INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

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Review Article

A Review On Development And Validation Of Ultra Performance Liquid Chromatography For Estimation Of Drug In Pharmaceutical Dosage Form By Quality By Design (Qbd) Approach

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ARTICLE INFO

Received: 12 June 2023
Accepted: 14 June 2023
Published: 04 July 2023

Keywords:
Quality by Design, Method development, Validation, Drug, UPLC, HPLC

DOI:
10.5281/zenodo.8112767

ABSTRACT

The pharmaceutical business, system optimization, stationary phase vs mobile phase, detector design, data processing, and data integrity control have all seen notable technical advancements in recent years. The individual accomplishments in each subject have led to a step-function increase in technique and performance when technology and quality are combined. A new method of using liquid chromatography for sample detection is provided by ultra-performance liquid chromatography (UPLC). UPLC primarily improves the resolution, speed, and sensitivity of liquid chromatography. UPLC approach uses Quality by Design to improve performance.

With the help of a more recent paradigm known as Quality by Design (QbD), the pharmaceutical industry is now able to create durable and reliable medicinal products that consistently meet the predetermined quality standards. The QbD strategy stresses establishing quality from the beginning of research and development and aids in the creation of goods and medicine samples that adhere to consumer and regulatory standards. QbD is already popular and has had incredible success in pharmaceutical formulation in many forms. However, the scientific community has not yet accepted and given genuine significance to the application of QbD in analytical research and development, also known as Analytical Quality by Design (AQbD). Using a strong design space to operate within, AQbD helps to enable the creation of high-quality analytical methods with better method performance that comply with regulatory requirements. This helps to prevent failures during the method transfer process and has a high degree of detection. The goal of the opening review article is to raise general understanding of the UPLC method's development and validation information using Quality by Design (QbD) and associated methodologies in order to evaluate medicinal active substances throughout the last ten years. The essay also concentrated on a broad review of the QbD approach to UPLC technique development.

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

For the identification and assessment of drugs in bulk and pharmaceutical dose form, the UPLC technique was developed and validated using the QbD methodology.

INTRODUCTION

The study of analytical chemistry involves creating strategies, tools, and techniques to gather information on the make-up and kind of substance. Pharmaceutical analysis encompasses matter with pharmaceutical uses since it is concerned with the chemical characterization of matter [1]. Ultra-Performance Liquid Chromatography is referred to as UPLC. Activity resolution, speed, and sensitivity analysis all get improvements. It employs small particles, saves time, and requires less solvent. The UPLC is based on the idea of using stationary sections made up of particles smaller than a pair of m. The van Demeter equation, a real formula that specifies the relationship between linear rate (flow rate) and plate height, governs the fundamental ideas of this development (HETP or column efficiency). HPLC precedes UPLC [3]. The packing materials used to effect the separation have evolved with HPLC [4]. Van Deemter equation,

$$H = A + B/u + Cu,$$

The Van Demeter curve, which is regulated by a three-part formula, demonstrates that the useful flow range is substantially broader for tiny diameter particles than for larger dimensions. The UPLC entailed developing a novel instrumental method for liquid chromatography that is coherent with pressures (around 8000 to 15,000 PSI, compared to 2500 to 5000 PSI in HPLC) and may benefit from partition performance (by lowering dead volumes) [5]. Efficiency is inversely correlated with particle size and directly correlated with column length. Consequently, without sacrificing resolution, the column size may be decreased by the same amount as the particle size. More drug metabolites, contaminants, greater separation, and increased spectral quality are identified using UPLC [6].

