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Design Of Experiments In Metformin Analytical Method Development: A Critical Review Of Aqbd Strategies And Optimization Approaches

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Abstract
Metformin is widely used therapeutically and presents analytical challenges that make it an ideal candidate for applying Analytical Quality by Design (AQbD) and Design of Experiments (DoE) approaches in pharmaceutical analysis. This review focuses on reverse-phase high-performance liquid chromatography (RP-HPLC) and capillary electrophoresis (CE) methods for metformin and its fixed-dose combinations, emphasizing optimization strategies using Box-Behnken Design (BBD) and Central Composite Design (CCD). It evaluates method performance in terms of sensitivity, accuracy, and analysis time, comparing seven validated procedures for analytical efficiency, regulatory compliance, and environmental sustainability through greenness metrics like AGREE and Eco-Scale. The role of experimental design in controlling critical method parameters, risk assessment, and defining the design space is highlighted, while limitations such as inconsistent lifecycle management and underutilization of CE-MS are discussed. This synthesis offers practical recommendations to enhance analytical robustness, sustainability, and regulatory adherence, supporting the broader application of AQbD in pharmaceutical quality control.

Keywords: *Metformin, Design of Experiments (DoE), Analytical Quality by Design (AQbD), HPLC, Capillary Electrophoresis, Green Analytical Chemistry, Risk Assessment, Method Validation, Bioanalysis.*

1. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a long-lasting and gradually worsening metabolic disorder characterized by the body's resistance to insulin and a decline in insulin secretion, leading to persistently elevated blood sugar levels [1]. Type 2 Diabetes Mellitus (T2DM) has emerged as a leading non-communicable disease globally, driven largely by shifts in lifestyle habits, increased urbanization, genetic predispositions, and the growing proportion of aging populations [2]. In India, over 101 million people currently live with diabetes, and this number is expected to rise further due to rapid urban migration, shifts in diet, and increasingly sedentary lifestyles. The significant socioeconomic burden posed by this disease underscores the urgent need for effective treatments and stringent pharmaceutical quality control measures to ensure that medications are both safe and effective [3; 4].
Metformin is the primary treatment for type 2 diabetes mellitus (T2DM) due to its effectiveness in reducing liver glucose production, enhancing insulin sensitivity, minimizing risk of low blood sugar and weight gain, and its additional benefits for heart health and metabolic complications [5]. As part of the biguanide class, metformin helps lower blood sugar primarily by reducing glucose production in the liver, improving the body's uptake of glucose in tissues, and decreasing how much glucose is absorbed from the intestine [6; 7]. It is commonly prescribed either alone as initial therapy or combined in fixed-dose formulations with other antidiabetic agents such as sulfonylureas, DPP-4 inhibitors, and SGLT2 inhibitors [8]. Beyond diabetic management, new data indicates metformin's therapeutic advantages in oncology, cardiovascular health, aging, and polycystic ovarian syndrome, hence broadening its clinical applicability [9]. As metformin’s therapeutic importance grows, so does the need for rigorous, dependable, and reproducible analytical techniquesto ensure the quality, purity, and stability of metformin-containing formulations [10]. High-performance liquid chromatography (HPLC), particularly reverse-phase HPLC (RP-HPLC), has established itself as the preeminent analytical method owing to its exceptional resolution, precision, and sensitivity in pharmaceutical analysis [11; 12]. Likewise, sophisticated techniques such as capillary electrophoresis (CE) have arisen as supplementary procedures that provide high efficiency and minimal solvent usage [13; 14]. These procedures are essential not only for standard quality control

and batch release but also for bioanalytical applications, including pharmacokinetic research and therapeutic medication monitoring.

The traditional development methodology frequently experiences difficult, time-consuming trial-and-error methods that are susceptible to variation. The pharmaceutical sector has progressively adopted the Quality by Design (QbD) framework, which offers a scientific and systematic methodology for method development [15; 16]. In this context, Design of Experiments (DoE) is essential for optimizing analytical parameters, facilitating the concurrent assessment of several variables, comprehending their interactions, and assuring resilience to tiny fluctuations [17]. The application of DoE approaches, including Box-Behnken Design (BBD), Central Composite Design (CCD), and Response Surface Methodology (RSM), enables the establishment of analytical design spaces, hence promoting method optimization within specified parameters [18; 19]. These tactics not only diminish the number of experimental runs necessary but also enhance method performance by focusing on Critical Method Parameters (CMPs) and Critical Quality Attributes (CQAs) [20; 21]. The amalgamation of DoE with risk-based instruments such as Analytical Failure Mode and Effect Analysis (AFMEA) enhances methodological reliability and adherence to regulatory standards, including ICH Q8(R2), Q9, and ISO/IEC 17025 [22]. Despite the growing application of DoE in analytical research, there is still a necessity for thorough studies that integrate these methodologies specifically for the analysis of metformin, encompassing both its individual and combination dose forms, as shown in **Fig 1** [23]. Current research frequently emphasizes singular method development initiatives, neglecting comparative assessments, critical evaluations, or forward-looking insights. This review seeks to address the gap by offering a comprehensive overview of DoE-based analytical methods for metformin, emphasizing chromatographic, electrophoretic, and bioanalytical approaches, while examining their comparative advantages, limits, and regulatory compliance [24; 25]. The paper develops information from both landmark and recent studies to record developments in the application of DoE for metformin analysis, while also providing essential insights into methodological efficiency, method validation methods, and practical problems as listed in **Table 1**. This review comprehensively integrates and critically assesses DoE-based analytical methodologies for metformin and its fixed-dose combinations. It addressed the shortcomings in current literature by analysing principal forms of Design of Experiments (e.g., Box-Behnken Design, Central Composite Design), evaluating proven chromatographic and electrophoretic techniques, and scrutinizing their sensitivity, sustainability, and compliance with regulations.

It examines the function of risk assessment instruments such as Ishikawa diagrams and AFMCEA, while emphasizing new prospects in green chemistry, CE-MS integration, and AI-driven technique optimization. The review's findings may inform future AQbD implementation by highlighting design-specific strengths, sustainability metrics, and integration strategies across chromatographic and electrophoretic methods.

