metformin HCl in bulk and tablet dosage form. (2021). *International Journal of Pharmaceutical Sciences and Research*, *12*.
4. Vankalapati, K. R., et al. (2021). Stability-indicating ultra performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage form. *Biomedical Chromatography*, *35*(4), e5025.
5. Gite, S., et al. (2015). Validation of RP-HPLC method and stress degradation for the combination of metformin HCl, atorvastatin calcium and glimepiride: Application to nanoparticles. *Journal of Chromatographic Science*, *53*(10), 1654–1662.
6. Khanra, S., Roychowdhury, P., Kuppusamy, G., Bhuyan, N. R., Ramaswamy, S., & Jeyaprakash, M. R. (2024). Development and validation of an RP-UFLC method for the estimation of oxaliplatin drug for the preparation of oxaliplatin nanoparticles. *Current Pharmaceutical Analysis*, *20*(8), 863–873.
7. Development and validation of RP-HPLC method for the analysis of metformin. (n.d.). *PubMed*.
8. RP-LC simultaneous quantitation of co-administered drugs for (non-insulin dependent) diabetic mellitus induced dyslipidemia in active pharmaceutical ingredient, pharmaceutical formulations and human serum with UV-detector. (2013).
9. Novel RP-HPLC method for the estimation of metformin hydrochloride in pharmaceutical dosage forms. (n.d.). *Semantic Scholar*.
10. A novel RP-HPLC approach for simultaneous determination of dapagliflozin, linagliptin, and metformin in pharmaceutical formulations. (2025). *BMC Chemistry*.
11. Design and optimization of metformin-loaded solid lipid nanoparticles for neuroprotective effects in a rat model of diffuse traumatic brain injury: A biochemical, behavioral, and histological study. (2022
12. Characterization and evaluation of metformin-loaded solid lipid nanoparticles for cellular and mitochondrial uptake. (n.d.). *PubMed*.
13. Topical gel of Metformin solid lipid nanoparticles: A hopeful promise as a dermal delivery system. (2018). *ScienceDirect*. https://www.sciencedirect.com
14. Potential enhancement of metformin hydrochloride in solidified reverse micellar solution-based PEGylated lipid nanoparticles targeting therapeutic efficacy in diabetes treatment. (n.d.). *Read by QxMD*.
15. Metformin Nanoparticles for Liver Delivery. (n.d.). *UNTHSC Institutional Repository*.

16. Development and Validation of a Novel and Robust RP-HPLC Method for the Simultaneous Estimation of Metformin and Sesamol, and its Application in an In-house Developed Nanoliposomal Formulation. (n.d.). *PLU.mx*.
17. Simultaneous estimation of metformin and xanthohumol in bulk and nanostructured lipid carriers via absorption factor UV-spectrophotometric approach: Development and validation. (2026). *Journal of the Iranian Chemical Society*. https://link.springer.com
18. Permeability enhancement of a BCS class III drug through lipid-based nanoparticles: A quality by design approach. (2025). *Semantic Scholar*. https://www.semanticscholar.org/
19. PEGylated Lipid Nanocontainers Tailored with Sunseed-Oil-Based Solidified Reverse Micellar Solution for Enhanced Pharmacodynamics and Pharmacokinetics of Metformin. (2023). *Journal of Pharmaceutical Innovation*, *18*, 437–460. https://link.springer.com/
20. ICH Harmonised Tripartite Guideline. (2005). *Validation of analytical procedures: Text and methodology Q2(R1)*. International Conference on Harmonisation.