According to the widely used Van Deemter equation, efficiency increases significantly when particle size drops to less than 2.5 m and doesn't decline at higher linear velocities or flow rates. Speed and peak capacity may be increased to unprecedented heights by the use of tiny particles, a process known as Ultra Performance [7]. High Performance Liquid Chromatography (HPLC) is a common segregation technique that has numerous benefits including robustness, usability, excellent selectivity, and tunable sensitivity. Due to low liquid phase diffusion coefficients and sluggish analyte diffusion in the stationary phase, its principal drawback is lack of efficiency when compared to gas chromatography or capillary electrophoresis. The Van Deemter equation demonstrates that efficiency may be increased by using smaller size particles, but doing so causes a rapid rise in back pressure, even though the majority of HPLC systems only operate up to 400 bars. In order to speed up the analysis without compromising effectiveness or load, small columns packed with particles of around 2 m in length are employed with these systems [9]. The following can be done to improve HPLC separation efficiency: - a) working in hotter environments; b) using monolithic columns. UPLC offers the chance to increase the usefulness of traditional HPLC, a popular separation science. The first instrument of its kind to use Intelligent Device Management technology is the ACQUITY UPLC System [10]. Quality by design (QbD), a methodical strategy for product development based on good science and quality risk management, stresses product, process understanding, and process control. QbD starts with established objectives. The goal of the QbD effort is to show that pharmaceutical processes can be understood and controlled in order to offer high-quality pharmaceutical products while providing chances for ongoing improvement [11]. The QbD technique assures a higher likelihood of

successful downstream method validation and method transfer through report to site and offers a better knowledge of the capabilities and limits of the method. At various phases of the life cycle of developing a pharmaceutical product, analytical methodologies must be established. The two most popular method development methodologies in the QbD are the one-factor-at-a-time (OFAT) and analytical quality-by-design approaches (AQbD). One variable is altered in this technique, which uses the one-factor-at-a-time approach, yielding an appropriate solution. This strategy may result in a workable technique, but it offers a limited knowledge of the capabilities, constraints, and resilience of the method [12].The AQbD is a thorough, organised, and risk-based method development technique. The development of the analytical approach starts with predetermined objectives and offers reasoned knowledge. This method screens several chromatographic and method variables to give a better understanding of how the elements under study affect the effectiveness of the method [13].The method operable design region (MODR), which refers to the multivariate/multidimensional combination of variables that have been confirmed to fulfil the method performance criteria, is established using this tested parameter information. The AQbD approach will be a suitable, well-designed, clear, and reliable technique that provides the anticipated performance over the course of its lifecycle[14].The AQbD method development technique using RP-UPLC, on the other hand, was especially targeted for pharmaceutical development in a QbD environment for pharmaceutical dosage form, which has never been documented anywhere. To assure the quality of the method throughout the product lifetime, it is therefore partially necessary to examine a systematic methodology for RP-UPLC method development for pharmaceutical development employing QbD principles. This study's goal was to apply the quality by design (QbD) methodology to develop and validate an RP-UPLC method that could separate drugs in bulk, formulated forms, and human urine with a thorough understanding of the method and build in the method's quality during method development to ensure optimum method performance over the lifetime of the product with appropriate degradation data[15].These days, software-based method development is employed, and an AQbD approach with software assistance is used to create a method for drugs and drug products. An ACQUITY UPLC H-Class PLUS, ACQUITY Arc System, which has a solvent choose valve and a column heater to enable automated exploration of a variety of conditions, was used for this development [16] Fusion QbD Software, S-Matrix This Platform for Analytical QbD is a thorough combination of powerful statistical tools and chromatography-focused software that is consistent with regulatory QbD guidelines. Since it is anticipated that such performance-based routine procedures can be altered within the analytical target profile without regulatory resubmission and approval, all worldwide drug administration authorities countersign the QbD approach.

ADVANTAGES OF UPLC[17, 18]:

Various advantages of UPLC are as follows:

- Shortens the running time and improves sensitivity.
- Lower usage of solvents
- Offers the LC analysis's selectivity, sensitivity, and dynamic range.
- Related and unrelated chemicals can be quantified rapidly thanks to UPLC's quick resolving power.
- Quicker analysis thanks to the employment of a brand-new, extremely-fine-particle separation material.
- Ensures the quality of the final product, including testing before release.

- Shortens process cycle times, allowing for increased production with already available resources.
- Provides real-time analysis that is in sync with production procedures.