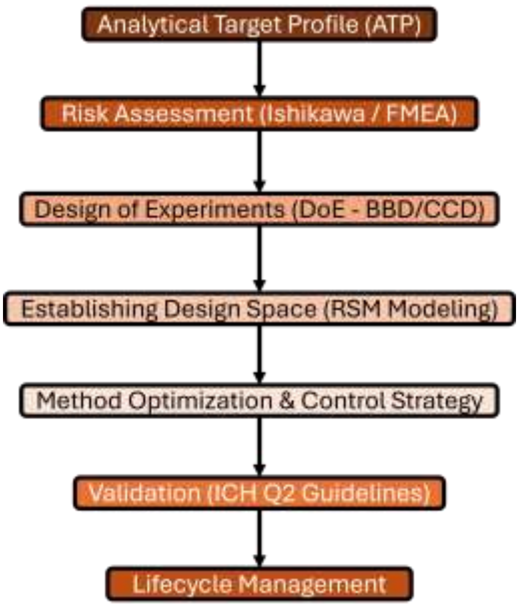

Fig 1. Overview of Analytical Workflow for Metformin

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2. FUNDAMENTALS OF DOE AND AQBD IN ANALYTICAL METHOD DEVELOPMENT

The integration of DoE into the Analytical Quality by Design (AQbD) framework signifies a transformative change in pharmaceutical method development, highlighting systematic, science-based, and risk-oriented strategies that promote analytical robustness, regulatory adherence, and efficiency [26]. In contrast to traditional one-factor-at-a-time (OFAT) methodologies DoE facilitates the concurrent assessment of multiple variables and their interactions, markedly improving the reliabilityand reproducibility of analytical techniques [27]. Within the framework of AQbD, DoE functions as a fundamental instrument for defining a Design Space (DS), a multidimensional area where method parameters can fluctuate without adversely affecting CQAs [28]. The ICH Q8(R2) rule asserts that activities within the design space are not deemed alterations and hence do not necessitate post-approval regulatory reports. This regulatory flexibility is significantly beneficial in the management of analytical method lifecycles [29; 30].

Table 1. Additional Analytical Studies on Metformin

Analytical Technique	**Matrix**	**Key Features**	**Application**	**Citation**
HPLC	Bulk and Pharmaceutical Dosage Forms	Simultaneous determination of metformin and gliclazide; validated method	Quality control of combination antidiabetic formulations	[31]
HPLC, HPTLC, Spectrophotometry, Electrochemical, Capillary Electrophoresis, LC-MS	Pharmaceutical Formulations and Biological Matrices	Comprehensive review of various analytical methods for metformin estimation	Method selection guidance for researchers and analysts	[32]
Various Techniques	Pure Forms, Pharmaceutical Formulations, Biological Samples	Review on determination methods for metformin and glimepiride in different matrices	Analytical method development and validation	[33]
HPLC, UV Spectrophotometry, HPTLC, LC-MS	Pharmaceutical Formulations and Biological Matrices	Review of analytical methods for metformin estimation	Reference for method development and validation	[34]
UV Spectrophotometry, HPTLC, RP-HPLC	Tablet Dosage Forms and Plasma	Development and validation of methods for simultaneous estimation of metformin and alogliptin	Routine analysis and bioanalytical studies	[35]
UV Spectrophotometry, TLC, HPTLC, HPLC	Pharmaceutical Dosage Forms	Review on analytical methods for estimation of empagliflozin, linagliptin, and metformin	Quality control and formulation analysis	[36]
Conductometry	Pharmaceutical Formulations	Green chemistry approach for conductometric determination of metformin	Environmentally friendly quality control method	[37]
HILIC-MS/MS	Herbal Supplements	Optimization of solid-phase extraction and hydrophilic interaction liquid chromatography for metformin detection	Detection of adulteration in herbal products	[38]
HILIC-MS/MS	Wastewater	Development and validation of method for quantifying metformin and other pharmaceuticals	Environmental monitoring of pharmaceutical contaminants	[39]
RP-HPLC	Active Pharmaceutical Ingredients (API) and Tablets	Simultaneous assay of benfotiamine and metformin; validated method	Quality control of combination formulations	[40]

RP-HPLC (Central Composite Design)	Tablet Dosage Forms	Quality by Design (QbD) approach for method development and validation; high sensitivity	Precision-focused quality control	[41]

2.1. Comparative Assessment of BBD and CCD in AQbD

BBD mitigates extreme conditions reduces solvent consumption and upholds GAC principles. CCD enhances model precision via central and axial points, hence augmenting technique robustness. **Table 2** delineates the performance of both designs regarding sensitivity, limit of detection (LOD), and runtime[42]. Numerous experimental designs are encompassed under the framework DoE, each customized for distinct phases of analytical technique development. Full Factorial Design is a thorough methodology that assesses all potential combinations of chosen components and is optimal for exhaustive screening when resources permit [43]. Fractional Factorial Design minimizes the number of experimental runs while effectively capturing essential interactions, rendering it appropriate for preliminary studies.

The Box-Behnken Design (BBD) is a three-level design that omits extreme values, making it particularly suitable for method optimization and response surface modeling in chromatographic analysis [19; 30; 44]. Central Composite Design (CCD), which enhances factorial design by integrating center and axial points, is optimal for constructing resilient quadratic models in optimization [30; 29; 45]]. The Plackett-Burman Design is effective for screening numerous factors; nevertheless, it fails to account for interaction effects.These designs facilitate the modeling of both primary effects and interactions among variables and are extensively employed for optimizing chromatographic and electrophoretic techniques. Randive et al., (2024) utilized BBD to adjust essential parameters, including flow rate, mobile phase composition, and detection wavelength in an HPLC technique for metformin, resulting in enhanced sensitivity and diminished variability, as shown in **Fig 2** [46].

A further work by Krishnan and Mishra (2020) employed CCD in technique development for the concurrent quantification of sitagliptin and metformin, resulting in improved accuracy, precision, and resilience of the method [41]. Their utilization of Design-Expert® software for optimization highlights the significance of statistical techniques in contemporary analytical development [47]. the comprehensive implementation of AQbD through Method 5 (Krishnan and Mishra), encompassing the development of the Analytical Target Profile (ATP), risk assessment via the Ishikawa diagram, design space exploration utilizing CCD, and the establishment of control strategies based on ANOVA results and robustness data [41].

Table 2. DoE Designs and Their Applications in Analytical Method Development

DoE Design	Application	Advantages	Typical Use Case	References
Full Factorial Design	Examines all factor combinations	Comprehensive, but resource-intensive	Best for preliminary screening	[43]
Box-Behnken Design (BBD)	Efficient 3-level design without extreme combinations	Reduces number of runs	Method optimization	[44]
Central Composite Design (CCD)	Includes center and axial points	Builds robust quadratic models	Response surface modelling	[45]
Plackett-Burman Design	Identify significant factors with minimal runs	Ignores interactions	Factor screening	[32; 52]

The efficacy of AQbD is augmented by risk assessment instruments like Ishikawa fishbone diagrams and Failure Mode and Effect Analysis (FMEA), which facilitate the identification of CMPs and their correlation with CQAs [53]. Prajapati et al. (2022) applied Analytical-Failure Modes and Critical Effect Analysis (AFMCEA) combined with Design of Experiments (DoE) to develop a versatile RP-HPLC method for metformin fixed-dose combination products [54]. This hybrid methodology established a systematic framework to control variability, mitigate risks, and enforce adherence to ICH Q9 risk management standards. CE techniques also benefit from DoE-based AQbD methodologies. Chiarentin et al. (2024) illustrated the utilization of BBD to optimize a CE approach for metformin and its contaminants employing carboxymethyl-β-cyclodextrin (CM-β-CD) as a modifier [28]. Their research developed a reliable methodology with reduced runtime, confirmed in compliance with ICH requirements.

