A REVIEW ON ANALYTICAL QUALITY BY DESIGN

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ABSTRACT:

Analytical Quality by Design (AQbD) is a systematic and science-based approach that improves the development and validation of analytical methods. When combined with Ultra-Performance Liquid Chromatography (UPLC), AQbD enables rapid, accurate, robust, and reliable analysis of pharmaceutical compounds. The approach begins with defining the Analytical Target Profile (ATP), followed by identifying Critical Method Parameters (CMPs) and Critical Analytical Attributes (CAAs). Risk assessment and Design of Experiments (DoE) are applied to optimize method performance and establish a Method Operable Design Region (MODR).

KEYWORDS: Quality by Design (AQbD), Ultra-Performance Liquid Chromatography (UPLC), Design of Experiments (DoE), Method Operable Design Region (MODR), Analytical Target Profile (ATP), Method Robustness.

INTRODUCTION:

Analytical Quality by Design (AQbD) is a scientific and systematic risk-based approach[1] to analytical method development that ensures methods are accurate, reliable and robust throughout their entire life cycle. It emphasizes the identification, understanding and control of potential sources of variability and risk from the earliest stages of method development. AQbD principles can also be applied during method transfer between laboratories or analytical instruments, where a thorough understanding of critical method parameters[2] (CMPs) and the establishment of a validated design space help maintain method performance and consistency across different settings. Furthermore, AQbD supports the continuous monitoring, periodic review and reassessment of analytical methods to ensure they remain suitable for their intended purpose over time. By adopting AQbD, analytical laboratories can improve method development, validation, transfer and routine operation, resulting in enhanced analytical performance, greater operational efficiency, stronger regulatory compliance and more reliable and accurate analytical testing.

Ultra-Performance Liquid Chromatography (UPLC) is an advanced chromatographic system designed to operate at pressures of up to **15,000 psi (approximately 1000–1034 bar)[3]**. It employs columns packed with **sub-2 µm particles**, which significantly enhance chromatographic efficiency, resulting in faster analysis, higher resolution, improved sensitivity, and greater reproducibility compared with conventional High-Performance Liquid Chromatography (HPLC). Additionally, UPLC reduces solvent and sample consumption, making it a cost-effective and environmentally friendly analytical technique.

Traditionally, analytical methods were developed using the One-Factor-at-a-Time (OFAT) or One-Variable-at-a-Time (OVAT) approach, in which a single experimental parameter was varied while all other variables were kept constant. Although straightforward, this approach is labor-intensive, time-consuming, and incapable of detecting interactions between multiple variables. Consequently, methods developed using OFAT often exhibit limited robustness and require repeated optimization during routine quality control and method transfer.

Regulatory authorities, including the International Council for Harmonization (ICH), strongly advocate the implementation of AQbD through guidelines such as **ICH Q8 (Pharmaceutical Development), ICH Q14 (Analytical Procedure Development), and ICH Q2(R2) (Validation of Analytical Procedures)**.[4] These guidelines emphasize that analytical quality should be designed into the method rather than verified solely through end-product testing. AQbD begins with defining the **Analytical Target Profile (ATP)**, followed by identifying **Critical Method Parameters (CMPs)** and **Critical Analytical Attributes (CAAs)** that influence method performance. Quality risk management tools, including Ishikawa (fish-bone) diagrams and risk assessment matrices, are employed to identify potential sources of variability. Subsequently, **Design of Experiments (DoE)** is used to statistically evaluate the effects and interactions of critical variables such as mobile phase composition, pH, flow rate, column temperature, and detection wavelength. The experimental data are then utilized to establish a **Method Operable Design Region (MODR)**.[5]

The integration of AQbD with **Ultra-Performance Liquid Chromatography (UPLC)** has transformed modern pharmaceutical analysis by combining systematic method development with advanced chromatographic technology. Utilizing stationary phases packed with sub-2 µm particles, UPLC provides significantly higher chromatographic resolution, sharper peak shapes, enhanced sensitivity, and faster separations compared with conventional High-Performance Liquid Chromatography (HPLC). Furthermore, UPLC substantially reduces analysis time, solvent consumption, and sample requirements while improving precision and reproducibility. When coupled with AQbD, UPLC enables efficient optimization of analytical conditions through statistical modeling, resulting in highly robust, stability-indicating, and cost-effective analytical methods.

In oncology research and pharmaceutical manufacturing, AQbD-based UPLC methods have been successfully applied to several anticancer agents, including cisplatin, paclitaxel, docetaxel, imatinib, sorafenib, bevacizumab, and other targeted therapies.[6] These methods offer exceptional sensitivity, reproducibility, and robustness while supporting efficient quality control, regulatory compliance, and reliable method transfer across manufacturing sites. The establishment of a scientifically justified MODR also provides operational flexibility, allowing minor adjustments in chromatographic conditions without compromising analytical performance or requiring complete method revalidation.

