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Review Article

Quality by Design in RP-HPLC Method Development: Optimizing Performance and Robustness

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ABSTRACT

Quality by Design (QbD) is a science-based approach to method development that is built around the concept of designing in quality with crosstalk into all stages of the product lifecycle. In this review we discuss the use of QbD principles in RP-HPLC for Design space optimization and increasing both the performance and the robustness. The QbD framework includes the following steps: identification and definition of Quality Target Product Profile (QTPP), determination of Critical Quality Attributes (CQAs) critical method parameters (CMPs), risk assessment (FMEA), method optimization (Design of Experiments [DoE]), establishment of method-operable design region (MODR), control strategy development, method validation, and continuous method monitoring. For method optimization, experimental design techniques such as Box-Behnken and central composite face designs have been used. Pharmaceutical industry experienced successful case studies of QbD in RP-HPLC method development which resulted in better performance, robustness, and efficiency of the method. In the future, processes will be governed by advanced technologies integrated into ultra-high-performance liquid chromatography (UHPLC) combined with process analytical technology (PAT) and quality by design (QbD) principles. Note: on the need to enhance QbD, the agencies are actively pushing the adoption of QbD in analytical method development. However, barriers concerning cost, training, and allocation of resources should be tackled to facilitate large-scale deployment. These principles, when applied to RP-HPLC, facilitate a more robust, flexible and regulatory-compliant methodology.

INTRODUCTION

Overview of RP-HPLC

RP-HPLC is a high-throughput analytical method that we commonly use in pharmaceutical drug development and manufacturing. It a key part of the quality assurance of bulk drugs and pharmaceutical formulations and the analysis of drugs in biological specimens (Yabré et al., 2018). RP- High performance liquid chromatographic method (HPLC) is a technical

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tool used for the separation & determination of compounds based on the differences of their hydrophobicity by using the nonpolar stationary phase & polar mobile phase. RP-HPLC is performed with C18 column as the stationary phase and a mobile phase comprising a mixture of water and organic solvents (acetonitrile or methanol) (Jadhav et al., 2017; Shamim et al., 2023). Due to its high resolution, sensitivity, and reproducibility, these features make the technique ideal for analytical method development. It has also been utilized for the simultaneous analysis of several drugs (Shamim et al., 2023) and quantitation of steroidal saponins from crude plant material (Jadhav et al., 2017) as well as the estimation of impurities of drug (Ganzera et al., 2001). RP-HPLC, for example, is a very powerful technique, but has some downsides as well, especially related to the use of large volumes of organic solvents, which is environmentally unfriendly. Green RP-HPLC methods are now analyzed by replacing toxic organic solvent with greener solvent or 100% aqueous MP (Yabré et al., 2018). Even so, RP–HPLC is still widely used for the development of analytical methods and applicable for the efficient analysis of a varied array of analytes from small, low-molecular-weight drugs to peptides and proteins (Tarr & Crabb, 1983).

Method Development A Short Introduction

In industries such as pharmaceutical and biotechnological, analytical chemistry techniques and their applications are used, which serve as the foundation of quality control, product characterization, and compliance with regulatory requirements. This covers the development, validation and servicing of the analytical procedure to determine that accurate and OK assessments of the items of interest, potentially ions, macromolecules, or biological tests, in complicated mixtures. Method development plays a key role in the pharmaceutical industry to guarantee the quality, purity and effectiveness of drugs. The use of computational tools and the application of specific artificial intelligence methodologies have dramatically accelerated the process to discover and/or develop drug candidates, which may lead to greater numbers of recently approved drugs in the near future (Olmedo et al., 2024). Advanced analytical techniques including biotechnological approaches yield critical information on biological activity, clinical efficacy, and safety, all of which constitute key components of drug quality (Chen et al., 2022). The development of smart nanomaterials prior to this in pharmaceutical analysis due to their excellent thermal, electronic, optical, and mechanical properties (Sharma & Hussain, 2018). These materials enable potential improvements of analytical methodologies used and approaches to address shortcomings in drug product quality assessment. Also, novel particle analysis techniques will be needed to distinguish particulate active pharmaceutical ingredients from impurities, especially for new product classes (Roesch et al., 2021). Method development is indispensable for the pharmaceutical and biotechnological industries as it permits the correct characterization, quality control and regulatory compliance. Various advanced technologies (e.g., artificial intelligence, biotechnological releasing strategies, and smart nanomaterials) have been utilized over the years to dramatically improve analytical methods. In addition, the adoption of Process Analytical Technology (PAT) and Quality by Design (QbD) principles has advanced real-time product release, shortening cycle time and production costs, in addition to guaranteeing product quality (Gerzon et al., 2021).

Introduction to Quality by Design (QbD)

Quality by Design (QbD) is a systematic approach to pharmaceutical development, emphasizing quality built-in, and not tested in. Analytical Quality by Design (AQbD) was suggested in 2010 as a process which applied this paradigm in analytical method development (Bastogne et al.,

2022). Similar to the concept vested within the pharmaceutical industry (Bastogne et al., 2022), this analysis strategy minimses risk from key variables at each step including sample prep, instrument and statistics providing an opportunity for validation of the predictive power and robustness of analytical results by adopting aggergate quality by design (AQbD). In the context of method development, the implementation of AQbD can be viewed as an extension of the risk-management principles provided in ICH Q9 as applied to methods in the pharmaceutical quality system (ICH Q10) (Peraman et al., 2015). Critical to developing a method operable design region (MODR) to enable to the analytical method to be altered and not influence performance. Due to its method robustness, it is one of the approach to reduce out-of-trend (OOT) and out-of-specification (OOS) (Peraman et al., 2015). AQbD is composed of several concepts and tools: Identify the Analytical Target Profile (ATP) and Critical Quality Attributes (CQAs), a risk assessment, method optimization (Design of Experiments (DoE)), the MODR, the control strategy, method validation and continuous method monitoring (Raman et al., 2015). The amalgamation of AQbD, QbD, will enhance product quality, mitigate and mitigate risk, provide major inputs for process analytical technology (PAT) processes (Raman et al., 2015).

