CHAPTER 1

Analytical Procedure Development

1.1 Introduction

Drug analysis serves as the foundation for determining the properties of a product. Often, there is a time gap between a drug's introduction to the market and its inclusion in pharmacopoeias. This delay can occur due to factors such as uncertainties regarding widespread usage, reports of new toxicities, development of patient resistance, and the introduction of superior drugs by competitors. Consequently, standard analytical procedures for these drugs may not be available in pharmacopoeias, necessitating the development of new analytical methods.

The reasons for developing novel analytical methods are as follows:

1. The new drug/drug combination may not be officially recognized in any pharmacopoeias.
2. Adequate analytical procedures for the drug may not be documented in the literature due to patent regulations.
3. Analytical methods may not be available for the drug's formulation excipients.
4. Analytical methods for the drug in combination with other drugs may be lacking.
5. Analytical methods for quantifying the drug in biological fluids may not exist.
6. Existing analytical procedures may require expensive reagents and solvents and involve complex extraction and separation techniques, which can compromise reliability.

An effective analytical approach not only ensures that the drug attains the desired therapeutic quality but also functions as a means to verify purity throughout every phase of the product development process. The diagram presented below (Fig. 1.) offers a comprehensive outline of the process involved in developing an analytical method, highlighting the method goals, analytic goals, validation requirements, and documentation requirements at various stages of drug development.

Drug Development Stage	Goal		Requirements	
	Method	Analysis	Validation	Documentation
Early Development	Effective, reliable	Robustness	Not required[#]	GDP
Non-Clinical Studies	Rigorous	Validation elements[@]	Not required[$]	GDP
Manufacturing (Phase-I & II)	Validated	Precision & Robustness	Required*	Protocols & Reports*
Manufacturing (Phase-III & VI)	Validated	Precision & Robustness	Required	Protocols & Reports

#-unless requested by Client; @-appropriate; $- unless requested by Study director; *-by Phase-II

Fig. 1. Analytical method development process – Goals & Requirements.

ICH Q2 defines the analytical procedure as the way of performing the analysis. Its description should include in detail the steps necessary to perform each analytical test. Analytical procedures or methods are mainly intended to determine the identity, purity, and effectiveness of drug substances or pharmaceutical formulations. A variety of procedures are used for analysis, from simple weighing to advanced techniques using highly specialized instrumentation. Analytical methods are classified into two types: Classical and Instrumental.

1.2 Classical Methods of Analysis

They refer to traditional techniques that have been used for a long time in chemical analysis. These methods rely on various chemical reactions and observations to determine the composition or concentration of analytes in a sample. Some common classical methods include volumetric and gravimetric analysis.

1. **Volumetric Analysis:** Volumetric methods involve measuring the volume of a reagent solution required to react with the analyte.
   These methods are often based on stoichiometric relationships between the analyte and the reagent, allowing for the determination of the analyte's concentration.
2. **Gravimetric Analysis:** This method involves the determination of analyte concentration based on the measurement of mass.
   It often involves the formation of a precipitate, which is then weighed to calculate the analyte's concentration.

1.3 Instrumental Methods of Analysis

They involve the use of advanced instruments and equipment to measure various physical or chemical properties of analytes. These methods offer increased sensitivity, precision, and speed compared to classical methods. Some common instrumental methods include spectroscopy, chromatography and electroanalytical methods.

1. **Spectroscopy:** Spectroscopic methods involve the measurement of the interaction between analytes and electromagnetic radiation. Examples include UV-Vis spectroscopy, infrared spectroscopy (IR), atomic absorption spectroscopy (AAS), and nuclear magnetic resonance spectroscopy (NMR).
2. **Chromatography:** Chromatographic methods separate and analyze analytes based on their distribution between a stationary phase and a mobile phase. Common types of chromatography include gas chromatography (GC), liquid chromatography (LC), and high-performance liquid chromatography (HPLC).
3. **Mass Spectrometry:** Mass spectrometry measures the mass-to-charge ratio of ions produced from analytes. It provides information about the molecular weight, structure, and composition of analytes. Techniques like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are widely used.
4. **Electrochemical Methods:** Electrochemical techniques measure the electrical properties of analytes. Examples include voltammetry, potentiometry, and coulometry. These methods are often used in the analysis of electroactive species or to study redox reactions.

Instrumental methods offer advantages such as high sensitivity, selectivity, and the ability to analyze complex mixtures. They often require specialized instrumentation and data analysis techniques but provide accurate and precise results.