Overall, the combination of AQbD and UPLC represents one of the most advanced paradigms in contemporary pharmaceutical analytical science. By integrating scientific understanding, quality risk management, statistical optimization through DoE, life-cycle management, and advanced chromatographic technology, AQbD-guided UPLC methods deliver highly robust, efficient, economical, and regulatory-compliant analytical procedures. This integrated strategy supports the development, validation, transfer, and continuous improvement of analytical methods while ensuring the quality, safety, and efficacy of pharmaceutical products.[7]

Instrumentation:

Ultra-Performance Liquid Chromatography (UPLC) is an advanced chromatographic technique that plays a pivotal role in the implementation of Analytical Quality by Design (AQbD) for pharmaceutical method development, optimization, and validation. Compared with conventional High-Performance Liquid Chromatography (HPLC), UPLC utilizes columns packed with sub-2 µm particles and operates at high pressures of up to 15,000 psi (approximately 1034 bar), enabling rapid, highly efficient, and reproducible chromatographic separations.

The enhanced performance of UPLC provides significantly shorter analysis times, higher chromatographic resolution, improved sensitivity, sharper peak shapes, and reduced solvent and sample consumption.These advantages make UPLC an ideal analytical platform for AQbD-based method development, where a systematic understanding of method variables is essential to ensure analytical robustness and consistent performance.

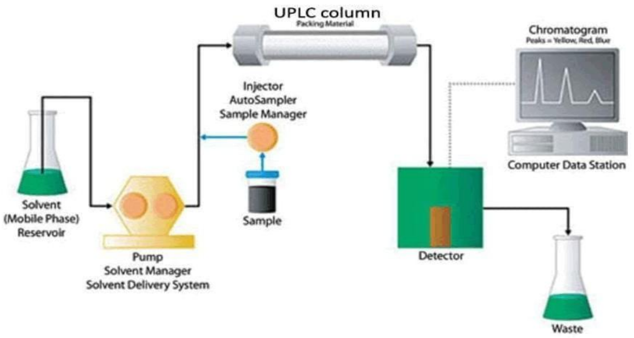


Fig 1: UPLC INSTRUMENT

Ultra-Performance Liquid Chromatography (UPLC) is an advanced chromatographic system designed to operate at pressures of up to **15,000 psi (approximately 1000–1034 bar)**. It employs columns packed with **sub-2 µm particles**, which significantly enhance chromatographic efficiency, resulting in faster analysis, higher resolution, improved sensitivity, and greater reproducibility compared with conventional High-Performance Liquid Chromatography (HPLC). Additionally, UPLC reduces solvent and sample consumption, making it a cost-effective and environmentally friendly analytical technique.

1. Solvent Manager (Binary Pump System):

The solvent manager is responsible for delivering the mobile phase through the chromatographic system with exceptional precision and accuracy. Unlike conventional HPLC systems that commonly utilize[8] Quaternary pumps, UPLC employs **high-pressure binary solvent management systems** consisting of two serial flow pumps that generate precise gradient elution at pressures up to 15,000 psi. These pumps incorporate **electronic compressibility compensation**, ensuring stable, pulse-free flow rates despite solvent compression under high-pressure conditions. Accurate solvent delivery is essential for achieving reproducible retention times and consistent chromatographic performance.

2. Sample Manager (Autosampler/Injector):

The sample manager introduces precise volumes of the sample into the chromatographic system while minimizing sample carryover and band broadening. Since UPLC typically uses very small injection volumes (generally 0.1–10 µL), the auto sampler is designed with **high-pressure injection valves** and **low-dead-volume flow paths** [9]to maintain excellent injection precision and reproducibility. Advanced needle-in-needle injection mechanisms allow accurate sample loading and reliable operation even at maximum system pressures.

3. Column Manager and Column Heater:

The chromatographic column is the core of the UPLC system and is packed with **sub-2 µm porous particles** (commonly 1.7 µm), which provide superior separation efficiency and peak resolution. The **column manager** maintains a constant column temperature, typically up to **65°C**, reducing mobile-phase viscosity, improving mass transfer, and enhancing chromatographic reproducibility. In UPLC systems coupled with **Mass Spectrometry (UPLC-MS)**,[10] specialized column managers position the column outlet close to the mass spectrometer inlet to minimize extra-column dispersion and maximize analytical sensitivity.

4. Detectors:

UPLC systems can be coupled with a variety of detectors depending on the analytical application.

