APPLICATION OF ANALYTICAL TOOLS AND TECHNIQUES FOR ESTIMATION OF ATAZANAVIR AND COBICISTAT

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ABSTRACT

Atazanavir (ATV) and Cobicistat (COBI) have emerged as essential components in combination antiretroviral therapy (cART) for the treatment of HIV infection. Despite their widespread clinical adoption, there exists a paucity of bioanalytical data regarding the accurate measurement of these compounds in plasma and other biological matrices. This review comprehensively evaluates the bioanalytical applications of liquid chromatographic techniques coupled with mass spectrometry (LC/MS), focusing on the most relevant methodologies for quantifying ATV and COBI. The document begins with an overview of the pharmacological properties and clinical applications of these drugs, followed by a detailed discussion of LC-MS and LC-MS/MS methodologies, emphasizing sample extraction and analysis from plasma. ATV functions as a selective HIV-1 protease inhibitor, while COBI serves as a potent cytochrome P450 3A4 inhibitor, specifically utilized to enhance the pharmacokinetics of ATV in a fixed-dose combination tablet. This fixeddose combination is notable since COBI alone lacks antiviral efficacy, underscoring the complementary roles of both agents in HIV treatment. The recent introduction of these drugs into the market necessitates the development of reliable and sensitive analytical methods to support clinical and preclinical pharmacokinetic studies, thereby advancing the therapeutic management of HIV infection.

KEYWORDS: Atazanavir, Cobicistat, CART, HPLC, UV, Spectroscopy

1. INTRODUCTION TO ATAZANAVIR AND COBICISTAT

Atazanavir (ATV) and Cobicistat (COBI) have recently become widely accepted. They are commonly prescribed as part of combination antiretroviral therapy (cART) regimens, which patients continue to take throughout their lives. Nevertheless, there is still only limited bioanalytical data available regarding the measurement of these drugs in plasma and other biological matrices, although several assays have been developed for drug quantitation in urine and accumulated hair [1-3]. A thorough examination of the bioanalytical uses of liquid chromatographic techniques combined with mass spectrometry (LC/MS) was conducted, resulting in the compilation of the most pertinent methodologies.

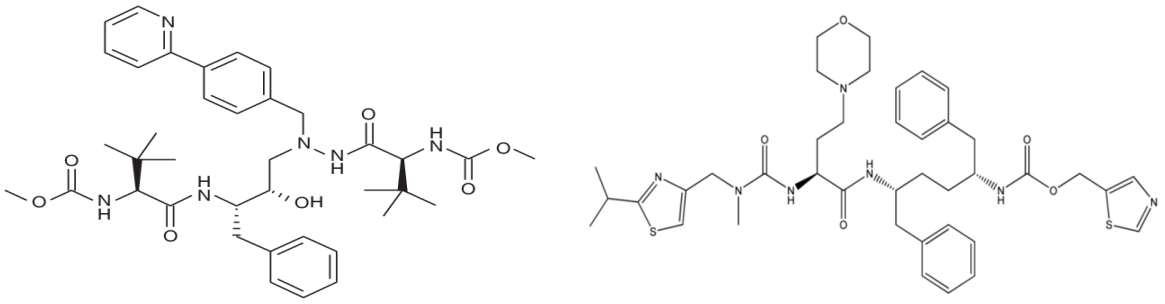
This review starts with a brief introduction of the analytes and their main applications followed by the description of LC-MS and LC-MS/MS methodologies from a practical and regulatory point of view. Due to the more systemic use of drugs, special attention is paid to the sample extraction and analysis from plasma [4-9].