Purpose of the Review

The Quality by Design (QbD) principles established in this work are very reliable and can be effectively employed to optimize the performance characteristics and robustness of RP-HPLC for pharmaceutical applications.

Therefore, the use of QbD for RP-HPLC method development has been widely studied . For example, an RP-HPLC method for simultaneous estimation of ciprofloxacin hydrochloride and rutin was developed using box-behnken design and demonstrated better analysis quality with lesser number of experimental runs (Shamim et al., 2023). In another case, a response surface design of experiments approach was utilized to investigate the significance of the critical method parameters and their relationship with critical method attributes for developing the stability-indicating RP-HPLC method for linagliptin and its related substances (Jadhav et al., 2017). Similarly, response surface design and Monte Carlo simulation were used to define the design space (Tome et al., 2020) for an HPLC method to quantify process-related impurities in celecoxib based on the Analytical Quality by Design (AQbD) approach. Analyses that incorporate QbD principles have begun to expand beyond method development into the realm of environmental impact in pharmaceutical analyses. "HPLC method greening" is an increasing popular paradigm to promote the reduction in the usage of toxic and environmental hazardous solvents as well as increasing operational safety (Yabré et al., 2018). Overall, QbD principles have been successfully applied to RP-HPLC method development for pharmaceuticals, leading to better method performance, robustness and efficiency. Through this scientific investigation, however, we not only try to optimize some analytical methods but also establish more environmentally sustainable practices in pharmaceutical R& D and quality control of Active pharmaceutical ingredients.

2. Principles of Quality by Design (QbD) (1.5 pages)

Definition and Key Concepts

This approach is referred to as Quality by Design (QbD), which focuses on the understanding of the product and process as a tool for delivering consistent quality of the product. A risk-based proactive methodology is introduced by the FDA to promote the flexibility of manufacturing and system understanding (Bastogne et al., 2022; Laky et al., 2019). These are the principles of QbD:

Quality Target Product Profile (QTPP): This defines the critical quality attributes (CQAs) of the

drug product, thus laying the groundwork for development (Yu et al., 2014). Design Space: This multidimensional space of input variables and process parameters, for which product quality is guaranteed. This enables operational flexibility within a tolerated range (Laky et al., 2019).

Process Understanding: The understanding of how the critical material attributes (CMAs) and critical process parameters (CPPs) are related to the CQAs. They include risk assessment, mechanistic models, design of experiments (DoE) and process analytical technology (PAT) tools (Su et al., 2019; Yu et al., 2014). (The other two points have been added later)Risk Management: Quality by design involves risk management to assess and control important parameters throughout the development, (Jayagopal & Murugesh, 2020; Pallagi et al., 2019) Control Strategy: these appear from specifications for drug substances, excipients, final products and control of each step of the manufacture (Yu et al., 2014). Continuous Improvement: QbD promotes continuous improvement via process capability evaluation (Su et al., 2019; Yu et al., 2014).

In fact, the QbD concept has also been applied to analytical method development under terms Analytical Quality by Design (AQbD) to improve accuracy and robustness of the quality of the results obtained from analytical methods (Bastogne et al., 2022). To summarize, QbD is a transformational change in pharmaceutical development from a rigid empirical approach to a flexible knowledge-based strategy. The incorporation of QbD principles allows pharmaceutical companies to improve product quality, accelerate development time lines, whilst ensuring that regulatory requirements are met in a cost-effective manner (Kovács et al., 2021; Pedro et al., 2023).

QbD versus Traditional Method Development

Quality by Design (QbD) was a foundational shift away from standard method-development approaches in pharmaceutical R&D. Compared to the empirical optimization methods and trial-and-error approaches, quality by design (QbD) is a proactive, risk-based, systematic methodology aiming to provide control and design of the entire development chain to guarantee product quality (Bastogne et al., 2022; Kovács et al., 2021).

Conventional methods generally involve one-factor-at-a-time experiments or small factorial designs^[5^], resulting in lengthy and expensive method development^7. Traditionally, the combination of parameters in the previously described study plan was investigated, but in QbD, multivariate design of experiments (DOE) with statistical analysis is employed to quickly study the multidimensional space of critical process parameters and interactions between them (Gurba-Bryśkiewicz et al., 2023; Pallagi et al., 2019). This also helps in gathering more information on the process and its effect on critical quality attributes. An important difference is the QbD approach that focuses on designing a quality product instead of performing a test (quality assurance) at the end of the process. This highlights the importance early in development to define what the quality target (substance) QTPP and critical quality attributes (CQAs) should be (Kovács et al., 2020; Kovács et al., 2021). By getting more responsive to this control dial early, we identify potential sources of variability enabling improving methods robustness. Additionally, risk assessment tools that assist in identifying the critical attributes and guiding the design of experiments, such as the failure mode effect analysis (FMEA) are executed in the QbD approach Jayagopal & Murugesh, (2020) In conclusion, although the traditional methods apply simultaneously and end up producing valid processes, QbD offers an organized, effective, and suitable data-based way of performing method development. Thus allows to improved overwiew of the entire process, better quality of the product and even increased flexibility from the regulators perspective (Luciani et al., 2015; Peraman et al., 2015). The QbD approach also conforms to regulation as increased method robustness may

lead to lower out-of-trend and out-of-specification results (Peraman et al., 2015).