Additionally, in bioanalytical settings, Patel et al. (2021) utilized a BBD methodology to enhance protein precipitation characteristics in plasma samples for the measurement of metformin and alogliptin [52]. Their approach exhibited elevated extraction recoveries and sensitivity, confirming the interdisciplinary use of Design of Experiments in pharmacological and therapeutic contexts.

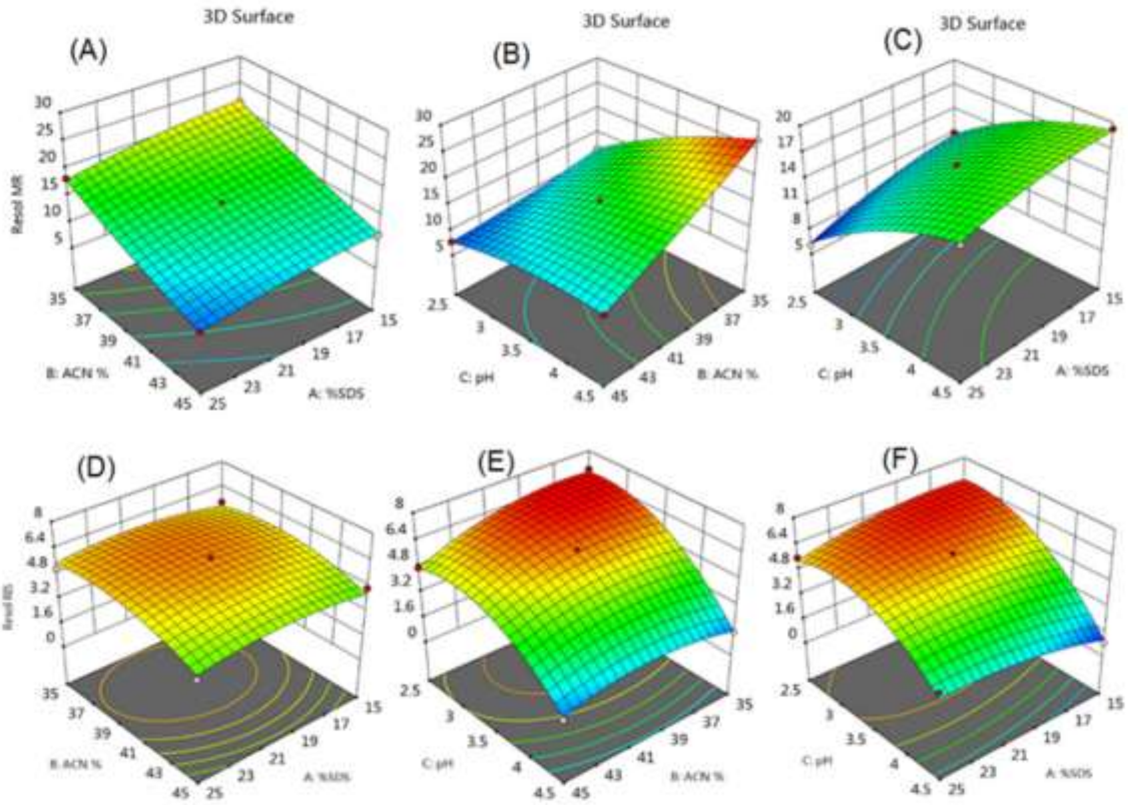

Fig 2. Box-Behnken Design Matrix for RP-HPLC Optimization for Metformin, Response surface models illustrating the effects of SDS concentration, acetonitrile percentage, and mobile phase pH on the resolution between MFH and RGE (A–C), and between RGE and IS (D–F). Courtesy [55]

3. CHROMATOGRAPHIC METHODS FOR METFORMIN ANALYSIS

Chromatographic methods, especially HPLC, are the benchmark in the pharmaceutical analysis of metformin owing to their exceptional sensitivity, specificity, precision, and reliability, as listed in **Table 3**. The incorporation of DoE into the AQbD framework has markedly enhanced method development procedures, improving method performance, diminishing variability, and conforming to regulatory standards, as shown in **Fig 3** [26; 56]. The BBD and RSM are the most employed DoE techniques for optimizing essential method parameters, including mobile phase composition, flow rate, pH, and detection wavelength. These instruments have facilitated the identification of CMPs and CQAs in metformin assays.

Table 3. Comparative Summary of DoE-Based Chromatographic Methods for Metformin

Technique	Retention Time (min)	LOD (μg/mL)	Accuracy (%)	DoE Design	Column Used	Remarks	Study
RP-HPLC	2.96	0.05	99.80	BBD	C18, 250 mm	Fast, cost-effective	[44]
RP-HPLC	3.5	0.02	100.10	CCD	Monolithic C18	Highest sensitivity	[41]
RP-HPLC	2.1	0.06	99.50	BBD + AFMCEA	Shim-Pack C18	Eco-friendly	[54]
RP-HPLC	3.0	0.1	98.70	CCD	C18	Good reproducibility	[57]

Suryawanshi and Palled (2022) enhanced an RP-HPLC method for metformin by applying Box-Behnken Design (BBD) and Analytical Quality by Design (AQbD) principles [44]. The authors focused on fine-tuning key factors like flow rate, the composition of the mobile phase, and detection wavelength. They assessed the method's performance in terms of linearity, accuracy, and precision, achieving high sensitivity with a retention time of 2.96 minutes. The study highlighted how using Design of Experiments (DoE) effectively reduces the number of experimental trials needed while optimizing crucial chromatographic conditions. Sundhani et al. (2020) developed an HPLC method using Box-Behnken Design and Response Surface Methodology (BBD-RSM) for simultaneously measuring metformin and glimepiride in spiked plasma samples [58]. The study optimized factors such as pH, flow rate, and buffer composition to improve separation efficiency. Using a phenyl C18 column, the method showed outstanding precision, accuracy, and resolution. Its successful application in plasma highlights both its importance in bioanalysis and its potential clinical relevance.