Atazanavir (ATV) is a potent and selective human immunodeficiency virus (HIV)-1 protease inhibitor (PI) [10]. Cobicistat (COBI) is a potent and selective cytochrome P450 3A4 inhibitor used as a booster in combination with ATV [11]. Both ATV and COBI received approval as part of a fixed combination tablet containing ATV 300 mg, COBI 150 mg, and emtricitabine 200 mg and tenofovir alafenamide 25 mg, marketed as a treatment for patients with HIV infection. This fixed-dose combination (FDC) is interesting considering that monotherapy with COBI is not successful in managing HIV infection, while ATV is an effective HIV-1 PI that is not effective in the inhibition of CYP3A4; therefore, both drugs have complementary roles for the FDC [12]. Furthermore, COBI is the first booster approved that does not have any antiviral effect and is used solely for pharmacokinetic enhancement of another antiretroviral agent. The recent market launch of both drugs has increased the need for reliable and sensitive quantitative methods to facilitate their clinical and preclinical pharmacokinetic studies.

1.1. CHEMICAL STRUCTURES AND PROPERTIES

Atazanavir and cobicistat are antiretroviral agents used in the treatment of HIV-1 infection. Atazanavir is an azapeptide protease inhibitor of HIV-1 and HIV-2 viruses. It is chemically described as methyl N-[(1S)-1-{N'-[(2S,3S)-2-hydroxy-3-[(2S)-2-[(methoxy carbonyl) amino]-3,3-dimethyl butanamido]-4-phenyl butyl]-N'-{[4-(pyridin-2yl) phenyl] methyl} hydrazine carbonyl}-2,2-dimethyl propyl] carbamate. It has a molecular weight of 675 g/mol. Atazanavir, a potent protease inhibitor containing a reversible covalent bond, was shown to inhibit replication of both HIV-1 and HIV-2 in MT-2 cells with EC50 values (early effect, 0-12 h) of 0.67 and 0.52 nM, respectively. Cobicistat co-administered with atazanavir in fixed-dose combination reduces gastric pH, allowing greater drug absorption [13].

Cobicistat is a selective cytochrome P450 3A (CYP3A) inhibitor used in combination with another antiretroviral drug. It is the only HIV protease inhibitor substrate to be detected in human plasma, urine, and other biological matrices. Cobicistat is identified as a widely used non-catalytic chemical scaffold inhibitor of CYP3A4. Cobicistat is chemically described as 1,3-thiazol-5-ylmethyl (2R,5R)-5-{[(2S)-2-({[(2-isopropyl-1,3-thiazol-4-yl)methylbutanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate. It's molecular weight is 569.73 g/mol. Cobicistat constitutes a 1:1 margin of safety for oral drug displacement studies designed to exhaustively establish the interactions of drug candidates in clinical development [14].



A **B**

Fig: - Chemical Structure for Atazanavir (A) & Cobicistat (B)

2. IMPORTANCE OF ANALYTICAL ESTIMATION

Analytical method development and validation (AMDV) are critical processes in the pharmaceutical industry and other scientific fields, ensuring that analytical techniques are robust, reliable, and suitable for their intended purposes. This discussion highlights the importance of AMDV, its key components, and its implications for drug development and quality assurance [15-16].

Importance of Analytical Method Development

- **Establishing Methodology**

Analytical method development involves creating and refining techniques to accurately measure and analyze the components of a product. This can involve both creating brand-new techniques and refining already-existing ones. Making sure the techniques are suitable for the particular properties of the substances being tested—such as their identity, purity, potency, and stability—is the aim[17-19].

- **Regulatory Compliance**

Validation of these methods is not just a best practice; it is a regulatory requirement. Regulatory authorities worldwide mandate that analytical methods used in clinical trials and for marketing authorization must be validated to demonstrate their accuracy, specificity, precision, and robustness. This is essential for gaining approval for new drugs and ensuring that they are safe and effective for patient use [20].

- **Quality Assurance**

The reliability of analytical methods directly impacts the quality of pharmaceutical products. Validation establishes that a method consistently produces results that meet predefined criteria, which is vital for quality control and assurance throughout the drug development process. This includes assessing active pharmaceutical ingredients (APIs), excipients, and degradation products to ensure that they meet safety and efficacy standards [21-22].