QbD In Method Development: Benefits

Benefits of QbD in method development: Method robustness, reliability, and reproducibility.
Adoption of a QbD approach to the development of analytical methods, known as Analytical Quality by Design (AQbD), leads to a method adjustment that takes place in an operable design region (MODR) of a particular method, which significantly reduces the occurrence of out-of-trend (OOT) and out-of-specification (OOS) results that are otherwise obtained as a consequence of method variability, as a benefit of enhanced robustness of the method using this methodology (Peraman et al., 2015). It also increases for this analytical method reproducibility by validating that the analytical method continues system condition-variable. The QbD approach provides more information about the critical process parameters and their interactions with critical quality attributes during the method development. This gives manufacturers more versatility for a less wasteful approach to manufacturing without sacrificing product quality (Laky et al., 2019). Moreover, in a framework of formulation development project like liposomes, the application of QbD principles improves the formulation process as well as increases the success of product preparation and reduces the number of experimental projects (Pallagi et al., 2019). Takeaway: The application of QbD principles to the method development process increases method robustness and reliability, as well as enables a systematic approach to understanding and controlling critical contributors. That time will result in better development processes, better products, and better outcomes for patients. As you gain a better understanding of critical quality attributes, risk management, and continuous improvement (Pedro et al., 2023), QbD principles support your organizations in meeting regulatory requirements.

3. QbD Framework for RP-HPLC Method Development

Development of analytical methods can be either a scheduled event or a rush event, however, the Quality by Design (QbD) approach is one such approach that is successfully implemented for developing robust and efficient reverse phase high performance liquid chromatography (RP-HPLC) methods for the analysis of various pharmaceuticals and natural products. Column temperature, composition of mobile phase, and pH were classified as critical method parameters (CMPs) which were optimized initially in the preparation of RP-HPLC methods following the principles of QbD, so as to produce the targeted critical method attributes (CMAs) such as resolution of the peaks, time of retention and theoretical plates (Mohammed et al., 2023). Method development, validation and robustness evaluations were performed using factorial experimental designs. Thank you for your answer, very helpful. It is important to point out that the second stage of the QbD approach was applied for the design of green RP-HPLC methods using less harmful solvents (e.g. ethanol and aqueous mobile phases) or ionic liquids (Yabré et al., 2018) instead of dangerous solvents. This has been done as the awareness of the environmental impact of analytical methodology in pharmaceutical analysis is increasing. Thus, the QbD principles provide a scientific approach which can develop RP-HPLC methods and it can provide a robust, accurate and reproducible method. This includes identification of analytical target profiles, assessment of critical quality attributes, methods development using design of experiments, establishing method-operable design spaces and implementation of control strategies (Raman et al., 2015). This not only reduces qquality and risk to methods, it also satisfies regulatory expectations and makes it easier to further develop methods continuously.

Specifying the Quality Target Product Profile (QTPP)

The initial critical element for QbD based pharmaceutical Product development is quality target product profile (QTPP). This is known as a list of quality attributes that must be included in the final product (Namjoshi et al., 2020). The Quality Target Product Profile (QTPP) describes the CQAs (Key Quality Attributes) of a drug product required to ensure qualification of safety, efficacy and quality (Yu et al., 2014).

The QTPP varies from product to product. E.g., drug product development objective for topical semi-solid products where the QTPP includes physiochemical properties, stability characteristics, and performance attributes (Chang et al., 2013; Namjoshi et al., 2020). The QTPP for microneedles includes penetration capability (if applicable, dissolves) or the dissolution and local efficacy (Sartawi et al., 2023). The QTPP for nanostructured lipid carrier includes size, surface charge (zeta potential), entrapment efficiency and in vitro release (Beg et al., 2017). The most important activity in QbD is defining the Quality Target Product Profile (QTPP) because this dictates the whole product development plan. CQAs are likewise employed in guiding the de-fining of related critical material attributes (CMAs) and critical process parameters (CPPs) (Raman et al., 2015; Yu et al., 2014). It is done as needed and output product quality is as per required.

Critical Method Parameters (CMPs)

RP-HPLC optimization is imperative to optimize and separate analytes at the best possible use during detection.CMPs. Several studies have characterized and refined various CMPs to enhance the method's functionality and robustness. The critical parameters considered during the development of RP-HPLC methods are mobile phase composition, column type, flow rate, temperature, etc. For celecoxib impurity analysis, central composite face response-surface design was used along with a CMP-based approach which was informed using ratios of acetonitrile in the mobile phase, flow rate and columnn temperature (Tome et al., 2020) wherein it was concluded that this state of cellular proliferation made cell density division impossible. Likewise, the mobile phase solvent percentage ratio, pH and column temperature of the mobile phase were defined as the analysis CMPs for curcuminoids (Mohammed et al., 2023). The appropriate stationary phase also plays an important role, for example the application of immobilized Chiralpak IA-3 column in RP mode, yielded a better resolve and separation of celecoxib impurities (Tome et al., 2020). Even minor changes in CMPs have been proven to create substantial divergences in method performance (interesting, right?). In fact, special mobile phase composition is deemed necessary to obtain very low limits of detection for the determination of the renin inhibitor CP-80,794 in HPLC (Fouda et al., 1991). Gradient elution is used to separate the other three which adopts using gradient elution for multiple components and performed at the optimum wavelength of the particular impurities (Buck, 2013, Jadhav et al., 2017).