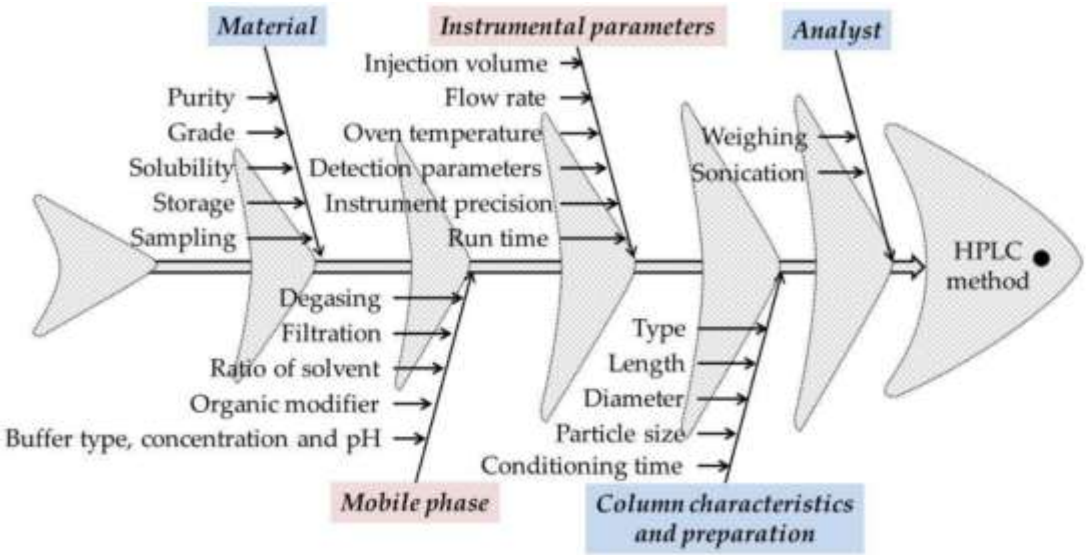

Fig 3. Ishikawa (Fishbone) Diagram of Analytical Method Risk Factors for metformin. Courtesy [59]

Khan et al. (2023) showcased a thorough application of Quality by Design (QbD) principles by developing an HPLC method for metformin hydrochloride tablets using a Central Composite Design (CCD) approach [60]. The optimization process focused on crucial variables such as the composition of the mobile phase—specifically, the balance of acetate buffer and methanol—as well as pH and the amount of organic modifier. This method showed outstanding reproducibility and satisfied all system suitability standards. To strengthen the method's reliability, a risk assessment was carried out using an Ishikawa fishbone diagram, which allowed for the identification and mitigation of factors that could introduce analytical variability, ultimately enhancing overall robustness.

Prajapati et al. (2022) further developed Analytical Quality by Design (AQbD) principles by pioneering a new approach that combines Analytical Failure Modes and Critical Effect Analysis (AFMCEA) with Box-Behnken Design (BBD) [54]. Their study focused on developing an RP-HPLC method tailored for different fixed-dose combination (FDC) formulations containing metformin, using Shim-Pack C18 columns and a mobile phase composed of acetonitrile and triethylamine, which was adjusted with perchloric acid. That approach highlighted traceability and accuracy, rendering it particularly suitable for multi-analyte pharmaceutical formulations. Statistical techniques such as ANOVA and response surface plots were used to examine how different factors interact and to confirm the reliability of the approach.

In a significant study, Krishnan and Mishra (2020) utilized a Central Composite Design (CCD) to refine an isocratic RP-HPLC method for simultaneously quantifying metformin and sitagliptin in both bulk substances and tablet forms [41]. The method utilized a monolithic C18 column, achieving excellent precision along with low limits of detection (LOD) and quantification (LOQ), and maintained an optimal retention time. This study demonstrated the effectiveness of Central Composite Design (CCD) in fine-tuning analytical parameters and minimizing variability, thereby meeting the ICH Q2B validation standards. Collectively, these studies highlight a transition from traditional empirical method development to more systematic, reproducible approaches using Design of Experiments (DoE). The benefits include reduced experimental workload, improved predictability of methods, and stronger compliance with regulatory requirements. Moreover, the integration of risk-based tools and software platforms such as Design-Expert® facilitates real-time data analysis and decision-making in method development [47].

Furthermore, the application of QbD-based chromatographic techniques is congruent with the ICH's focus on method lifecycle management, thereby enabling post-approval change control and ongoing enhancement. Methods developed using DoE inherently exhibit superior comprehension, facilitating improved control over variability and increased robustness in routine quality control. While RP-HPLC prevails in metformin analysis, other chromatographic advancements such green chromatography, micro-HPLC, and ultra-performance liquid chromatography (UPLC) present opportunities for future investigation. These strategies, when integrated with Design of Experiments frameworks, could further diminish solvent consumption, limit operational duration, and enhance sustainability in analytical laboratories.

4. ELECTROPHORETIC AND BIOANALYTICAL APPROACHES

Although chromatographic methods like HPLC have historically prevailed in pharmaceutical analysis, electrophoretic and bioanalytical techniques have garnered increasing recognition for their superior resolution, less sample consumption, and compatibility with intricate biological matrices, as listed in **Table 4**. CE and bioanalytical RP-HPLC have been substantially improved via the incorporation of DoE and QbD approaches. Orlandini et al. (2014) exemplified a complete application of DoE in electrophoretic techniques by developing a cyclodextrin-modified CE method for the concurrent investigation of metformin hydrochloride and its associated impurities [61]. Their methodology employed CM-β-CD in Britton-Robinson buffer and implemented a BBD to optimize essential parameters including capillary length, injection duration, and pH. This technique achieved highly efficient separation in 9 minutes, validated according to ICH norms. The study highlighted capillary electrophoresis (CE) as an effective tool for impurity profiling and recognized it as a greener, more environmentally friendly alternative to conventional chromatography methods.

Table 4. Comparative Evaluation of Analytical Techniques

Technique	Sensitivity	Precision	Cost	Solvent Use	Notes	Application
RP-HPLC	High	High	Moderate	Low	Widely accepted	Routine QC
CE	Moderate	Moderate	Very Low	High	Eco-friendly	Impurity profiling
UV Spectrophotometry	Low	Low	High	Moderate	Limited precision	Initial screening
LC-MS/MS	Very High	High	Low	Very Low	High sensitivity	Bioanalysis
Conductometry	Moderate	Moderate	Very High	High	Green method	Simple QC

The use of capillary electrophoresis (CE) has been strengthened by applying Quality by Design (QbD) risk assessment tools such as Plackett-Burman and Doehlert designs, which have enhanced the robustness of analytical methods [62]. Orlandini's study demonstrated how Monte Carlo simulations combined with Response Surface Methodology (RSM) effectively define the design space, helping to reduce method variability and meet regulatory standards [61]. Patel et al. (2021) developed a dependable method for simultaneously measuring metformin hydrochloride and alogliptin benzoate in plasma, using protein precipitation (PP) as the extraction technique [52]. Their study used a Box-Behnken Design (BBD) to optimize plasma preparation parameters such as centrifugation speed, time, and plasma volume. This approach achieved a recovery rate exceeding 93% and demonstrated strong linearity, making it suitable for pharmacokinetic studies. The method was validated following USFDA bioanalytical validation guidelines.