DISADVANTAGES OF UPLC[19]:

- The life of these sorts of columns is shortened as a result of greater pressure, which also necessitates additional maintenance. Until yet, without the negative effects of high pressure, performances comparable to or even greater have been achieved employing stationary phases of size approximately 2 m.
- In addition, the utilisation of phases smaller than 2 m is constrained since they often aren't regenerable.

INSTRUMENTATION[20]:

Figures 1 and 2 illustrate the instrument's schematic diagram and different components, respectively.

Parts of ultra-performance liquid chromatography (UPLC)

- Reservoir for Solvents
- Degasser
- Pump High Pressure
- Port for injecting samples
- Column
- Detector
- Processing and management of data
- Waste

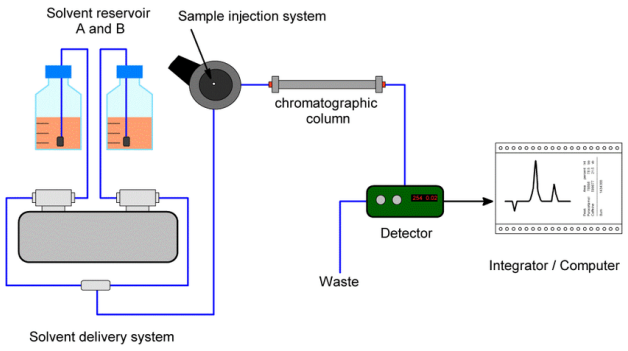

Figure 2: Schematic diagram of UPLC

Figure 3: UPLC instrument

Analytical RP-UPLC method development

Pharmaceutical manufacturing and drug discovery both heavily rely on the development and validation of analytical RP-UPLC methods. These techniques are used to quickly, precisely, and sensitively guarantee the identification, purity, potency, and performance of medicinal goods.

When constructing techniques, there are several things to take into account21. The first step in developing a technique is to collect data on the physicochemical characteristics of the analyte in the sample, including its solubility, log P, and pKa. The RP-UPLC method's major goal is to resolve and quantify the principal active substance, any reaction impurities, all readily available synthetic intermediaries, and any degradants separately [22].

Developing an RP-UPLC approach involves the following steps:

1. Recognize the physical characteristics of the drug's molecule.
2. Set up the RP-UPLC parameters.
3. sample solution preparation for method development.
4. Method improvement.
5. testing the procedure.

1) physicochemical properties of drug molecule

A drug's physicochemical characteristics have a significant impact in the creation of a technique. The physicochemical characteristics of the drug

molecule, such as its molecular formula, solubility, polarity, pH, and pKa, must be studied in order to build a method. The analyte's polarity is used to determine the mobile phase's solvent and chemical makeup. The electrons inside two atoms are distributed evenly in a nonpolar covalent bond [23] The choice of diluents should be aided by the drug's molecule's solubility. It operates on the same premise as solubility, which states that substances with comparable polarities are soluble in one another. The choice of diluents depends on how soluble the analyte is. The diluents' constituents must not react with the analyte, which must be soluble in them. To prevent peak distortion, particularly for early eluting components [24], the diluent should be equal to the beginning eluent composition of the assay.

pKa and pH are crucial in the development of UPLC methods. The pH value provides the most consistent definition of a substance's acidity or basicity. For ionizable analytes, choosing the right pH typically results in symmetrical and acute peaks in UPLC. The quantity of [H3O+] ions in an aqueous solution determines its acidity. As a result, a solution's pH reveals the amount of hydrogen ions present. The hydrogen ion concentration can be represented by the symbol [H+] or by its solvated version, [H3O+], whose value typically ranges from 0 to 14. The more the pH drops, the more acidic the fluid is. Simple addition of an acid or base should modify the pH of a solution. The pKa is a property of a certain chemical that indicates how easily it releases a proton. Analysis of the ratio is possible if the pH is known. This ratio reflects the acid's analytical concentration on an individual basis. Calculating the degree of dissociation and pH of a monoprotic acid solution is simple when the pKa and analytical concentration of the acid are known [25].