CONCLUSIONS: The selection and optimization of CMPs is a crucial way in developing a better RP-HPLC method with robustness and efficiency. Screening of the effects of CMPs on the performance of a method can be accomplished by experimental designs such as Box-Behnken or central composite face response-surface designs (Mohammed et al., 2023; Shamim et al., 2023; Tome et al., 2020). Hence these factors need to be tuned optimally to operationally maximise selectivity, resolution and sensitivity of the RP-HPLC used by researchers.

Understanding the Risk and Impact on Offerings

New step by step map and unique schedule to Failure Mode and Effects Analysis (FMEA) Failure Mode and Effects Analysis (FMEA) is a method of assessment of risk that has been applied across industries to discover, estimate and mitigate

possible failure modes in the products, process and systems (Aguirre et al., 2021; Chang et al., 2019). FAT is a systematic tool to assess the risks and the impact, so it can increase organization reliability, safety, and quality. A conventional way of FMEA implements the Risk Priority Number (RPN) for risk assessment, which is constrained by uncertain data computation and subjective expert measurements (Lv et al., 2019; Yucesan et al., 2021). To counter these challenges, the researchers proposed some modifications in FMEA methodology. Hybrid approaches incorporate MADM methods such as rough best worst method (R-BWM) and rough technique for order preference by similarity to an ideal solution (R-TOPSIS) with FMEA to enhance the accuracy of risk assessment (Chang et al., 2019). A recent approach to mitigate uncertainties in failure data and to model dependencies between failure events is the combination of fuzzy set theory and Bayesian networks (Yucesan et al., 2021) The integration of Game Theory principles and BWM for multi-criteria decision making enhances the accuracy of risk evaluation process in complex systems (Yazdi, 2023). Utilization of the D numbers theory to model non-exclusive fuzzy evaluations and create a multi-sensor information fusion approach for risk assessment (Deng & Jiang, 2017; Liu & Deng, 2019). The latest advanced FMEA methodologies provide stronger and clearer outputs to aid the decision maker and R&D department in making multimillion dollar decisions regarding product failure rates and system safety. Although the Design of Experiments (DOE) does not appear in the given context, it is still necessary to mention it as another method that could also be used in addition to the FMEA for optimization of the method parameters. In summary, the utilization of advanced mathematical and decision-making techniques has been applied to enhance the effectiveness of the regular FMEA through its combination within integrated FMEA for risk assessment and control. These enhanced methodologies offer organizations more precise and trustworthy methods to discover, rank, and manage potential threats across a wide range of sectors and uses.

Design Space

RP-HPLC method development can be visualized through input variables and process parameters that reside in a multidimensional design space that has been demonstrated in favor of quality assurance. One of the key decisive principles is integrated into Analytical Quality by Design (AQbD) initiative (1) to develop a robust and reproducible analytical method. The design space aids in understanding method robustness, by exploring the relationships between critical method parameters (CMPs) and method performance. For instance, Tome et al. (2020), stated the ratio of acetonitrile of the mobile phase, flowrate, and temperature of target column as critical manufacturing parameters (CMPs) and specified them by a corresponding composite axial face response-surface design. That is, the mathematical models are able to define the factor-response relationships and improve understanding of the influence of these parameters on the performance of the system (Tome et al., 2020).
Experimental designs and statistical tools are commonly employed to establish a design space. Marie et al. (2023) screened CMPs using a fractional factorial design (FFD) followed by a Box-Behnken design to obtain the MODR. Thus, it enables systematic interrogation of parameter interactions and impact, both method performance and usability robustness (Marie et al, 2023).
To summarize, The method design space is a graphical or quantitative illustration of the acceptable values for the method parameters that continuously yield favourable outcomes. This not only ensures robustness of methods but also flexibility in performing methods in designated space, thus potentially minimizing regulatory post-approval changes (Alanazi et al., 2023; Elkady et al., 2022).

4. Experimental Design and Method Optimization

Adaptive optimal experimental design methods utilize existing data and outcomes to inform the selection and design of future experiments, prioritizing active reporting regions of interest (Wilkinson et al., 2015) and behavioral and neural sciences (Kim et al., 2014). The goal is to extract as much information as possible with as few observations or experiments as possible. Fun fact: many optimization methods assume you have perfect control and reproducibility over your experimental conditions, which is not always the case in practice however! Golem, an algorithm developed by (Aldeghi et al., 2021) addresses this challenge, identifying optimal solutions that are robust to input uncertainty, and thus guarantees the reproducible performance of optimized experimental protocols and processes. The value of this approach is especially relevant for experiments where there is significant noise present in the conditions/processes of the experiment, and where they could potentially be deployed under different operating conditions in the future. As a final point, effective experimental design techniques can drastically enhance efficiency and impact to many scientific fields. Sequential optimization methods (Lei et al., 2008), Bayesian methods (Kim et al., 2014; Lei et al., 2021), and robust optimization approaches (Aldeghi et al., 2021) are among these novel solution methods. There are many DE approaches, but the specific application leads to the choice of the appropriate one because some functions are more informative than others based on the dimensionality of the design space, the smoothness of the objective functions, and whether there is uncertainty in the inputs (e.g., parameter uncertainties in process and design).