- **Photodiode Array (PDA) Detector:** The most widely used detector in UPLC, capable of simultaneously monitoring multiple wavelengths while providing UV-visible spectral information for qualitative and quantitative analysis.
- **Mass Spectrometry (MS):** Frequently coupled with UPLC for highly sensitive detection, structural characterization, impurity profiling, metabolomics, proteomics, and trace-level analysis.
- **Fluorescence Detector (FLD):** Offers excellent sensitivity and selectivity for naturally fluorescent compounds or analytes derivatized with fluorescent reagents.
- **Refractive Index (RI) Detector:** Primarily used for compounds that lack UV absorption, such as carbohydrates, sugars, polymers, and other non-volatile analytes.

5. Data Acquisition and Control System:

Modern UPLC instruments are equipped with sophisticated chromatography software that controls all instrument functions, including solvent delivery, sample injection, gradient programming, detector operation, data acquisition, peak integration, and quantitative analysis. The software also supports method development, system suitability testing, validation, and regulatory-compliant data management[11].

MATERIALS:

Analytical Quality by Design (AQbD) is a systematic and science-based approach to analytical method development that emphasizes predefined objectives, risk assessment, and process understanding. When implemented with Ultra-Performance Liquid Chromatography (UPLC), AQbD enables the development of robust, reliable, and regulatory-compliant analytical methods. Since UPLC operates at very high pressures (up to 15,000 psi or approximately 1034 bar) using columns packed with sub-2 µm particles, the selection of appropriate materials is critical. These materials are regarded as **Critical Material Attributes (CMAs)** because their physical and chemical properties directly influence **Critical Quality Attributes[12] (CQAs)** such as chromatographic resolution, retention time, peak symmetry, sensitivity, and method reproducibility.

1. Stationary Phase Materials (UPLC Columns):

The stationary phase is the most important material used in AQbD-guided UPLC method development. UPLC columns are typically packed with **1.7–1.8 µm porous spherical particles**, which provide high separation efficiency, narrow peak widths, and rapid analysis.

Common stationary phase materials include:

- **Bridged Ethyl Hybrid (BEH) Particles:** BEH technology combines an inorganic silica matrix with organic ethylene bridges, providing excellent mechanical strength and chemical stability over a wide pH range (pH 1–12). These particles minimize secondary silanol interactions, resulting in improved peak symmetry and reproducible chromatographic performance.
- **Charged Surface Hybrid (CSH) Particles:** CSH columns possess a controlled positive surface charge that improves the separation of basic compounds by reducing peak tailing and increasing sample loading capacity. They are particularly suitable for low-ionic-strength mobile phases and LC–MS applications.
- **High-Strength Silica (HSS) Particles:** HSS columns consist of mechanically strengthened silica particles that provide excellent retention of highly polar compounds and metabolites while maintaining high efficiency under elevated pressures. The small particle size of UPLC columns significantly reduces band broadening and improves chromatographic resolution according to the Van Deemter equation, enabling faster analyses without compromising separation efficiency.

2. Mobile Phase Materials:

The mobile phase is another critical material attribute that directly affects chromatographic selectivity, retention behavior, and detector performance.[13]

- **Organic Solvents**: UPLC methods employ **UPLC-grade or LC–MS-grade solvents**, including: Acetonitrile (ACN) and Methanol (MeOH)

These solvents possess high purity, low UV absorbance, and minimal trace-metal impurities, ensuring low baseline noise and reproducible chromatographic performance.

- **Aqueous Solvent**: Type I ultrapure water (18.2 MΩ·cm resistivity) is used for preparing the aqueous component of the mobile phase. The water is filtered through a **0.22 µm membrane filter** to remove particulate matter and microbial contamination.
- **Buffers and pH Modifiers**: Volatile buffers are commonly selected because they are compatible with both UPLC and LC–MS analysis. Frequently used buffers include: Ammonium formate, Ammonium acetate, Ammonium bicarbonate.

Common pH modifiers include:

- Formic acid
- Acetic acid
- Ammonium hydroxide

Within AQbD, mobile phase pH and buffer concentration are considered critical method parameters because they strongly influence analyte ionization, selectivity, peak shape, and retention time.[14]

3. Instrument Fluidic Materials:

UPLC instrumentation is constructed from high-strength, chemically inert materials capable of withstanding extreme operating pressures.