2) UPLC Chromatographic conditions

i. Selection UPLC Column

The column is the center of a UPLC system. During technique development, the column change will have the most impact on the resolution of analytes. In general, the stationary phase's characteristics have the biggest an impact on elution [26], selectivity, capacity factor, and efficiency. Silica and polymers are two examples of the several kinds of matrices that may be used to sustain the stationary phase. In a 1.7 m particle packed column, resolution is improved due to superior efficiency. This column provides greater molecular separation since its particles range in size from 1.7 m to 2.5 m. A bonded phase that offers selectivity and retention is necessary for the segregation of a sample's constituent parts. Four bonded stages are possible for the UPLC separation. Shield RP18 (an embedded polar group column), C18 and C8 (straight chain alkyl columns), and Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl). The properties, silanol activity, hydrolytic stability, and chemical interactions with analytes that each column chemistry offers are distinct [27].Due to its ability to provide the broadest range of hydrogen ion concentration, C18 and C8 columns are the standard columns used for many UPLC separations. They use tri-practical matter bonding chemistries to create excellent low hydrogen ion concentration stability. Long column lives and exceptional peak shape are provided by this factor in combination with proprietary finish capping procedures that are identical to those used for the C18 and C8 columns. The 1.7 m BEH particle's unique composition of matter and finish capping produces a replacement dimension in property that enables a quick fit to the current HPLC column [28]. It uses a 2.1 metric linear unit column internal dimension (ID). Choose a length of 100 metric linear units for the highest resolution, and a column of 50 mm for a speedier analysis and better sample output. According to the detection technique, the improvement in sensitivity of

UPLC detection should be 2–3 times more than that of HPLC separations. UPLC significantly improves MS detection, and higher peak concentrations with smaller action dispersion at lower flow rates encourage higher supply ionization efficiencies[29].

UPLC Columns have been manufactured and developed by the below organizations:

1. **Waters:** Vanguard Pre-columns and Acquity UPLC columns have been produced.
2. **Agilent technology:** provides quicker, repeatable results with better-performing columns. For instance, ZORBAX Rapid Resolution High-Definition Columns, ZORBAX Eclipse Plus Columns, ZORBAX Rapid Reduction High Throughput Columns.
3. **Altech Assosciate**
4. **Phenomenex:** great performance and efficiency Kinetex® Coreshell HPLC/UHPLC columns.

In the development of UPLC methods, many types of columns filled with minute particles made using various preparation methods are employed. The following techniques are listed:

1. the technique of charged-surface hybrid [CSH] particles,
2. The use of Ethylene Bridged Hybrid (BEH) particle technology,
3. The use of High Strength Silica [HSS] particles,
4. Technology for Separating Peptides (PST).

- **Steps for installation of UPLC Column on ACQUITY UPLC System using Active Column Preheater-**
    1. Squeeze the tabs together to remove the active preheater.
    2. Remove the ferrule, compression screw, and lock nut from the tubing's end by unscrewing the lock nut from the compression screw's rear.
    3. While tightening the compression screw, insert the tube into the column.
    4. Tighten the gold screw's gold lock with the stainless-steel lock.
    5. Attach the tubing with a finger-tight fitting of the peek one piece.
    6. After tightening the fitting, insert the tube into the column.
    7. Squeeze the plastic tubs on the active preheater to secure the column into place.
    8. Start the system flow, then check for leaks.
    9. Connect the eCord reader to the eCord, then start utilizing the column.

Selection of Mobile Phase

Chromatography works on the basis of adsorption, and real separation only happens when the adsorbate is rinsed with a mobile phase with the appropriate polarity. The selection of the column is always made concurrently with the selection of the mobile phase (stationary phase). The following elements must be taken into account while choosing and refining the mobile phase [30].

- The appropriate buffer selection and its eluting effectiveness
- Mobile phase pH and buffer pH
- A blend of binary and tertiary solvents used in nature's mobile phase

Buffer selection

Peak symmetries (shapes) and peak spacing are greatly influenced by the buffer and its effectiveness. To achieve the requisite separation, a variety of organic/inorganic buffers are used [31]. The buffers that are most often utilised are:

- Acetate buffers, such as sodium acetate and ammonium acetate.
- Buffers made of triethylamine and diethylamine.
- Phosphate buffers, such as KH2PO4, K2HPO4, NaH2PO4, and H3PO4.
- Buffers containing various ion-pair reagents, such as hexane sulfonic acid, butane among others, sulfonic acid, and heptane sulfonic acids.