Design of Experiments (DoE)

RP-HPLC Method Development Using Design of Experiments RP-HPLC method development entails a systematic approach to optimizing various experimental conditions. In the RP-HPLC context, DoE was utilized to systematically investigate the effect of critical method parameters on chromatographic performance and obtain a robust method in the design space. For example, in the development of an RP-HPLC method, response surface methodology was used, the Box-Behnken design was applied to the optimization of ciprofloxacin hydrochloride and rutin separation (Shamim et al., 2023). By using fewer runs, statistically significant values are obtained, enhancing the quality of analysis and allowing testing factors and responses for exploration of their inter-relationships. On the other hand, a central composite face response-surface design can be utilized in the method development, as it was done in celecoxib impurity determination by RP-HPLC (Tome et al. 2020). This design was used to explore key method parameters, including acetonitrile content of mobile phase, flow rate and column temperature. Using the experimental data fitted the mathematical models with multiple linear regression and established factor-response relationships with Monte Carlo simulation to define the design space. In conclusion, DoE is the systematic way with which we would develop an RP-HPLC method in terms of identifying the critical parameters, their optimized settings and a design space wherein the method will be robust. By providing quantitative information about all relevant parameters in the system, their optimized conditions as well as the effect on the method, this approach not only improves the method performance, but also allows for deeper understanding within the chromatographic system and thus more reliable and reproducible analytical methods.

Factors Influencing RP-HPLC Method Performance

Column chemistry is a direct interpreter for separation efficiency. Some frequently used columns, like C18 for many analytes (Carranco et al., 2018; Sarmento et al., 2006), versus specialized ones, e.g. polysaccharide based columns provide unique selectivity for certain

compounds (Dobó et al., 2024). In which the column impacts analyte retention, selectivity, and resolution. The mobile phase composition had a great influence on the separation. Typical buffers are aqueous buffers with organic modifiers (e.g. Acetonitrile, Methanol) (Carranco et al., 2018; Li et al., 2018; Sarmento et al., 2006). In addition, by tuning the pH of the mobile phase also enhances the resolution/peak shape for ionizable species (Li et al., 2018; Shamim et al., 2023); Retained fractions and ratios between the retained fractions (the ratio of water to phosphate) also play a role in selectivity and retention (water vs organic).

The science behind the principles of gradient elution has played a part in the development of many of the modern means of separation of difficult mixtures. This approved enhances the resolution of compounds with different polarities and also publish the analysis time (Carranco et al., 2018; Li et al., 2018; Sarmento et al., 2006). The gradient profile (the rate of change in the mobile phase composition) also impacts the shape and resolution of the peaks. The temperature affects the separation, because the changes in temperature cause differences in retention time and selectivity (Dobó et al., 2024; Li et al., 2018). Interestingly, when it came to these factors but the studies mentioned experienced surprising phenomena. For example, (Dobó et al., 2024) reported on the hysteresis of retention times and enantioselectivity in the organic polar to reversed-phase transition on amylose-type columns. So the composition of the mobile phase is a pnma=.ise(cn that structures the column, radion separation. RP-HPLC is optimally performed by the suitable selection of column chemistry, mobile phase composition and gradient elution and temperature. These aspects are inter-related and demanding systematic method development strategies (e.g. quality-by-design (Shamim et al. 2023)) for the optimum separation and quantification of analytes.

Optimization Techniques

This goes on until we reach some ideally optimal value returned by our solution, dataset or simulation system. Statistical methods and tools are employed to develop protocols to estimate the interaction between input parameters and output responses through systematic derived experiments, which helps calculate and optimize process parameters (Veza et al., 2023). RSM has proved to be a potent tool in optimizing chemical and biological processes and in obtaining the most costeffective options of environmentally friendly and sustainable methods (Zaid et al., 2022).

RSM has the fundamental benefits that it can optimize multiple objectives at the same time. For example, in the area of incremental sheet forming, RSM has been combined with genetic algorithms to optimize the forming angle and thickness reduction of AA5052 sheets (Xiao et al., 2020). Compared to other applications, similar RSM has been used in the optimization of many performance metrics, including cogging torque, average torque, torque ripple, back-EMF harmonics, etc. in surface-mounted and interior PM synchronous motors as well (Si et al., 2018).

However, RSM has its own limitations. As the problem becomes larger, however, the number of necessary experimental runs increases exponentially, making it time- and resource-prohibitive (Tachibana et al., 2023). Although RSM is the most ubiquitous, it is not the only such design that has been considered; in fact, other types of experiment design have been studied by researchers such as Bayesian optimization algorithms, which have been demonstrated up to many orders of magnitude more efficient than RSM on certain problems (Tachibana et al., 2023). Furthermore, RSMs were susceptible to experimental noise, and approaches such as coefficient clipping were developed to make them more robust (Kim et al., 2023). So RSM is a great multivariable optimization tool that comes with its own timing and a drawback, but people have been trying to find faster and more effective methods. These include leveraging machine learning methods—including Gaussian process

regression (Tang et al., 2009)—and fitting the RSM to observational data (Hadiyat et al., 2022). These methods are surfacing as powerful problem-solving tools that are rapidly evolving for more complex optimizations, and will be determinant in the evolution of many applications, ranging from biofuel production to wind-generator design (Asef et al., 2018).