1.4 Analytical Procedure Development

Along with Validation and transfer, analytical procedure development plays a crucial role in the process of drug development and manufacturing. Its primary objective is to establish the identity, purity, physical properties, and potency of drugs, including their bioavailability and stability. This involves ensuring that the analytical procedures are adequate for assessing drugs, particularly the active pharmaceutical ingredient (API). Analytical procedures are designed to test specific characteristics of substances against predetermined acceptance criteria. Analytical techniques widely include the estimation of physical, chemical, physicochemical, and/or biological

parameters of the analyte substance. Therefore, analytical method development entails evaluating and selecting the most precise assay procedures for determining the composition of a drug. When developing a new analytical procedure, the choice of analytical instruments and methodologies should align with the intended purpose and scope of the method. The release of product into the market is decided based on the final quality control results of the finished product accompanied by other data of the batch. Chromatographic methods, including High-performance liquid chromatography (HPLC), Gas chromatography (GC), High-performance thin layer chromatography (HPTLC), and supercritical fluid chromatography (SFC), are extensively recognized for their numerous benefits compared to non-chromatographic approaches. These techniques are versatile, resilient, and demand smaller sample quantities. Through automation, they also mitigate the potential for human errors.

1.5 Approaches to Analytical Procedure Development

The foremost concern of an analytical chemist is to formulate an analytical procedure that precisely fulfills its intended purpose. This procedure's development holds immense significance within the drug development journey. Two distinct approaches, OFAT and AQbD, are employed for crafting these analytical methods. A comparative overview of these approaches' key characteristics is provided in Table 1.1. To avert potential risks or shortcomings, it is vital to implement the Quality by Design (QbD) principles as outlined in the ICH guideline Q8 (R2). Analytical Quality by Design (AQbD) refers to the process of integrating QbD principles into the development of analytical procedures. AQbD assists in establishing a dependable and cost-effective analytical protocol that can be consistently utilized throughout the product's lifecycle to facilitate regulatory adaptability. By embracing AQbD principles, a deeper comprehension of the impact of diverse factors on procedure performance can be achieved.

Table 1.1. OFAT & AQbD Key features.

Approach 1 (OFAT) ONE FACTOR AT A TIME	Approach 2 (AQbD) ANALYTICAL QUALITY BY DESIGN
It relies on the trial-and-error approach, as observed in OFAT studies, where a single parameter is optimized for the desired response, while keeping other variables constant.	It systematically applies scientific knowledge to method implementation, beginning with method quality, linking risk assessment in method selection, considering method parameters and outcomes, and ultimately identifying a robust and cost-effective approach.

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Approach 1 (OFAT) ONE FACTOR AT A TIME	Approach 2 (AQbD) ANALYTICAL QUALITY BY DESIGN
It results in a highly robust method for the instrumental variables employed during the method development phase.	DoE is a component of AQbD, illustrating the interplay among input variables that ultimately influence the method response and outcomes.
A revalidation protocol is necessary whenever there's a method transfer or during the development of an alternative method.	It doesn't necessitate a revalidation protocol post method transfer or during alternative method development.
It carries a high risk of method failure.	It carries a low risk of method failure,
It leads to an escalation in method costs.	It diminishes the necessity and expense of method redevelopment and revalidation.
It is not a favored strategy for method development, as it fails to achieve regulatory flexibility and can lead to undesirable outcomes.	It is a recommended and preferred strategy in method development to achieve regulatory flexibility and mitigate occurrences of Out of Specification (OOS), Out of Trend (OOT), and out-of-control (OOC) results.

1.6 Minimal Approach

In recent times, method failure has become more prevalent, particularly during method transfers and within quality control departments. This trend is thought to stem from the leniency in robust test compliance granted by the ICH Q2 guidelines. In present-day procedures, chromatographic methods are the dominant choice for accurate analytics at all stages of the product life cycle. Common analytical techniques for content uniformity, assay, impurity profiling, and stability-indicating assays rely on HPLC, UPLC, or rapid resolution liquid chromatography (RRLC) methods.

In the context of chromatography, the intricate parameters involved in method development, combined with challenges like low sensitivity, selectivity, and a limited grasp of the relationship between method performance and parameters, have led to the consistent recommendation of revalidation protocols. Conversely, current practices often hinge on the implementation of analytical methods through one-Variable-at-a-time (OVAT) approaches, where only one parameter is optimized while others remain constant. This approach consistently results in a narrow robust behavior of the method concerning the instrumental variables used during the method development phase.

Analytical procedure development should encompass the following essential elements:

- Identification of the specific attributes of the drug substance or drug product that require testing through the analytical procedure.
- Selection of an appropriate analytical procedure technology and the associated instruments or suitable apparatus.
- Execution of comprehensive development studies to assess the performance characteristics of the analytical procedure. This evaluation should cover aspects like specificity, accuracy, and precision across the reportable range, including calibration model, limits at both lower and higher range extremes, and robustness.
- Establishment of a comprehensive analytical procedure description, which includes the analytical procedure control strategy. This encompasses parameter settings and system suitability criteria.

1.7 Enhanced Approach

The current analytical method development strategy, i.e., One-Variable-at-a-Time (OVAT), carries a substantial risk of method failure. It consistently necessitates revalidation protocols post method transfer or when alternative methods are developed, thereby escalating method costs.

In contrast, Analytical Quality by Design (AQbD) employs scientific insights throughout method implementation, commencing with product quality assessment that links risk assessment in method selection. It extends to the correlation between method parameters and expected outcomes, culminating in the identification of a zone for a robust and cost-effective approach. Design of Experiments (DoE) is a component of AQbD, illustrating how input variables interact to impact method responses and results.