Patel et al.'s method showcased the effectiveness of combining gradient RP-HPLC with Box-Behnken Design (BBD) to develop a sensitive and selective bioanalytical technique. By using 1-octane sulfonic acid, phosphate buffer, and acetonitrile in the mobile phase, the method achieved efficient separation along with low limits of detection (LOD) and quantification (LOQ) [52; 53]. Additionally, statistical methods like Bartlett's test were used to evaluate data variance, thereby enhancing the reliability of the method for clinical use. These studies show that Design of Experiments (DoE)-based electrophoretic and bioanalytical techniques are not only cost-effective and eco-friendly but also highly adaptable to meet regulatory standards [63]. Their ability to handle complex sample matrices and multiple analytes with high sensitivity makes them indispensable tools in pharmacokinetic studies and routine quality control.

5. COMPARATIVE ANALYSIS OF REVIEWED METHODS

A comparative evaluation of Design of Experiments (DoE)-based analytical methods for metformin provides valuable understanding of their practical use, effectiveness, and compliance with regulatory requirements. Among the reviewed studies, seven key approaches stand out: five chromatographic techniques, one electrophoretic method, and one bioanalytical procedure, each optimized using DoE within Quality by Design (QbD) frameworks [56; 28]. This section compares various methodologies regarding sensitivity, accuracy, robustness, regulatory compliance, and operational practicality.

5.1. Chromatographic Methods (HPLC/RP-HPLC)

Among chromatographic techniques, Method 1, devised by Suryawanshi and Palled (2022), utilized a Box-Behnken Design to optimize a reverse-phase high-performance liquid chromatography (RP-HPLC) approach for metformin. It attained a rapid retention time (2.96 min), significant linearity, and little tailing, accompanied by outstanding system appropriateness metrics. The primary strength was the resilience of the technique, underpinned by statistical modelling of essential factors including flow rate and detecting wavelength. Nevertheless, the moderate retention duration of this approach renders it somewhat less appropriate for ultra-high-throughput settings [44].

Method 2, developed by Kalpana et al. (2025), concentrated on the concurrent measurement of metformin and glimepiride in spiking plasma utilizing RSM and BBD. Despite exhibiting superior linearity and technique precision, its retention time (~5.5 minutes) and elevated limits of quantification renders it less efficient than alternative methods. However, it retains significance in bioanalytical applications necessitating dual-analyte measurement [64].

Method 3, as described by Chiarentin et al. (2024), utilized a CCD for the AQbD-based development of a metformin assay in tablet formulations. Notwithstanding its commendable reproducibility and incorporation of risk assessment (e.g., Ishikawa diagram), it exhibited significantly elevated LOD/LOQ values, constraining its utility in ultra-trace detection. Its efficacy is rooted in cost-effectiveness and reproducibility within standard quality control settings [28].

The evaluated research employed BBD and CCD according to their experimental requirements. BBD was preferred in situations emphasizing optimization efficiency, especially where fewer iterations and the absence of extreme values were required. Conversely, CCD facilitated extensive examination of variable interactions, enhancing resilience in high-precision scenarios. The comparative efficacy of various designs, as indicated by sensitivity, run duration, and environmental impact, is summarized in **Table 5**.

Table 5. Overall Comparison and Method Selection

Method	Strengths	Limitations	Best Use Case
Method 1	Balanced performance, rapid analysis, good precision	Slightly less sensitive than Method 5	Routine QC and batch release
Method 2	Dual-analyte quantification in plasma	High retention time, moderate sensitivity	Bioanalytical studies
Method 3	Cost-effective, reproducible	High LOD/LOQ	Generic drug testing
Method 4	Fastest run time, eco-friendly, multi-analyte	Lower sensitivity	High-throughput labs

Method 5	Highest accuracy, robust, low LOD/LOQ	Slightly longer analysis time	Reference method in validation
Method 6	Green, efficient, impurity profiling	Moderate precision	Sustainability-focused labs
Method 7	High recovery, suitable for PK studies	Long retention time, lowest accuracy	Clinical bioanalysis

Method 4, developed by Prajapati et al. (2022), incorporated Analytical-Failure Mode and Effect Analysis (AFMCEA) into the DoE for the development of metformin-based fixed-dose combination (FDC) products using reverse-phase high-performance liquid chromatography (RP-HPLC). This approach was both expeditious (little retention time) and environmentally sustainable. Still, its reduced theoretical plates and sensitivity compared to Method 1 render it somewhat less suitable for formulations necessitating high resolution. Nonetheless, its relevance to various APIs and focus on risk-based method validation render it a thorough, GMP-compliant strategy [54].
Method 5, established by Krishnan and Mishra (2020), is distinguished as the most accurate among the evaluated approaches. It attained the utmost accuracy (100.18%) and the minimal LOD/LOQ values. Although exhibiting a moderate retention duration, the implementation of monolithic columns and CCD tuning yielded elevated sensitivity and robustness. It met ICH validation criteria, rendering it optimal for high-precision quality assurance settings [41].

5.2. Electrophoretic Method (Capillary Electrophoresis)
Method 6, as delineated by Orlandini et al. (2014), employed a BBD-QbD strategy to construct a cyclodextrin-modified CE technique for metformin and its impurities. This technique exhibited excellent separation efficiency, brief duration (<9 min), and minimal solvent usage. The primary restriction was a little decrease in precision relative to HPLC-based approaches [61]. Nonetheless, its benefits in green chemistry and impurity profiling render it a significant alternative, especially for laboratories pursuing sustainable practices.

5.3. Bioanalytical Method (RP-HPLC for Plasma Samples)
Method 7, as described by Patel et al. (2021), concentrated on enhancing a protein precipitation (PP)-based RP-HPLC bioanalytical technique for metformin and alogliptin utilizing BBD. The study attained a recovery rate of 93.35%, exhibiting strong sensitivity and commendable linearity [52]. Nonetheless, it exhibited the greatest retention duration among all approaches, accompanied by marginally reduced precision. Its efficacy is rooted in clinical pharmacokinetic applications and conforms to USFDA bioanalytical method validation requirements, rendering it essential for in vivo studies.
Comparative studies indicate that Method 5 (Krishnan & Mishra) and Method 1 (Suryawanshi & Palled) are the most effective analytical procedures for metformin estimation. Method 5 delivers superior precision, but Method 1 presents a cost-effective and efficient alternative. Method 4 provides a feasible solution for high-throughput situations, whereas Methods 6 and 7 cater to particular requirements in impurity profiling and bioanalysis, respectively. Finally, the choice of approach must correspond with the analytical objective, regulatory framework, and resource accessibility.