5. Method Validation and Performance Characterization (1.5 pages)

Validation Parameters

Several studies in this regard have reported some details about validation of RP-HPLC methods, some of the important parameters being accuracy, precision, specificity, linearity, range and robustness. Recovery studies are typically used to establish bias for example by spiking know levels of analytes in the samples. An example is (Siddique et al., 2023), recovery values ranging from 98.44% to 99.96% for Eletriptan hydrobromide and Itopride hydrochloride (Siddique et al., 2023). Precision was determined by repeatability (intraday) and reproducibility (inter-day) studies as per (Nakurte et al., 2012) 4 and (Kim et al. (2016), and Nakurte et al. (2012).
In the presence of other components, specificity guarantees that the technique is able to accurately quantify the analytes. This is often illustrated by the selectivity studies (Dewani et al., 2013) (Dewani et al., 2013). Linearity was evaluated by generating calibration curves across various concentration ranges. The linear calibration curves ($r > 0.999$) of aflatoxins, ochratoxin A, and zearalenone have been reported by Rahmani et al., 2010 (Rahmani et al., 2010). The applicable concentration range of the method is defined by the lowest and higher concentrations of the analyte for which the precision, accuracy and linearity is previously established. Robustness tests how reliable the method remains under purposely perturbations of the method parameters. (Abd El-Hay et al., 2016) described their method development without an explicit mention of validating robustness as a component of their development process (Abd El-Hay et al., 2016).
These are the validation parameters that are essential for the reproducibility and reliability of RP-HPLC methods across laboratories and conditions. This is accompanied by just two short studies demonstrating the need to validate these parameters according to ICH and FDA, in addition to the presented results (Kim et al., 2016; Shamim et al., 2023).

Importance of Robustness

The validation of analytical and pharmaceutical methods on the whole partially depends on the methodology robustness approach which helps establish that the method is robust and will consistently deliver temporally changing conditions. The systematic identification and control of critical parameters lead to enhanced robustness as per Quality by Design (QbD) principles. However, the maintenance of the reliability and accuracy of analytical results requires robustness. This ensures that minuscule yet intentional differences in method parameters do not heavily impact the performance of the method. QbD approaches(QbD (Jovanović et al., 2015)), including the design of experiments (DoE) methodology to develop links between critical process parameters and critical quality attributes. This step helps to establish a strong design space in which the method performance is relatively invariant to small changes in the operating conditions (Jovanović et al., 2015). QbD principles involve a detailed understanding of critical quality attributes, risk management based on understanding of the process, and continuous improvement which helps in enhancing robustness (Pedro et al., 2023). Horizontal models of uncertainty account for the above assumptions due to its robustness characteristics where benefits of loss function such as Taguchi loss functions and other robustness criteria into the stochastic optimization formulations can yield optimum designs meeting the observed processes with

resilient operating policies that optimize expectation of the process performance (Bernardo et al., 2001). Furthermore, Analytical Quality by Design (AQbD) has been adopted as part of the methodology to maximize the accuracy and robustness of the analysis results via the identification and control of critical analytical variables and method parameters throughout the whole protocol (Bastogne et al., 2022). Ultimately, the robustness of analytical methods is indispensable to control the reliability and consistency of the methods. QbD approaches provide a systematic framework for method development where risk assessment is performed and a design space within which the method performs robustly is determined, significantly contributing to robustness of the developed method. Overall, such an approach not only improves the quality of analytical outcomes, but also minimizes the probability of OOT and OOS, ensuring that pharmaceutical processes are more reliable and darn-strict (Peraman et al., 2015).

Case Studies and Examples

Various studies have been employed successfully to implement the quality-by-design (QbD) principles to establish and validate RP-HPLC methods for pharmaceutical analysis.

In this work, we report a novel RP-HPLC method developed using a quality by design (QbD) approach for the simultaneous estimation of ciprofloxacin hydrochloride and rutin. Here we use a Box-Behnken approach to develop a statistical analysis that optimized the factors and responses to gain statistical significant and quality-enhanced analysis. Validation of the developed method was performed in compliance with ICH Q2 R(1) guidelines, and all validation parameters were found to be within acceptable ranges (Shamim et al., 2023). Also, in another work, quality by design (QbD) was integrated with Green Chemistry's principles, where the aim was to develop a comparative study between the two respective methods — a green RP-HPLC method for the quantitative determination of impurities in artesunate and amodiaquine drugs. The green solvent used in this study was ethyl alcohol. The critical method attributes and parameters were determined, with a three-level full factorial design applied to optimize the method. This developed method was validated using an accuracy profile methodology and met the ICH Q2(R1) requirements (Yabré et al., 2020). A stability-indicating high-performance liquid chromatography (HPLC) method was developed and validated by adopting Quality by Design (QbD) principles for the simultaneous quantitative determination of curcuminoids in various formulations. Factorial experimental designs were used for the method development, validation and robustness evaluation. They are indicating their specificity, linearity, precision, and accuracy were within an acceptable range and such an approach is emphasizing the QbD application in method development for an improved analytical detection and quantification method (Mohammed et al., 2023). Overall, these case studies demonstrate, through the principles of QbD and experimentation, a pathway to developing and validating RP-HPLC methods that are robust for analytical use in the pharmaceutical industry.

6. Implementation Of QbD In RP-HPLC (Case Studies)

Industry Examples

RP-HPLC method development in a pharmaceutical analysis context has been effectively accomplished by utilising these same quality by design (QbD) principles and while those concerns are primarily not relevant here, general empirical experience suggests they work.