The molar concentration of the buffer salt and its acid or base is directly proportional to the buffer's capacity, which is its ability to resist pH shifts. The buffering capacity increases as the pKa of the buffer approaches the required pH of the mobile phase. For instance, if the acidic analyte has a pKa of 4.5, the mobile phase's pH needs to be almost the same to stay deprotonated. A phosphate buffer with a pKa of 2.1 and an effective pH range of 1.1 to 3.1 is a good buffer in this pH range. The purpose of using a buffer is to lower the tailing factor for each splitting peak that develops because of varying ionic strength. When a highly concentrated buffer is employed, the retention time of the analyte(s) is slowed down and becomes separated well. When the utilised buffer's molarity is between 0.05 and 0.20 M, superior separation occurs. By carefully selecting the organic mobile phase's composition, the buffer concentration is chosen [32].The pKa value of the analyte and the intended pH of the mobile phase are the main determinants of the buffer selection. Therefore, it is optimal to use a buffer whose pKa value is as close as feasible to the appropriate pH level of the mobile phase.

compatibility of the detector, particularly when a mass spectrometer is used as the detector The eluate exiting the column has to be volatile in order to produce enough ions in the ion source for the mass detector to be able to detect them. This is a need for mass processing. Because of this, inorganic buffers like phosphate don't function with mass spectrometers. Modifiers are often used in LCMS with the goal of enhancing analyte signal, reducing undesired signal, or focusing on a specific compound's signal in a mixture. Since formic acid, ammonium salts, and di- or tri-ethyl amine are the most often utilised additives when using a mass detector, the buffers and additives used in conjunction with it should also be volatile. The UV cutoff limit of the buffer is another crucial consideration, particularly when operating at shorter wavelengths. Baseline noise will be present because some buffers may have an absorbance at lower wavelengths, which may impact the analyte's absorption range and diminish the detector's sensitivity. The strength of the buffer can be adjusted between 10 and 20%, depending on the requirements of the selected mixture for separation, in order to obtain the correct separation. The effects of variation must be thoroughly examined before to use. But it should be checked that, either during operation or during storage in a refrigerator, increased or reduced buffer strength shouldn't cause precipitation or turbidity in the mobile phase. Test experiments must be performed to optimize the separation in order to prevent peak tailing, provide greater separation, and ensure reproducibility [33] before employing the chosen buffer of particular strength to run a column.

pH of buffer

In order to achieve chromatographic detection, pH is crucial since it regulates the elution qualities by regulating the ionization characteristics. According to the pKa of the analyte or test combination, which is dependent on the molecule's structure, the pH of the buffer or mobile phase should be preferred. Drug molecules vary retentions based on the pKa; for example, acids exhibit an increase in retention when the pH is dropped, whereas bases show a decrease [34].Low pH or an acidic mobile phase must be chosen if the compound's pKa is high since this will prevent unintended interaction with the stationary phase. For effective separation, using a high pH or basic mobile phase for basic compounds and a neutral mobile phase for neutral compounds is highly recommended [35].Since most columns cannot sustain a pH that is outside of the range, it is crucial to keep the fixed pH of the appropriate mobile phase in the range of 2.0 8.0. Due to the breakage of siloxane bonds, the commonly employed silica column deactivates at high pH (2) and low pH

(>8). If it is determined that a pH outside the range of 2.0 8.0 is necessary, a stationary phase that can survive the range must be chosen [36].