6. FUTURE PERSPECTIVES AND RESEARCH GAPS
Future AQbD strategies should incorporate AMLM principles to support post-approval change management and real-time method monitoring. The integration of DoE with AQbD has markedly enhanced the analytical framework for metformin and its FDCs; nonetheless, various developing domains present additional prospects for innovation, optimization, and wider use. These future strategies emphasize the prospective enhancements of existing procedures and significant deficiencies that necessitate attention to conform to advancing pharmaceutical quality requirements. Few studies apply AQbD across full lifecycle stages. This review encourages future research in AI-DoE hybrids and AMLM-compliant validations.
A significant domain for future advancement is the broader implementation of green analytical chemistry (GAC) concepts. Current DoE-optimized HPLC methodologies, although efficient, frequently necessitate the utilization

of organic solvents such as methanol and acetonitrile, which present environmental and health hazards. Although integration of green solvents in DoE-based methods has been limited, it is increasingly recognized as essential for sustainable analytical practices. Future research should emphasize scalable, eco-conscious method development aligned with AQbD principles. Prajapati et al. (2022) and Orlandini et al. (2014) underscored the necessity for continued exploration of approaches integrating green chemistry, particularly in the pursuit of scalable and sustainable solutions [54; 61].

A further underexamined domain is the utilization of DoE in electrophoretic and hybrid methodologies, such Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Liquid Chromatography-Mass Spectrometry (LC-MS). Although CE is excellent for impurity profiling and low-volume sample analysis, its scalability and regulatory acceptance are constrained. The research conducted by Orlandini et al. (2014) established the viability of DoE in CE; nevertheless, limited subsequent investigations have applied similar methodologies to mass spectrometry platforms, which are essential for trace-level and metabolomic analyses [61]. This indicates a deficiency in converting refined CE procedures to high-sensitivity hybrid instruments necessary for modern pharmaceutical applications.

Moreover, there exists the possibility to amalgamate machine learning (ML) and artificial intelligence (AI) with DoE to improve predictive modeling of chromatographic reactions. While tools such as Design-Expert® and MODDE are utilized for modeling under specified experimental parameters, machine learning algorithms have the potential to evaluate extensive historical information to create dynamic, self-optimizing models. AI-augmented methodologies have been suggested in recent pharmaceutical technology literature but remain predominantly unexamined in metformin analytic workflows.

From a regulatory perspective, another essential domain for future advancement is analytical method lifecycle management (AMLM) [65]. Contemporary research frequently emphasizes technique creation and validation stages while overlooking ongoing performance assessment and post-approval adjustments. The lifetime approach, as recommended in ICH Q14 and Q12 standards, must be integrated into the AQbD framework to facilitate ongoing method enhancement while ensuring compliance [66].

Ultimately, a notable deficiency exists in the restricted practical implementation of these improved techniques inside regulatory submissions and commercial quality control laboratories. Although fully DoE-optimized approaches demonstrate stability and efficiency. in academic contexts, their adoption in industry is inconsistent. Addressing this gap necessitates cooperative endeavors among academic researchers, industry partners, and regulatory authorities to facilitate technology transfer, standardize validation processes, and educate personnel.

CONCLUSION

This review highlights the role of Design of Experiments (DoE) within Analytical Quality by Design (AQbD) in optimizing methods for evaluating metformin. Of the seven evaluated methods, RP-HPLC procedures utilizing Box-Behnken and Central Composite Designs exhibited superior sensitivity, accuracy, and adherence to regulatory standards. Electrophoretic and bioanalytical techniques provided environmentally sustainable and clinically pertinent alternatives, respectively. The analysis highlights the efficacy of DoE in improving technique robustness, minimizing variability, and adhering to ICH recommendations. This analysis highlights prospective potential for merging green chemistry, CE-MS platforms, and AI-assisted modeling, alongside the implementation of lifecycle management strategies to conform to changing regulatory requirements. This work provides a comparative assessment and identifies methodological deficiencies, serving as a valuable resource for academics, analysts, and regulatory experts seeking to establish dependable, efficient, and sustainable analytical techniques for metformin and its formulations..

STATEMENTS AND DECLARATIONS

The authors declare that they have no competing interests to disclose.

ABBREVIATIONS

T2DM Type 2 Diabetes Mellitus
NIDDM Non-Insulin Dependent Diabetes Mellitus
HPLC High-Performance Liquid Chromatography

RP-HPLC	Reverse Phase High-Performance Liquid Chromatography
CE	Capillary Electrophoresis
AQbD	Analytical Quality by Design
QbD	Quality by Design
DoE	Design of Experiments
BBD	Box-Behnken Design
CCD	Central Composite Design
RSM	Response Surface Methodology
CMP	Critical Method Parameter
CQA	Critical Quality Attribute
ATP	Analytical Target Profile
FDC	Fixed Dose Combination
AFMCEA	Analytical Failure Modes and Critical Effect Analysis
FMEA	Failure Modes and Effects Analysis
ICH	International Council for Harmonisation
FDA	Food and Drug Administration
LOD	Limit of Detection
LOQ	Limit of Quantification
SPE	Solid Phase Extraction
HILIC	Hydrophilic Interaction Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
CE-MS	Capillary Electrophoresis-Mass Spectrometry
QC	Quality Control
API	Active Pharmaceutical Ingredient
UV	Ultraviolet
PDA	Photodiode Array
PK	Pharmacokinetics
AMLM	Analytical Method Lifecycle Management
ML	Machine Learning
AI	Artificial Intelligence
GAC	Green Analytical Chemistry

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Authors contributions (CRediT)

Dharanagar Prateek Singh: Conceptualization, Investigation, Writing – original draft. **S. Shakir Bhasha**, Adabala Janardhanaswamy: Writing – review & editing, Resources. **K. Vinod Kumar**: Supervision, Writing – review & editing.

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The authors declare that they have no competing interests to disclose.

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CONSENT FOR PUBLICATION

All authors have reviewed the manuscript and consent to its submission for publication.