Material Component	Critical Material Attribute (CMA)	Affected Critical Quality Attribute (CQA)
Column packing	Particle size (1.7–1.8 µm)	Resolution, efficiency, run time, back pressure
Column chemistry	C18, C8, Phenyl, BEH, CSH, HSS	Selectivity, peak symmetry, retention behavior
Mobile phase	Solvent type, purity, pH, buffer concentration	Retention time, peak shape, analyte ionization
Organic modifier	Acetonitrile or methanol	Elution strength, viscosity, baseline noise
Instrument fluidics	Stainless steel, titanium, PEEK	Peak tailing, adsorption, carryover
Sample containers	Glass vial quality and septa material	Sample stability, contamination, reproducibility

- **Titanium and stainless steel** are used for pump heads, capillary tubing, injector components, and high-pressure fluidic systems due to their excellent mechanical strength and corrosion resistance.
- **PEEK (Polyether Ether Ketone)** and titanium-PEEK hybrid materials are employed in sample loops, tubing, and bio-inert flow paths to minimize adsorption of proteins, peptides, oligonucleotides, and monoclonal antibodies, thereby reducing carryover and improving analytical accuracy.[15]
- **Synthetic sapphire and ruby** are used in pump pistons, check valves, and valve seats because of their exceptional hardness, wear resistance, and chemical stability, ensuring long-term performance under high-pressure conditions.

4. Sample Containment Materials

Proper sample storage and handling materials are essential for maintaining sample integrity during chromatographic analysis.

- **Type I borosilicate glass vials** are widely used because they exhibit excellent chemical resistance and prevent ion leaching that may alter sample composition
- **Silanized (deactivated) glass vials** are preferred for peptides, proteins, and highly polar analytes to minimize adsorption onto glass surfaces.
- **PTFE-lined silicone septa** provide excellent chemical inertness, prevent solvent evaporation, and allow repeated needle punctures without leakage during automated sample analysis.

5. Critical Material Attributes (CMAs) in AQbD

In AQbD, all materials are evaluated through systematic quality risk assessment using tools such as **Ishikawa (Fishbone) diagrams**, **Failure Mode and Effects Analysis[16] (FMEA)**, and **Design of Experiments (DoE)**. The influence of each material on chromatographic performance is assessed before method optimization to ensure robust and reliable analytical performance.

METHODS:

Analytical Target Profile (ATP):

The **Analytical Target Profile (ATP)** is the foundation of the Analytical Quality by Design (AQbD) approach and defines the intended purpose and desired performance characteristics of an analytical method before method development begins. ATP establishes predefined objectives that guide the systematic development, optimization, validation, and lifecycle management of analytical procedures. It serves as a communication tool among analysts, researchers, quality assurance personnel, and regulatory authorities by clearly specifying the analytical requirements that the method must satisfy.

Components of the Analytical Target Profile:

The ATP generally includes the following key components:

- **Target Analyte:** Specifies the analyte of interest, which may be an active pharmaceutical ingredient (API), impurity, degradation product, metabolite, or any other component requiring analysis.
- **Measurement Parameters:** Defines the analytical characteristics to be measured, such as assay, concentration, purity, content uniformity, identification, or impurity profiling.
- **Performance Criteria:** Establishes predefined acceptance criteria for method performance, including accuracy, precision, specificity, selectivity, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), and analytical range.
- **Sample Matrix:** Identifies the sample type or formulation (e.g., bulk drug, pharmaceutical dosage form, biological fluids, or stability samples) in which the analytical method will be applied.
- **Method Attributes:** Specifies additional characteristics such as analysis time, simplicity, ruggedness, cost-effectiveness, automation capability, and compatibility with chromatographic instrumentation.

A well-defined ATP provides a scientific basis for selecting chromatographic conditions, identifying critical method parameters, performing risk assessment, and establishing appropriate experimental designs. Furthermore, it serves as the benchmark for evaluating method performance during validation and lifecycle management, ensuring that the analytical method remains fit for its intended purpose.

Example of an Analytical Target Profile

A typical ATP for a pharmaceutical assay may include the following performance requirements:

Parameter	Acceptance Criteria
Analyte	Drug X
Sample Matrix	Serum
Analytical Technique	High-Performance Liquid Chromatography (HPLC)
Analytical Range	5–500 ng/mL
Accuracy	±10% of the true value
Intra-day Precision	≤5% RSD
Inter-day Precision	≤10% RSD
Specificity	No interference from impurities or degradation products
Limit of Detection (LOD)	≤2 ng/mL
Limit of Quantification (LOQ)	≤5 ng/mL
Robustness	Unaffected by minor changes in pH, flow rate, mobile phase composition, and column temperature
Sample Stability	Stable throughout storage and analysis
Sample Throughput	Minimum of 50 samples per day
Method Suitability	Suitable for routine quality control with reliable data integrity

The ATP acts as the reference standard throughout analytical method development, optimization, validation, and routine application, ensuring that the method consistently satisfies predefined quality requirements.