General factors to take into account while choosing a buffer [37]

1. It is necessary to degas mobile phases.
2. Acetonitrile and THF are less soluble in phosphate than methanol and water.
3. Organic/water mobile phases often include ammonium salts that are more soluble.
4. TFA is volatile, can deteriorate over time, and absorbs short UV rays.
5. Buffered mobile phases with little to no organic modifier might experience fast microbial growth.
6. Phosphate buffer increases silica dissolution at pH levels over 7 and significantly shortens the lifespan of silica-based HPLC columns. When using organic buffers, pH levels greater than 7 must be preferred.
7. Ammonium bicarbonate buffers are often unstable for just 24 to 48 hours and are frequently subject to pH variations. The introduction of carbon dioxide tends to make the pH of this mobile phase more basic.
8. After preparation, buffers should be filtered using a 0.2-m filter.

Sample Injection

The injector is used to introduce a little quantity of solution containing the targeted sample in the mobile phase. The injection must be carried out regularly and accurately. To protect the column against greater pressure instabilities, typical injection valves can be used manually or automatically. The injection method should be largely pulse-free. The device's swept volume needs to be maintained to a minimum in order to reduce the possibility of band spreading[38].A quick injection cycle time is needed to properly capitalize on UPLC's speed. To achieve improved sensitivity, low volume injections with less carry over are necessary. The sample volume in UPLC is close to 2–5 L. Direct injection methods are increasingly often applied to biological samples [39].

Selection of detector

Any analytical method's development and finalization depend heavily on the detector. The majority of chemical and pharmacological compounds are often aromatic or unsaturated, which absorbs light in the UV-vis range. This is advantageous for measuring and examining the molecules and related contaminants. By examining the UV-visible spectrophotometer or diode array detector (DAD) of HPLC/UPLC, the accurate absorbance maximum of the chemical or sample must be acquired. The test compound should be quantified based on the area intensity of the test compound using calibration curves[40].In order to reduce the amount of segregated solute that is lost on the column, the employed UPLC detector should be capable of offering a high sampling rate with modest achievable compound peaks (1s half-height peak width) and low peak dispersion. Due to the detector sensitivity, the UPLC method provides two to three times the separation sensitivity of the HPLC method. Teflon AF provides an internally reflecting surface that improves light transmission efficiency by eradicating internal absorptions. Acquity photodiode array (PDA) and Tunable Vis-UV (TUV) detectors are employed in the UPLC.

For increased precision, mass spectrometry is also utilised in conjunction with UPLC for detection41.

For increased precision, mass spectrometry is also utilised in conjunction with UPLC for detection [41].

Preparation of sample solutions for method development

The analyst must consider the censorious stage of technique development known as sample preparation. The drug substance under analysis needs to be diluent stable. The creation of the solutions in amber flasks should be carried out

during the earliest stages of the development of a UPLC technique until it is shown that the active component is stable at room temperature and does not deteriorate under typical laboratory circumstances. Filtering the sample solution is necessary; for the removal of particles larger than 0.45 m, a 0.22 or 0.45 m pore-size filter is advised. For UPLC analyses, filtration is a key preventative maintenance tool [42].Syringe filters are most efficient when they are able to remove impurities and insoluble materials without releasing unwanted artefacts (extractables) into the filtrate. To ascertain whether a leachable component is coming from the syringe filter housing/filter [43], the diluent must be filtered if any additional peaks are seen in the filtered samples.

UPLC METHOD DEVELOPMENT BY QBD APPROACH:

The following parameters were identified as necessary for Analytical QbD's development of the UPLC method:

- **Selection of quality target product profile (QTPP)**

The establishment of a quality goal product profile serves as the foundation for the systematic development of analytical methods. It consists of all characteristics necessary to guarantee the application of the analytical procedure and the quality of its qualities. The QTPP is crucial in identifying the factors that influence the QTPP parameters. For the UPLC method[44], the retention duration, separation, and peak asymmetry were designated as QTPP.

- **Determine critical quality attributes.**

The method factors in QbD that have a direct impact on the QTPP are the important quality characteristics. CQAs are the variables that have an influence on how well a technique performs and can really change the outcome. For the impurity detection approach, more CQAs might come from the resolution between nearby peaks. To keep QTPP[45']s reaction within an acceptable range, two crucial technique parameters—content in the mobile phase and buffer pH—need to be regulated.