One of such examples is, an RP-HPLC (stability-indicating) method for the simultaneous determination of curcuminoids in extracts of Curcuma longa, tablets, capsules, and forced degradants was developed (Mohammed et al., 2023) A factorial experimental designs was used for the method development, validation, and

robustness evaluation. Critical method attributes (CMAs) were defined as peak resolution, retention time, and theoretical plate number while the derived critical method parameters (CMPs) were mobile phase composition, pH, and column temperature. QbD tool for developing a robust analytical method The analysis of CPX and RUT is another industrially utilized RP-HPLC method developed with QbD principles (Shamim et al., 2023). In this study, a Box-Behnken design was used for method optimization with comparatively lower number of experimental runs, thus resulting in a statistically significant high-quality analysis. The developed method was successfully validated in accordance with the ICH-Q2 guidelines and was successfully applied for the analysis of newly developed CPX-RUT-loaded bilosomal nanoformulations. This article invites the readers to find out the most timely examples of QbD principles that have been successfully implemented in the pharmaceutical industry to design a novel, successful, and dependable RP-HPLC Methods for drug analysis. These systematic identification of key parameters and attributes increases method performance and enhances alignment between drug development and regulatory review process (Yu et al., 2014).

Problems Encountered and How to Overcome Them

Although quality by design (QbD) is possible in drug development, it must be developed for the specific formulation approach; and while on an experimental level, this is possible at a laboratory scale, translating this to the hot-melt extrusion (HME) platform to develop amorphous solid dispersions (ASDs) and nanoparticle formulations can be challenging. A core challenge is to discover and quantify important material attributes and process parameters. Hybrid polymer-based nanoparticles: Critical material attributes and designs for high throughput screening. Pressurization and the number of cycles during homogenization are amongst the critical process parameters (Soni et al., 2020). Note that the understanding of drug-polymer miscibility and the optimization of formulation and process variables are key for HME-based ASDs as well (Butreddy et al., 2020). An important issue would be to choose and follow a suitable Design of Experiments (DoE) strategy to optimize and to screen the formulation and process variables. Particularly, it becomes more critical for maintaining the consistency of product quality and to mitigate the drawbacks of poor solubility and bioavailability of poorly soluble active pharmaceutical ingredients (Butreddy et al., 2020). There are a few proposed strategies to mitigate these issues. The increasing application of quality by design approaches has also paved way for the establishment of an efficient control strategy for the assured safety of anticancer drugs encapsulated in nanoparticles (Soni et al, 2020) Understanding these QbD elements such as critical quality attributes, risk assessment tools, and experimental designs would help in formulation and process optimization (Butreddy et al., 2020). Furthermore, intellect towards the prediction of drug-polymer miscibility and the use of several screening and optimization designs can offer an insight into the formulation and process variables that are experienced during ASD production (Butreddy et al., 2020). To summarize, there are indeed challenges in adopting QbD in pharmaceutical development but as is demonstrated in the HME and nanoparticle case studies to find ways around the obstacles through properly identifying critical attributes, suitable DoE methodologies, as well as extrapolating the principles of QbD to every facet of development will yield dividends in being able to consistently produce high quality medicines.

Outcomes and Benefits

Some general benefits of implementing QbD in pharmaceutical development can be listed.

By implementing QbD principles, we not only fulfil the regulatory requirements but also improve product performance and shorten development

times. Absorption modeling conducted before first-in-human studies influenced drug formulation design, resulting in formulations with reduced sensitivity to elevated gastric pH, thus minimizing drug-drug interactions during co-administration with PPIs and H2RAs (Kesisoglou & Mitra, 2015). It has also allowed for controlled release formulations with targeted release rates that meet trough plasma concentrations and provides for once-daily dosing (Kesisoglou & Mitra, 2015). Interestingly, QbD application is not only beneficial for the product development stage. In the case of analytical method development, QbD has improved the accuracy and robustness of the analysis results through the identification and control of critical analytical variables and other method parameters (Bastogne et al., 2022). A similar work needs to be carried out was performed using QBD approach to develop a much shorter run time UHPLC method for the estimation of degradation products as stability indicating and much well-characterised degradation impurity (Jayagopal & Murugesh, 2020). The end result is more robust and efficient processes for pharmaceutical development due to the quality by design approach. This allowed a more systematic development focusing on deep knowledge of critical quality attributes, risks and a culture of continuous improvement (Pedro et al., 2023). As a result, development time has decreased, product performance has improved, and analytical methods have improved, all contributing to safer and more effective medicines for patients.

7. Future Trends and Challenges (1 page)

Advances in RP-HPLC Technology

Advances in RP-HPLC technology show great potential for integration with quality-by-design (QbD) principles, resulting in improved analytical method development and optimization. However, the development of the HPLC method according to QbD approaches resulted in more robust, faster, and greener analytical methods (Mohammed et al., 2023; Shamim et al., 2023; Yabré et al., 2020). Significant developments have also been made in chromatographic technologies, such as the adoption of ultra-high-performance liquid chromatography (UHPLC), leading to better resolution, shorter run times, and decreased solvent usage compared to HPLC. While not specifically stated in the papers received, UHPLC lends itself to QbD principles because greater precision can be achieved in respect to critical method parameters (Cohen, 2023; Bhanot, 3032; Moore, 3035). For example, Mohammed et al. According to (Mohammed et al., 2023), QbD approaches can be applied to optimize the mobile phase composition, pH, and column temperature, which are equally applicable to UHPLC systems. Another notable progress is the combination between Process Analytical Technology (PAT) and HPLC. Raman spectroscopy has emerged as a tool for process analytical technology (PAT) in pharmaceutical production, potentially serving as a complement to high-performance liquid chromatography (HPLC) analysis (Esmonde-White et al., 2016). Using a combination of these technologies enables the real-time monitoring and control of critical quality attributes, an approach in line with the principles of QbD, where the vision is that everything would be improved and understood continuously throughout the process. The application of new HPLC technologies with the QbD approach improves in separation quality resulting in a more efficient and robust procedures. Taking the studies on curcuminoids (Mohammed et al., 2023) and antimalarial drugs (Yabré et al., 2020) as an example, in this manner provides the possibility for analytical methods to be more environmentally friendly and analyti-micro-biotically superior. Overall, these advancements in HPLC technology, combined with QbD principles and their practical application in pharmaceutical analysis and manufacturing, will continue to drive improvements in quality and efficiency.