REFERENCES

1. Lima JE, Moreira NC, Sakamoto-Hojo ET (2022) Mechanisms underlying the pathophysiology of type 2 diabetes: From risk factors to oxidative stress, metabolic dysfunction, and hyperglycemia. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 874:503437
2. Joseph A, Thirupathamma M, Mathews E, Alagu M (2022) Genetics of type 2 diabetes mellitus in Indian and Global Population: A Review. Egyptian Journal of Medical Human Genetics 23 (1):135
3. Nanda M, Sharma R, Mubarik S, Aashima A, Zhang K (2022) Type-2 diabetes mellitus (T2DM): spatial-temporal patterns of incidence, mortality and attributable risk factors from 1990 to 2019 among 21 world regions. Endocrine 77 (3):444-454
4. Tinajero MG, Malik VS (2021) An update on the epidemiology of type 2 diabetes: a global perspective. Endocrinology and Metabolism Clinics 50 (3):337-355
5. Yasin MT (2024) A review on metformin for the treatment of type II Diabetes Mellitus. Brac University,
6. Samson SL, Garber AJ (2015) Metformin and other biguanides: pharmacology and therapeutic usage. International Textbook of Diabetes Mellitus:641-656
7. Tulipano G (2021) Integrated or independent actions of metformin in target tissues underlying its current use and new possible applications in the endocrine and metabolic disorder area. International journal of molecular sciences 22 (23):13068
8. Kalra S, Das A, Priya G, Ghosh S, Mehrotra R, Das S, Shah P, Bajaj S, Deshmukh V, Sanyal D (2020) Fixed-dose combination in management of type 2 diabetes mellitus: expert opinion from an international panel. Journal of Family Medicine and Primary Care 9 (11):5450-5457
9. Moka MK, Sriram DK, George M (2024) Recent Advances in Individualized Clinical Strategies for Polycystic Ovary Syndrome: Evidence From Clinical Trials and Emerging Pharmacotherapies. Clinical Therapeutics
10. Costa M The regulatory challenge of determining acceptable intakes for nitrosamine drug substance-related impurities while ensuring medicinal product supply. MasterarbeitzurErlangung des Titels" Master of Drug Regulatory Affairs, MDRA
11. Pund S, Nalawade S, Rajurkar V, Jayatpal S, Deshmukh N, Tare H (2024) A brief review on recent advances in reverse phase HPLC. Multidisciplinary Reviews 7 (4):2024072-2024072
12. Bhalerao A, Shelke MS, Borkar V (2023) Advances, Applications, and Challenges in RP HPLC Method Development. International Journal of Advanced Research in Science, Communication, and Technology 3:111-123
13. Wu L, Wang L, Huang C, Gu C, Tan W, Tian Y, Ma J (2024) Advances in the detection of emerging contaminant metformin: A critical review. Microchemical Journal:110422
14. AlThikrallah MK, Idris AM, Elbashir AA, Elgorashe RE, Buzid A, Alnajjar AO (2023) Development of capillary zone electrophoresis method for the simultaneous separation and quantification of metformin and pioglitazone in dosage forms; and comparison with HPLC method. Molecules 28 (3):1184
15. Mishra V, Thakur S, Patil A, Shukla A (2018) Quality by design (QbD) approaches in current pharmaceutical set-up. Expert opinion on drug delivery 15 (8):737-758
16. Lee S-H, Kim J-K, Jee J-P, Jang D-J, Park Y-J, Kim J-E (2022) Quality by Design (QbD) application for the pharmaceutical development process. Journal of Pharmaceutical Investigation 52 (6):649-682
17. Jankovic A, Chaudhary G, Goia F (2021) Designing the design of experiments (DOE)–An investigation on the influence of different factorial designs on the characterization of complex systems. Energy and Buildings 250:111298
18. Malenga EN, Mulaba-Bafubiandi A, Nheta W (2022) Application of the response surface method (RSM) based on central composite design (CCD) and design space (DS) to optimize the flotation and the desliming conditions in the recovery of PGMs from mine sludge. Separation Science and Technology 57 (18):2960-2983
19. Silva V, Cardoso JS (2024) Response Surface Methods-Theory, Applications and Optimization Techniques: Theory, Applications and Optimization Techniques. BoD–Books on Demand,

20. Özcan S, Levent S, Can NÖ (2023) Quality by design approach with design of experiment for sample preparation techniques. Advances in Sample Preparation 7:100079
21. Ortiz M, Sarabia L, Sánchez M (2023) The inversion of multiresponse partial least squares models, a useful tool to improve analytical methods in the framework of analytical quality by design. Analytica Chimica Acta 1276:341620
22. Panagiotidou E, Chountalas PT, Magoutas AI, Georgakellos DA, Lagodimos AG (2025) Systematic Identification and Validation of Critical Success Factors for ISO/IEC 17025 Implementation. Administrative Sciences 15 (2):60
23. Prajapati P, Rana B, Pulusu VS, 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
24. Tricht Ev, Sänger-van de Griend CE (2025) A Practical Approach to Implementing ICH Q14: Tools for Analytical Quality by Design in Capillary Electrophoresis Method Development. Electrophoresis
25. Marie AA, Salim MM, Kamal AH, Hammad SF, Elkhoudary MM (2022) Analytical quality by design based on design space in reversed-phase-high performance liquid chromatography analysis for simultaneous estimation of metformin, linagliptin and empagliflozin. Royal Society Open Science 9 (6):220215
26. Bairagi A, Kothrukar R, Chikhale H, Kosanam S, Borse L (2024) AQbD-novel strategy for analytical methods. Future Journal of Pharmaceutical Sciences 10 (1):138
27. Mehdizadeh Allaf M, Trick CG (2019) Multiple-stressor design-of-experiment (DOE) and one-factor-at-a-time (OFAT) observations defining Heterosigma akashiwo growth and cell permeability. Journal of Applied Phycology 31 (6):3515-3526
28. Chiarentin L, Gonçalves C, Augusto C, Miranda M, Cardoso C, Vitorino C (2024) Drilling into "Quality by Design" approach for analytical methods. Critical Reviews in Analytical Chemistry 54 (8):3478-3519
29. Gameiro BMS (2022) Lifecycle management of process analytical methods for pharmaceuticals quality control. Universidade de Lisboa (Portugal),
30. Patil SD, Chalikwar SS A brief review on application of design of experiment for the analysis of pharmaceuticals using HPLC. In: Annales Pharmaceutiques Françaises, 2024. vol 2. Elsevier, pp 203-228
31. Gedawy A, Al-Salami H, Dass CR (2024) Simultaneous HPLC of Metformin, Glipizide and Gliclazide, and Metformin Uptake and Protection of HEI-OC1 from Hyperglycaemia. Applied Sciences 14 (22):10469
32. Patel D, Kumar P, Sharma S, Dwivedi J (2017) Analytical methods for metformin estimation. Critical reviews in analytical chemistry 47 (5):405-417
33. Abdel-Aziz LM, Abdelkhalek AS, Antar A, Abdelraziq EH, Farghaly EA, Elsayed EM, Metwally EY, Khalil AY, Khalifa AT, Sebaiy MM (2023) The Methods of Analysis for Determination of Metformin and Glimepiride in Different Matrices. Journal of Nanotechnology and Nanomaterials 4 (1):1-10
34. Patel T, Patel V (2024) Thiazolidinediones: Recent Development in Analytical Methodologies. Journal of chromatographic science 62 (8):789-802
35. Vijayalakshmi M (2017) Development and Validation of Analytical Methods for Estimation of Metformin and Alogliptin in Combined Dosage Form and From Plasma. PSG College of Pharmacy, Coimbatore,
36. Eswarudu M, Ouchitya G, Reddy NS, Deekshitha M, Babu PS (2023) Review on Analytical Methods for Estimation of Antidiabetic Drugs: Empagliflozin, Linagliptin and Metformin Hydrochloride. Asian Journal of Pharmaceutical Analysis 13 (1):42-46
37. Suryawanshi SS, Palled MS (2023) A green chemistry approach to establish a conductometric technique for quantifying Metformin HCl in pharmaceutical samples and its greenness assessment using an analytical greenness metric calculator. Journal of Applied Pharmaceutical Science 13 (10):238-244
38. Wu X, Zhu B, Lu L, Huang W, Pang D (2012) Optimization of a solid phase extraction and hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the determination of metformin in dietary supplements and herbal medicines. Food chemistry 133 (2):482-488
39. Oertel R, Baldauf J, Rossmann J (2018) Development and validation of a hydrophilic interaction liquid chromatography-tandem mass spectrometry method for the quantification of the antidiabetic drug metformin and six others pharmaceuticals in wastewater. Journal of Chromatography A 1556:73-80
40. Tandrima M, Lakshmi S, Yasodha A Scholars Academic Journal of Pharmacy (SAJP) ISSN 2347-9531 (Print).
41. Krishnan B, Mishra K (2020) Quality by Design based Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Metformin in Bulk and Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Investigation 10 (4)
42. Müller AL, de Oliveira JA, Prestes OD, Adaime MB, Zanella R (2020) Design of experiments and method development. In: Solid-phase extraction. Elsevier, pp 589-608
43. Kekatpure V, Subramaniam N, Sunny S, Nambiar S, Sarah T, Vasudevan V, Rao A, Murali A, Kolur T, Krishnamurthy A (2024) Two by two factorial design using metformin and curcumin for second primary head and neck cancer prevention trial. Asian Pacific Journal of Cancer Prevention: APJCP 25 (6):1935