- **Factorial design or Design of Experiment**

The central composite experimental design was used to optimise and select the two key components, mobile phase and pH of buffer, once the QTPP and crucial quality attribute were defined. Using a central composite statistical screening design[46], the various interaction effects and quadratic impacts of the mobile phase composition and pH of the buffer solution on the retention duration, theoretical plates, and peak asymmetry were taken into consideration.

- **MODR (Method Operable Design Region)**

The development of a multidimensional space using method parameters and settings is known as method operable design region (MODR); MODR can offer suitable method performance. Additionally, it is used to set up important method controls including system appropriateness, RRT, and RRF. To define the MODR[47] and prove ATP conformity, further method verification techniques may be used.

- **Control Strategy and Risk Assessment**

A high set of required controls, developed from an understanding of MODR and the nature of analytes, make up the control strategy. Detailed statistical information gathered throughout the design and MODR stages should be used to validate the method control approach. For the ability to achieve the ATP requirements, correlations between technique and analyte properties can be derived using statistical experimental or factorial data [48]. The method parameter anomaly will be fixed by the control strategy (e.g., reagent grade, instrument brand or type, and column type). Critical method parameters and CQA are inversely correlated. When compared to the direct traditional technique, the method control strategy under the AQbD approach does not seem significantly different. To establish a strong connection between the

method's goal and performance[49], method controls are validated based on important quality qualities, factorial or experimental design, and MODR experimental results.

- **AQbD Method Validation**

Analytical method validation using a variety of different samples is what is meant by the AQbD method validation technique. It calculates method validation for all types of modifications without revalidation using both design and MODR knowledge. The method offers the necessary components for ICH validation as well as details on interactions, measurement uncertainty, control strategy, and ongoing development. Compared to the traditional validation strategy, this method uses fewer resources without sacrificing quality [50].

- **Continuous Method Monitoring (CMM) and Continual Improvement**

A control approach for applying design space at the commercial stage is life cycle management. The CMM is the last component of the AQbD life cycle; it is an ongoing process of exchanging knowledge developed during the application and evolution of design space. The outcomes of risk analyses, presumptions based on prior information, statistical design considerations and bridge inside the design space, MODR, control strategy, CQA, and ATP are included. Once a method validation is complete, it may be utilized for routine analysis and the performance of the method can be tracked continuously. Control charts, tracking system appropriateness data, method-related final studies, and other tools can be used to figure this out. The analyst is able to spot and correct any out-of-trend performance using CMM [51].

METHOD VALIDATION

Multiple evaluations are conducted as part of the method validation process to ensure that an analytical test system is appropriate for its intended use and capable of producing useful and reliable analytical findings. The procedures required to carry out each analytical test should be thoroughly explained. The applicant's planned and methodical gathering of the validation data to support analytical procedures is the first step in the methods validation process for analytical procedures. All RP-UPLC techniques that are intended for use in drug sample analysis must be verified. According to ICH guidelines[52], analytical method validation is carried out.

Components of method validation

The following are typical analytical performance characteristics that can be tested during method validation:

- ❖ System suitability assessment
- ❖ Specificity
- ❖ Linearity
- ❖ Accuracy
- ❖ Precision
- ❖ Repeatability
- ❖ Intermediate accuracy
- ❖ Detection ceiling
- ❖ Quantitative restriction
- ❖ Robustness

System Suitability Determination

System Appropriateness Determination is the assessment of a parameter in an analytical system to demonstrate that the system's performance satisfies the method's acceptance criteria. These variables, including the resolution, tailing factor, capacity factor, relative retention time, and relative standard deviation, may be computed experimentally to set up a quantitative system appropriateness test (precision). On a peak attained in the system [53], they are measured.

Specificity

Being able to precisely and specifically measure the analyte of delight in the presence of other components that would be anticipated to be present in the sample matrix is a validation parameter. The specificity parameter makes sure that the procedure, including the heavy metals and organic volatile impurity limitations, enables an

accurate description of the impurity concentration. Specificity offers a peak purity result that demonstrates the analyte's specificity (54, 55).