Regulatory Perspectives

Quality by Design (QbD) principles in analytical method development are advocated to a significant degree by the regulatory agencies, especially the FDA and the EMA. Several New Drug Applications (NDAs) approved by the FDA also highlight regulatory flexibility regarding QbD-based analytical methods, which suggests that the agency is already embracing this new methodology (Peraman et al., 2015). The QbD trend also applies to method development in analytical chemistry, termed Analytical Quality by Design (AQbD), which enables the movement within the method operable design region (MODR), thereby reducing out-of-trend and out-of-specification results as a result of enhanced method robustness (Peraman et al., 2015).

Even though QbD is recommended by the FDA for submission, implementation of QbD and Process Analytical Technology (PAT) for sterile products, including the biopharmaceuticals and biotechnology industry are rare (Riley & Li, 2010). For this reason, there is room for more growth and application of MCS in very important Manufacturing area. EMA is also working toward this goal, scoring the first-ever marketing authorization for monoclonal antibodies developed through the application of extensive QbD principles in the EU (Luciani et al., 2015)

[8,10] Overall, regulatory agencies encourage the application of QbD and AQbD in pharmaceutical development and manufacturing. This strategy is in accordance with the ICH Q8-Q11 principles and strives to enhance the accuracy and robustness of the analytical methods (Bastogne et al., 2022). As companies implement these principles, we look forward to seeing more regulatory guidance and acceptance of QbD into method development/validation for a commercial method.

Challenges and Limitations

While there are advantages of QbD, challenges and limitations in the implementation of QbD are faced across industries. The major barriers are cost, training, and resource allocation.

The time-consuming and costly initial commitment for adoption is one of the major obstacles to QbD implementation. Such QbD applications based on principles may have high costs of new technological solutions, processes reengineering, and analytical equipment (Drobnjakovic et al., 2023) Not only the example of the pharmaceutical industry It may be quite expensive to implement Process Analytical Technology (PAT) and to develop the mathematical models, which will be used for the control of the bioprocesses (Drobnjakovic et al., 2023). In addition to the requirement of extensive experimentation for design space definition (which can be costly), there is a clear need for more efficient computational approaches (Laky et al., 2019). A much bigger roadblock is the training and resources required. QbD also comes with necessity of broad understanding of specific functions that need to be performed by personnel in differs departments. Such arrangements necessary extensive training programs and recruitment of dedicated staff (Pedro et al., 2023). In addition, to adopting QbD principles, it is a cultural change in the organisation to a more systematic and risk based approach for development and manufacturing (Bastogne et al., 2022; Butreddy et al., 2020). In general, although QbD does afford certain advantages, extensive adoption faces challenges around cost, education and resource utilization. Companies need to dedicate to a long-term commitment to overcome these challenges, but will require a better toolkit and methodologies to ease the pressure of implementation. Notwithstanding these challenges, QbD remains desirable to many industries due to the potential benefits of increased product quality and manufacturability and increased regulatory compliance (Dickinson et al., 2008; Luciani et al., 2015).

SUMMARY

Correspondence: This review investigates the implementation of Quality by Design (QbD) approach in reverse-phase high-performance liquid chromatography (RP-HPLC) to maximize

the performance and robustness. Quality by Design (QbD) is on a similar wavelength, where it is a systematic approach to product and process development that highlights designing quality into products and processes from the very beginning [11]. This includes setting quality target product profile (QTPP), defining critical quality attributes (CQAs), risk-based assessment and method optimization via design of experiments (DoE), establishment of method operable design region (MODR), development of control strategy, method validation and continuous monitoring of the method. Development of solutions for RP-HPLC analysis of biopharmaceuticals and pharmaceutical products, as well as the analysis of natural products, e.g. using a QbD framework have been successfully used to develop robust and efficient RP-HPLC methods[6,14,22,31]. Initial steps enabling design space = definition of the QTPP; determination of Critical Method Parameters (CMPs) associated with the requested value of the QTPP; risk assessment (e.g. failure mode and effects analysis (FMEA)) and then design space. The method was optimized using the experimental four-level Box-Behnken and central composite face designs. The analysis methods require validation, including accuracy, precision, specificity, linearity, range, and robustness. Moreover, there were also three case studies that highlighted the successful implementation of QbD in RP-HPLC method development in the pharmaceutical industry. Future Trends Recently, there have been increasing efforts to combine advanced technologies, such as ultra-high-performance liquid chromatography (UHPLC) and process analytical technology (PAT), with QbD concepts. QbD is being actively promoted by regulatory agencies for analytical method development. Nonetheless, obstacles concerning cost, training and resource utilization should be overcome to facilitate widespread implementation.

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