Risk Assessment:

Risk assessment is an integral component of the AQbD framework and forms the basis of quality risk management during analytical method development. It is a systematic, science-based process used to identify, evaluate, prioritize, and control potential sources of variability that may influence analytical performance. Risk assessment begins during method development and continues throughout the analytical method lifecycle to ensure consistent method robustness, reliability, and regulatory compliance.[17]

The primary objective of risk assessment is to identify **Critical Method Parameters (CMPs)** and **Critical Material Attributes (CMAs)** that significantly affect method performance and Critical Quality Attributes (CQAs). By understanding these relationships, appropriate control strategies can be implemented to minimize analytical variability and improve method reliability.

Risk Ranking System:

Risks are generally categorized into three levels:

- **Low Risk:** Acceptable risk requiring no additional investigation.
- **Medium Risk:** Acceptable with further evaluation or mitigation if necessary.
- **High Risk:** Unacceptable risk requiring immediate corrective actions and process optimization.

Steps in Risk Assessment:

The AQbD risk assessment process consists of the following stages:

- **Hazard Identification:** Potential sources of variability are identified, including sample characteristics, analytical instrumentation, chromatographic conditions, environmental factors, analyst variability, and sample preparation procedures.
- **Risk Analysis:** Each identified risk is evaluated according to its probability of occurrence and its potential impact on analytical performance.
- **Risk Evaluation:** Risks are prioritized based on their severity and likelihood to determine which variables require further investigation and optimization.
- **Risk Control:** Appropriate mitigation strategies are implemented to minimize the impact of critical risks. These may include optimization of chromatographic conditions, modification of analytical procedures, improved instrument control, personnel training, or implementation of additional quality control measures.
- **Risk Communication:** The identified risks, their potential impact, and implemented control measures are documented and communicated to all relevant stakeholders to ensure effective quality management.
- **Risk Review:** Risk assessment is a continuous process. Periodic reviews are performed throughout the analytical method lifecycle to evaluate the effectiveness of control strategies, monitor method performance, and identify newly emerging risks.

Systematic risk assessment enables analytical laboratories to improve method robustness, enhance accuracy and precision, reduce analytical variability, facilitate successful method transfer, and ensure compliance with regulatory guidelines. Furthermore, it provides the scientific foundation for Design of Experiments (DoE), establishment of the Method Operable Design Region (MODR),[18] and development of an effective analytical control strategy within the AQbD framework.

Risk Assessment Parameters:

1.Critical Quality Attributes (CQAs):

Critical Quality Attributes (CQAs) are the physical, chemical, biological, or microbiological characteristics of a pharmaceutical product or analytical method that must be maintained within predefined limits to ensure product quality, safety, and efficacy. Within the Analytical Quality by Design (AQbD) framework, CQAs are identified based on scientific knowledge, regulatory requirements, intended product use, and quality risk assessment.

CQAs are directly associated with the therapeutic performance of a pharmaceutical product and therefore require continuous monitoring and control throughout the product lifecycle. They are closely linked to Critical Process Parameters[19] (CPPs) and Critical Method Parameters (CMPs), as variations in these parameters can significantly affect product quality and analytical performance. Consequently, validated analytical methods and appropriate specifications are established to monitor CQAs and ensure compliance with regulatory standards. Risk assessment tools such as Failure Mode and Effects Analysis (FMEA) and Ishikawa (Fishbone) diagrams are commonly employed to identify potential risks affecting CQAs and to develop suitable control strategies.

For analytical methods, the major CQAs include:

- ✓ **Accuracy:** Ability of the analytical method to produce results that are close to the true or reference value.
- ✓ **Precision:** Degree of agreement among repeated measurements under specified conditions, expressed as repeatability and intermediate precision.
- ✓ **Specificity/Selectivity:** Ability of the method to accurately measure the analyte in the presence of impurities, degradation products, excipients, or other interfering substances.
- ✓ **Sensitivity:** Capability of the method to detect and quantify low concentrations of the analyte, expressed in terms of the Limit of Detection (LOD) and Limit of Quantification (LOQ).
- ✓ **Linearity:** Ability of the method to provide analytical responses directly proportional to analyte concentration over a specified range.
- ✓ **Robustness:** Capacity of the method to remain unaffected by small, deliberate changes in analytical conditions such as pH, flow rate, mobile phase composition, or column temperature.
- ✓ **Ruggedness:** Reproducibility of analytical performance under different operating conditions, including different analysts, laboratories, instruments, or days.[20]

Critical Method Parameters (CMPs):

Critical Method Parameters (CMPs) are the experimental variables that significantly influence the performance, reliability, and robustness of an analytical method. Proper identification and control of CMPs are essential to ensure that the analytical method consistently produces accurate, precise, and reproducible results throughout its lifecycle.