Linearity

The capacity to generate linearity test findings that are directly proportionate to the concentration of analyte in the analytical technique is characterized as linearity. If there is a linear connection, the test findings should be assessed using a suitable statistical technique, such as by computing a regression line using the least squares approach. It is advised to use a minimum of 5 concentrations to establish linearity. It is necessary to provide the correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares. The data should be plotted, as appropriate [55].

Accuracy

Trueness is another name for the accuracy parameter. The degree to which the measured and true values are similar when compared to the reference standard is known as accuracy or trueness. By adding a known concentration of analyte standard to the sample matrix of interest and then evaluating the sample, accuracy is determined. The standards for drug substances and goods are 98.0-102.0% and 97.0-103.0%, respectively, to report the accuracy. The range of 50 to 150% of average recovery can be used to accurately determine impurities [55].

Precision

Under the predetermined requirements of Repeatability, Intermediate precision, and Reproducibility, the analytical process for precision reflects the degree of agreement between a set of measurements acquired from successive sampling of the same homogenous sample. Typically, the RSD used to measure an analytical procedure's accuracy is 2%. RSD of 5–10% is often acceptable for low level contaminants. having a parameter [56] of repeatability, intermediate precision, and reproducibility.

Detection limit

The lowest quantity of analyte in a sample that can be detected but not always quantitated as an accurate number is expressed by the detection limit. A feature of limit testing is called the detection limit. The lowest concentration of an analyte in a sample that can be detected but not quantitated is known as the detection limit. This parameter indicates whether an analyte is more than or less than a certain value [56].

Quantitation limit

The least quantity of analyte in a sample that can be quantitatively identified with enough precision and accuracy is known as the quantitation limit of a particular analytical method. In quantitative tests for low levels of chemicals in sample matrices, the LOQ is a measure that is particularly useful for quantifying contaminants and degradation products. You may find it by using formula [56].

$LOQ = 10 \times \delta/S$

Where, = response standard deviation.

S = Median of the calibration curves' slopes.

Robustness

The ability of a technique to remain unaffected by purposeful, minor changes to the method parameters is known as robustness. The robustness of an approach gauges its dependability. The robustness parameter ought to be assessed in late development or early on in the course of technique validation. Using summary tables, bar and control charts, effect and probability plots, and other means of result comparisons, robustness may be demonstrated in a variety of ways[57].

CONCLUSION

The overall process of UPLC method development and analytical method validation is described in this paper. It was explored how to build a UPLC technique for separating medicinal chemicals. Small particle size UPLC increases liquid chromatography's resolution, speed, and sensitivity. The UPLC technique allows for high throughput analysis, reduced solvent use, shorter analysis times, and lower analysis costs. The

method's complexity expands along with the complexity of a medicine and pharmaceutical drug product, making its development challenging. Robustness is one of a technique's key characteristics, and it should be assessed as the method is being developed. Strong analytical techniques can deliver consistent, dependable outcomes. AQbD is a methodical approach to method development that starts with setting the ATP, determining CMAs with their specifications, and identifying crucial method parameters for a robust RP-HPLC method (factors). An essential component of AQbD is design of experiments, which employs statistical methods for both mathematical modelling and experiment design.It can be inferred from the literature review on the development and validation of UPLC methods using the QbD methodology that all classes of pharmaceutical pharmaceuticals may be examined using UPLC methods in a very short amount of time with a reliable technique and with little solvent usage. Chromatography gains and broadens its relevance thanks to UPLC. With AQbD, it is revolutionizing laboratories and lifestyles while also opening up new avenues for profitable corporate operations. The review of the literature reveals that work on UPLC method development and validation using QbD has been done effectively on all drug categories at both the national and international levels.

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HOW TO CITE: Kanchan Shinde*, Neha Gaikwad, Varsha Pangale, Charushila Bhangale, A Review On Development And Validation Of Ultra Performance Liquid Chromatography For Estimation Of Drug In Pharmaceutical Dosage Form By Quality By Design (Qbd) Approach, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 7, 123-138. https://doi.org/10.5281/zenodo.8112767