44. Suryawanshi SS, Palled MS (2022) Box-Behnken design assisted optimization and standardization of chromatographic methodology for quality assessment of metformin: analytical quality by design avenue. Indian J Pharm Educ Res 56 (2s):s152-s162
45. Shahbazi N, Zare-Dorabei R (2019) A facile colorimetric and spectrophotometric method for sensitive determination of metformin in human serum based on citrate-capped gold nanoparticles: central composite design optimization. ACS omega 4 (17):17519-17526
46. Randive KH, Maste MM, Kempwade AA (2024) Exploring Analytical Quality by Design (AQbD) Enabled RP-HPLC Method for Carvedilol. Ind J Pharm Edu Res 58 (2):651-660
47. Sopyan I, Gozali D, Kurniawansyah I, Guntina R (2022) Design-expert software (DOE): An application tool for optimization in pharmaceutical preparations formulation. International Journal of Applied Pharmaceutics 14 (4):55-63
48. Collins LM, Trail JB, Kugler KC, Baker TB, Piper ME, Mermelstein RJ (2014) Evaluating individual intervention components: making decisions based on the results of a factorial screening experiment. Translational behavioral medicine 4 (3):238-251
49. Alsadi J (2025) Optimization and Simulation of Extrusion Parameters in Polymer Compounding: A Comparative Study Using BBD and 3LFFD.
50. Bayuo J, Abukari MA, Pelig-Ba KB (2020) Optimization using central composite design (CCD) of response surface methodology (RSM) for biosorption of hexavalent chromium from aqueous media. Applied Water Science 10 (6):1-12
51. Vanaja K, Shobha Rani R (2007) Design of experiments: concept and applications of Plackett Burman design. Clinical research and regulatory affairs 24 (1):1-23
52. Patel H, Patel D, Christian J, Shah P, Patel K, Gandhi T (2021) Box-Behnken Design Assisted Protein Precipitation Optimization for Simultaneous Determination of Metformin Hydrochloride and Alogliptin Benzoate in Plasma along with Pharmacokinetic Application. Indian Journal of Pharmaceutical Sciences 83 (4):785-793
53. Tandel FB, Khandai M, Shah P, Panda DS, Patel VP (2024) Analytical quality by design. In: Introduction to Quality by Design (QbD) From Theory to Practice. Springer, pp 91-118
54. Prajapati PB, Mistry KY, Shah SA (2022) DoE-based analytical failure modes critical effect analysis (AFMCEA) to a multipurpose-RP-HPLC method for the estimation of multiple FDC products of metformin hydrochloride using an analytical quality by design approach. Journal of AOAC International 105 (4):986-998
55. Attimarad M, Elgorashe REE, Subramaniam R, Islam MM, Venugopala KN, Nagaraja S, Balgoname AA (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
56. Tome T, Žigart N, Časar Z, Obreza A (2019) Development and optimization of liquid chromatography analytical methods by using AQbD principles: Overview and recent advances. Organic process research & development 23 (9):1784-1802
57. Bandi S, Adikay S (2023) AQbD-based stability indicating development and validation of azilsartan medoxomil and cilnidipine by UPLC method. Journal of Applied Pharmaceutical Science 13 (5):095-106
58. Sundhani E, Lukitaningsih E, Nurrochmad A, Nugroho AE (2022) Potential pharmacokinetic and pharmacodynamic herb-drug interactions of Andrographis paniculata (Burm. f.) and andrographolide: A systematic review. Journal of Herbmed Pharmacology 11 (2):154-165
59. Sha'at M, Spac AF, Stoleriu I, Bujor A, Cretan MS, Hartan M, Ochiuz L (2022) Implementation of Qbd approach to the analytical method development and validation for the estimation of metformin hydrochloride in tablet dosage forms by HPLC. Pharmaceutics 14 (6):1187
60. Khan MZ, Yousuf RI, Shoaib MH, Ahmed FR, Saleem MT, Siddiqui F, Rizvi SA (2023) A hybrid framework of artificial intelligence-based neural network model (ANN) and central composite design (CCD) in quality by design formulation development of orodispersible moxifloxacin tablets: Physicochemical evaluation, compaction analysis, and its in-silico PBPK modeling. Journal of Drug Delivery Science and Technology 82:104323
61. Orlandini S, Pasquini B, Gotti R, Giuffrida A, Paternostro F, Furlanetto S (2014) Analytical quality by design in the development of a cyclodextrin‐modified capillary electrophoresis method for the assay of metformin and its related substances. Electrophoresis 35 (17):2538-2545
62. Santhanam MK, Nagarajan NC, Ponraj PB, Mohamed Hilurudeen MS (2023) A complete roadmap of analytical quality by design in various analytical techniques. Current Pharmaceutical Analysis 19 (3):184-215
63. Aboushady D, Samir L, Masoud A, Elshoura Y, Mohamed A, Hanafi RS, El Deeb S (2025) Chemometric Approaches for Sustainable Pharmaceutical Analysis Using Liquid Chromatography. Chemistry 7 (1):11
64. Kalpana P, Raj B, Ritika P, Devang T, Rajesh P, Tejal G (2025) Green metrics-based HPLC method for quantification of three antidiabetic drugs in tablet dosage form using Box–Behnken design. Accreditation and Quality Assurance 30 (2):141-152

65. Volta e Sousa L, Gonçalves R, Menezes JC, Ramos A (2021) Analytical method lifecycle management in pharmaceutical industry: a review. Aaps Pharmscitech 22:1-14
66. Verch T, Campa C, Chéry CC, Frenkel R, Graul T, Jaya N, Nakhle B, Springall J, Starkey J, Wypych J (2022) Analytical quality by design, life cycle management, and method control. The AAPS Journal 24 (1):34