CMPs are identified during method development using scientific understanding, prior knowledge, and quality risk assessment. Typical CMPs include mobile phase composition, pH, buffer concentration, flow rate, column temperature, injection volume, detection wavelength, and sample preparation conditions. Variations in these parameters can significantly affect chromatographic responses such as retention time, peak shape, resolution, sensitivity, and system suitability.

Within the AQbD approach, CMPs are systematically optimized using Design of Experiments (DoE) and continuously monitored through predefined control strategies and standard operating procedures (SOPs). During method validation, the influence of each CMP on analytical performance is evaluated to establish acceptable operating ranges. Continuous monitoring of CMPs during routine analysis ensures consistent method performance and facilitates reliable method transfer between laboratories.

Design of Experiments (DoE):

Design of Experiments (DoE) is a statistical and systematic methodology employed in AQbD to investigate the effects of multiple experimental variables simultaneously and to optimize analytical methods efficiently. Unlike the traditional one-factor-at-a-time[21] (OFAT) approach, DoE evaluates both the individual and interaction effects of critical factors, thereby providing a comprehensive understanding of the analytical process while minimizing the number of experiments required.

The implementation of DoE generally involves the following steps:

- ✓ **Problem Definition:** Define the Analytical Target Profile (ATP) and identify the analytical responses to be optimized.
- ✓ **Selection of Factors:** Identify critical variables and determine their operating ranges based on prior knowledge and risk assessment.
- ✓ **Experimental Design:** Select an appropriate statistical design for conducting experiments efficiently.
- ✓ **Experimental Execution:** Perform experiments according to the selected design while collecting analytical response data.
- ✓ **Statistical Analysis:** Analyze the results using statistical methods such as Analysis of Variance (ANOVA), regression analysis, and response surface plots to determine significant factors and interactions.
- ✓ **Method Optimization:** Establish optimum operating conditions and define the Method Operable Design Region (MODR).
- ✓ **Model Validation:** Confirm the optimized conditions through verification experiments before routine implementation.

Data Analysis:

The data generated during method validation are analyzed using appropriate statistical techniques to assess method performance and compliance with predefined acceptance criteria. Statistical parameters such as mean, standard deviation, relative standard deviation (RSD), confidence intervals, regression analysis, and analysis of variance[22] (ANOVA), where applicable, are used to evaluate the accuracy, precision, and reliability of the analytical method. The results are compared with the predefined Analytical Target Profile (ATP) to confirm method suitability.

Documentation and Reporting:

All validation activities, experimental observations, analytical results, and statistical evaluations are comprehensively documented in a validation report.The report includes the validation protocol, experimental procedures, acceptance criteria, statistical analysis, obtained results, deviations (if any), and final conclusions regarding method suitability. Proper documentation ensures traceability, regulatory compliance, and serves as a permanent record throughout the analytical method lifecycle.

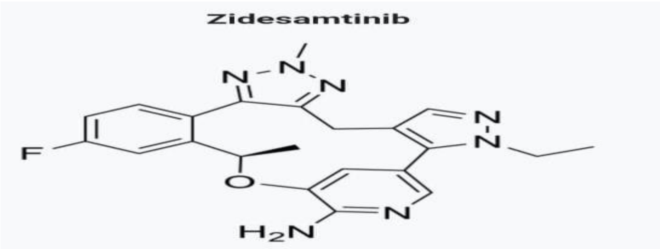
Drug Profile:

Zidesamtinib (JIDEYTRO®):

Zidesamtinib (JIDEYTRO®, formerly NVL-520)[23] is a next-generation, highly selective ROS1 tyrosine kinase inhibitor (TKI) developed for the treatment of **ROS1-positive non-small cell lung cancer (NSCLC)**. ROS1 gene rearrangements occur in approximately **1–2%** of patients with NSCLC and function as oncogenic drivers that promote uncontrolled cellular proliferation and tumor progression.

Although first-generation ROS1 inhibitors, such as crizotinib, demonstrate favorable initial clinical responses, many patients eventually develop acquired resistance, most commonly due to the **ROS1 G2032R resistance mutation**. Zidesamtinib was specifically designed to overcome these resistance mechanisms while maintaining high selectivity for ROS1 and minimizing inhibition of tropomyosin receptor kinase (TRK) proteins, thereby reducing treatment-associated.

STRUCTURE:



Analysis of Zidesamtinib Using UPLC:

Zidesamtinib is analyzed using **Ultra-Performance Liquid Chromatography (UPLC)** as the analyte of interest, while the UPLC system serves as the analytical platform for its separation, identification, and quantification. The drug itself does not perform chromatography; rather, it is the compound being measured to evaluate its purity, assay, degradation products, and related impurities.