At this juncture, adopting the AQbD paradigm as the preferred and recommended strategy for analytical procedure development offers several benefits. These include achieving regulatory flexibility, curbing occurrences of Out of Specification (OOS), Out of Trend (OOT), Out of Control (OOC), and Out of Statistical Control (OOSC) results, attaining a high level of robustness, and fostering cost-effective analytical methods.

The enhanced approach presents a systematic methodology for developing and refining knowledge about an analytical procedure. This approach incorporates additional elements alongside those described in the minimal approach. These elements include:

- Assessing sample properties and anticipated sample variability based on an understanding of the manufacturing process.
- Defining the Analytical Target Profile (ATP).
- Performing risk assessments and leveraging prior knowledge to identify analytical procedure parameters that could influence procedure performance.
- Conducting uni- or multi-variate experiments to explore the ranges and interactions of identified analytical procedure parameters.
- Defining an analytical procedure control strategy founded on a heightened comprehension of the procedure, encompassing appropriate set points and/or ranges for pertinent analytical procedure parameters to meet performance criteria.
- Outlining a lifecycle change management plan with clear definitions and reporting categories for Established Conditions (ECs), Proven Acceptable Ranges (PARs), or Method Operational Design Regions (MODRs) as applicable.
- Employing aspects of the enhanced approach during development can lead to more robust analytical procedures, a deeper grasp of the impact of analytical procedure parameters, and enhanced flexibility for lifecycle management, including broader operating ranges and a more suitable set of ECs and associated reporting categories for changes.

The enhanced approach brings several potential advantages, such as:

- Discerning the essential analytical procedure attributes for procedure performance.
- Utilizing predefined performance characteristics (e.g., within the ATP) linked to Critical Quality Attributes (CQAs) and their acceptance criteria, enabling purpose-oriented protocols for analytical procedure validation and future comparisons between current and new analytical procedures/technologies.
- Enhancing analytical procedure control, resulting in more dependable operation.
- Enabling preventive measures and facilitating ongoing improvement through greater analytical procedure knowledge.
- Reducing the effort required across the analytical procedure lifecycle.

The advantages of QbD in method development can be summarized as

- Increased understanding of method and its control.
- Moving beyond traditional ICH method validation protocols.
- Increased adaptability for analyzing Active Pharmaceutical Ingredients (API), impurities in dosage forms, stability samples, and metabolites in biological samples.
- Diminished variability in analytical attributes to enhance method robustness.
- Maintaining analytical attribute values within pharmacopoeial monograph limits, avoiding Out of Specification (OOS) thresholds.
- Streamlining method transfers to production scale.
- No need for re-validation within Method Operational Design Regions (MODRs).
- Eradicating batch failures, curtailing deviations, and minimizing resource-intensive investigations.

QbD has gained importance in the area of pharmaceutical processes like drug development, formulations, analytical procedure development and biopharmaceuticals. Table 1.2. enlist the differences between traditional method, product QbD and analytical QbD.

Table 1.2. Conventional approach vs product development QbD vs analytical QbD.

Parameter	Traditional	Product QbD	Analytical QbD
Approach	Based on empirical approach	Based on systematic approach	Based on built-in systematic approach
Quality	Quality assurance is achieved through end product testing.	Quality is instilled within the product and process through deliberate design and a scientific approach.	Robustness and reproducibility of the method are established during the method development stage.
FDA Submission	Incorporating solely data for submission.	Submitting with comprehensive product knowledge and process understanding.	Submitting with thorough product knowledge and ensuring through the Analytical Target Profile (ATP).

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Parameter	Traditional	Product QbD	Analytical QbD
Specifications	They are derived from the history of batches.	They are derived from the demands of product performance	They are determined according to method performance against ATP criteria.
Process	The process is locked in and resistant to changes.	A flexible process with defined design space enables ongoing enhancements.	Method adaptability using Method Operational Design Regions (MODR) facilitates continuous improvement.
Targeted Response	Prioritizing reproducibility while disregarding variation.	Prioritizing robustness, which comprehends and manages variation.	Emphasizing a method that is both robust and cost-effective.
Advantage	Limited and simple	It serves as an extended Process Analytical Technology (PAT) tool, eliminating the necessity for end-product testing.	Substituting the requirement for revalidation while minimizing occurrences of OOT and OOS outcomes.

Applications of QbD to analytical methods

QbD can be applied to the various analytical methods such as

- Chromatographic techniques like HPLC, HPTLC, UPLC (For method development, stability-indicating studies, and determination of impurities in pharmaceuticals).
- Hyphenated techniques like LC-MS, GC-MS etc
- Advanced techniques like mass spectrometry, and capillary electrophoresis.
- Karl Fischer titration for determination of moisture content in the samples.
- Vibrational spectroscopy (IR) for identification and quantification of compounds.
- Dissolution studies by Spectroscopy techniques.

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