Zidesamtinib is a next-generation macrocyclic **ROS1 tyrosine kinase inhibitor[24](TKI)** with a complex chemical structure that requires a highly sensitive and selective analytical technique. UPLC is the preferred method because it employs columns packed with sub-2 µm particles, enabling significantly higher chromatographic resolution, sharper peak shapes, improved sensitivity, and shorter analysis times compared with conventional High-Performance Liquid Chromatography (HPLC). In addition, UPLC minimizes solvent consumption while providing excellent reproducibility, making it well suited for pharmaceutical quality control and stability studies.

Sample Preparation

Before chromatographic analysis, Zidesamtinib must be extracted from its pharmaceutical formulation or biological matrix. For tablet or capsule formulations, the dosage form is accurately weighed, finely powdered, and dissolved in a suitable solvent such as methanol or acetonitrile. The resulting solution is sonicated to ensure complete dissolution and then filtered through a **0.22 µm membrane filter** to remove particulate matter that could damage the UPLC column or interfere with the analysis. The filtered solution is subsequently diluted to the required concentration and transferred into UPLC sample vials for injection.

Analytical Quality by Design (AQbD) Approach:

Analytical Quality by Design (AQbD) provides a systematic, science- and risk-based framework for developing robust analytical methods in accordance with **ICH Q14** and **ICH Q2(R2)** guidelines. In the case of Zidesamtinib, AQbD ensures that the analytical method consistently delivers reliable, accurate, and reproducible results throughout its lifecycle.

1. Analytical Target Profile (ATP):

The first step in AQbD is establishing the **Analytical Target Profile (ATP)**, which defines the intended purpose and required performance characteristics of the analytical method. For Zidesamtinib, the ATP specifies parameters such as assay accuracy, precision, specificity, sensitivity, linearity, robustness, and acceptable limits for quantifying the drug and its related impurities in pharmaceutical formulations.

2. Identification of Critical Method Variables (CMVs):

Potential sources of analytical variability are identified through structured risk assessment tools such as **Ishikawa (Fishbone) diagrams** and **Failure Mode and Effects Analysis (FMEA)**. Critical Method Variables (CMVs) include factors such as mobile phase composition, pH, buffer concentration, column temperature, flow rate, injection volume, gradient profile, and detection wavelength. These variables are evaluated based on their potential impact on chromatographic performance.

3. Design of Experiments (DoE):

Instead of varying one parameter at a time, AQbD employs **Design of Experiments (DoE)** to investigate the simultaneous effects and interactions of multiple analytical variables. Statistical experimental designs are used to optimize chromatographic conditions, ensuring adequate resolution, acceptable retention time, symmetrical peak shape, and high method sensitivity. DoE enables the identification of critical variables while minimizing experimental trials and development time.

4. Method Operable Design Region (MODR):

The optimized experimental data are used to establish the **Method Operable Design Region (MODR)**, which represents the multidimensional range of operating conditions where the analytical method consistently meets predefined performance criteria. Operating within the validated MODR provides regulatory flexibility and allows routine analytical adjustments without requiring complete method revalidation.

Mechanism of Action of Zidesamtinib:

Zidesamtinib exerts its therapeutic activity by selectively inhibiting the **ROS1 receptor tyrosine kinase**,[25] an oncogenic driver involved in the development of ROS1-positive non-small cell lung cancer (NSCLC). By blocking ROS1 phosphorylation, the drug suppresses downstream signaling pathways, including the **MAPK** and **PI3K/AKT/mTOR** pathways, thereby inhibiting tumor cell proliferation and inducing apoptosis.

Unlike first-generation ROS1 inhibitors, Zidesamtinib possesses a macrocyclic molecular structure that effectively inhibits resistance-associated mutations, particularly the **G2032R solvent-front mutation**, which commonly limits the effectiveness of earlier therapies. Furthermore, the drug exhibits excellent penetration across the blood-brain barrier, enabling effective treatment of central nervous system metastases.

An additional advantage of Zidesamtinib is its **TRK-sparing selectivity**. By selectively targeting ROS1 while avoiding significant inhibition of tropomyosin receptor kinase (TRK) proteins, the drug reduces the incidence of neurological adverse effects commonly associated with less selective kinase inhibitors.

Overall, the integration of AQbD principles with UPLC provides a robust, reliable, and regulatory-compliant analytical strategy for the quantitative determination of Zidesamtinib. This systematic approach ensures accurate assay performance, efficient method development, effective quality control, and lifecycle management while supporting the pharmaceutical development of this advanced targeted anticancer therapy.

PHARMACOKINETICS (PK):

Absorption:

Zidesamtinib is administered orally at a recommended dose of **100 mg once daily**

Distribution:

Following absorption, zidesamtinib is widely distributed throughout body tissues. Importantly, it demonstrates excellent penetration across the blood–brain barrier.

Metabolism:

Zidesamtinib is predominantly metabolized in the liver through the cytochrome P450 (CYP450) enzyme system

Excretion: The drug is through the biliary fecal route, with only a small proportion excrected via kidneys.

ADVERSE REACTIONS AND SIDE EFFECTS:

Zidesamtinib is generally well tolerated; however, treatment may be associated with several adverse effects ranging from mild to severe.

Common Adverse Effects:

Commonly reported adverse reactions include:

- ✓ **Peripheral edema:** Swelling of the lower limbs, ankles, and feet due to fluid retention.
- ✓ **Peripheral neuropathy:** Tingling, numbness, burning sensation, or paresthesia affecting the hands and feet.
- ✓ **Gastrointestinal disorders:** Constipation is the most frequently reported gastrointestinal adverse event, although nausea and diarrhea may also occur.
- ✓ **Fatigue:** Persistent tiredness, generalized weakness, and reduced physical endurance.
- ✓ **Dyspnea:** Shortness of breath during routine physical activity.
- ✓ **Musculoskeletal pain:** Pain, stiffness, or discomfort involving muscles, joints, or extremities.

Serious Adverse Reactions:

Serious adverse events require prompt medical evaluation and may necessitate dose interruption, dose reduction, or permanent discontinuation of therapy.

- ✓ **Neurological disorders:** Dizziness, vertigo, impaired balance (ataxia), and reduced motor coordination.
- ✓ **Cognitive impairment:** Memory loss, confusion, decreased concentration, and slowed cognitive function.
- ✓ **Psychiatric effects:** Anxiety, depression, mood alterations, hallucinations, sleep disturbances, and, in rare cases, suicidal ideation.
- ✓ **Interstitial Lung Disease (ILD)/Pneumonitis:** A potentially life-threatening inflammatory lung condition characterized by persistent cough, chest discomfort, and worsening dyspnea.
- ✓ **Hepatic and pancreatic toxicity:** Elevations in liver enzymes (ALT and AST) and pancreatic enzymes (lipase and amylase) may indicate drug-induced liver injury or pancreatitis, requiring regular laboratory monitoring.

Patient Risk Factors and Contraindications:

Careful patient selection and continuous clinical monitoring are essential during zidesamtinib therapy, particularly in individuals with pre-existing medical conditions.

- ✓ **Cardiovascular disorders:** Patients with congenital long QT syndrome, severe congestive heart failure, bradycardia, or other significant cardiac abnormalities have an increased risk of QT interval prolongation and potentially life-threatening arrhythmias.
- ✓ **Electrolyte abnormalities:** Hypokalemia and hypomagnesemia should be corrected before initiating therapy, as these conditions significantly increase the risk of QT prolongation.
- ✓ **Hepatic or pancreatic impairment:** Patients with active liver disease, impaired hepatic function, or a history of chronic pancreatitis require close monitoring because the drug is primarily metabolized in the liver and may induce hepatic or pancreatic toxicity.

Overall, zidesamtinib demonstrates favorable pharmacokinetic properties, including excellent oral absorption, extensive tissue distribution, effective CNS penetration, hepatic metabolism, and predominantly biliary excretion. Although generally well tolerated, appropriate monitoring for neurological, pulmonary, hepatic, pancreatic, and cardiovascular adverse effects is essential to ensure safe and effective treatments.

Conclusion:

The **Analytical Quality by Design (AQbD)** approach provides a systematic, science-based, and risk-oriented strategy for developing a robust **UPLC (Ultra Performance Liquid Chromatography)** method for the analysis of **Zedisamtinib**. By identifying the **Analytical Target Profile (ATP)**, **Critical Method Attributes (CMAs)**, and **Critical Method Parameters (CMPs)**, the method can be optimized to achieve consistent performance throughout its lifecycle.

Using **Design of Experiments (DoE)** allows the simultaneous evaluation of factors such as mobile phase composition, flow rate, column temperature, and pH, helping establish a **Method Operable Design Region (MODR)** where the method remains reliable. The optimized UPLC method offers **high sensitivity, excellent resolution, short analysis time, improved precision, and enhanced robustness**, making it suitable for routine quality control and stability studies.

Overall, the AQbD-based UPLC method for **Zedisamtinib** ensures compliance with regulatory expectations (such as ICH Q2(R2), ICH Q14, and USP guidance), minimizes method variability, reduces the need for revalidation, and supports efficient pharmaceutical development and quality assurance. This approach results in a reliable, accurate, precise, and cost-effective analytical method for the quantitative estimation of Zedisamtinib in pharmaceutical formulations.

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