Key Components of Analytical Method Validation

Analytical method validation involves several critical parameters:

- ✓ **Accuracy:** The closeness of the measured value to the true value.
- ✓ **Precision:** The extent to which measurements made again under the same circumstances yield identical findings.
- ✓ **Specificity: The method's capability of measuring the analyte when other components are present**.
- ✓ **Limit of Detection (LOD) and Limit of Quantification (LOQ):** the lowest analyte concentration that can be accurately measured or identified.
- ✓ **Robustness:** The method's ability to stay unaffected by slight changes in its parameters.
- ✓ **System Suitability Testing:** Prior to analysis, make sure the analytical system is operating properly.

Steps in Method Development and Validation

- ✓ **Assessment of Existing Methods:** Determine if current methods are sufficient or if new methods need to be developed.
- ✓ **Experimentation:** Conduct experiments to test the new or improved methods against established standards.
- ✓ **Theory Application:** Utilize theoretical frameworks to predict outcomes and analyze data.
- ✓ **Real-World Application:** Apply the methods to actual samples to validate their effectiveness

2.1. ROLE IN PHARMACEUTICAL INDUSTRY

In order to guarantee that medications are high-quality, safe, and effective, analytical technique development and validation are essential to the pharmaceutical sector.. These processes are integral to the overall drug development lifecycle, from initial research through to clinical trials and manufacturing [23]. An overview of their significance, processes involved, and regulatory considerations are narrated below.

Regulatory Guidelines

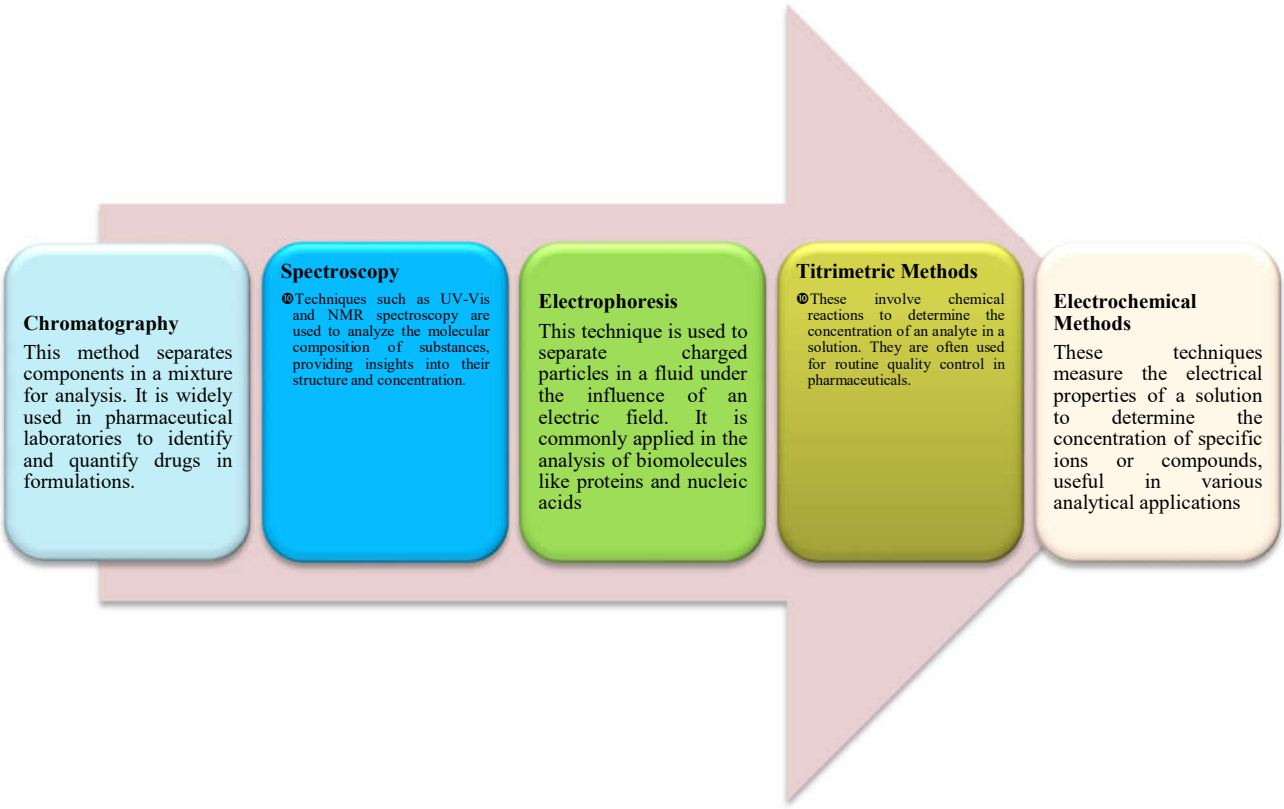
The validation of analytical methods is governed by guidelines established by various regulatory bodies. These include:

- ❖ **ICH Q2(R1):** Offers instructions for confirming analytical methods.
- ❖ **FDA Guidance for Industry:** outlines standards for drug and biologic analytical processes and method validation.

These guidelines help standardize the validation process across the industry, ensuring that all pharmaceutical products meet safety and efficacy standards.

3. ANALYTICAL TECHNIQUES FOR ESTIMATION

Pharmaceutical Analysis Techniques:



The choice of analytical technique for estimation depends on the specific requirements of the project or analysis being conducted. Each method has its strengths and limitations, and often, a combination of techniques is employed to achieve the most accurate and reliable results [24-25]. Understanding these techniques is crucial for effective project management, data analysis, and pharmaceutical research.

3.1. CHROMATOGRAPHIC TECHNIQUES

Sl No.	Drug	Method	Description	Reference
1	Atazanavir	RP-HPLC	Range of linearity: 120-360 ìg/ml %RSD: 0.083% Detection limit: 0.06ìg/ml Quantification limit: 0.18ìg/ml	26

	Cobicistat		**Range of linearity: 60-180 ìg/ml %RSD: 0.054% Detection limit: 0.075 ìg/ml Quantification limit: 0.225 ìg/ml**	
2	**Atazanavir**	**RP-HPLC**	**Range of linearity: 10-30 µg/mL Correlation co-efficient: 0.998 %RSD: 0.816**	**27**
	Cobicistat		**Range of linearity: 5-15 µg/mL Correlation co-efficient: 0.998 %RSD: 1.063**	
3	Atazanavir	**RP-HPLC**	**Range of linearity: 30-90 mcg %RSD: 98-102% Retention Time: 3.576 min**	**28**
	Cobicistat		**Range of linearity: 15-45 mcg %RSD: 98-102% Retention Time: 6.592 min**	
4	Atazanavir	**RP-HPLC**	**Range of linearity: 45-135 µg/mL %RSD: 0.290-0.401% % Recovery: 99.311-100.342%**	**29**

	Cobicistat		**Range of linearity: 22.5-67.5 µg/mL %RSD: 0.290-0.401% % Recovery: 99.311-100.342%**	
5	Atazanavir	**RP-HPLC**	**Range of linearity: 100-1200 µg/ml %RSD: 0.054% Detection limit: 0.5 µg/ml Retention Time: 6.113 min**	**30**
	Cobicistat		**Range of linearity: 50-600 µg/ml %RSD: 0.054% Detection limit: 0.25 µg/ml Retention Time: 3.606 min**	
6	Atazanavir	**UPLC**	**Range of linearity: 75 µg/ml-450 µg/ml %RSD: 0.04 % Recovery: 99.74% Detection limit: 0.76 Quantification limit: 2.30 Retention Time: 0.536 min**	**31**
	Cobicistat		**Range of linearity: 37.5 µg/ml-225 µg/ml %RSD: 0.6 % Recovery: 99.34% Detection limit: 0.5 Quantification limit: 1.11**	

			Retention Time: 1.366 min	

3.2. SPECTROSCOPIC TECHNIQUES

Sl No.	Drug	Method	Description	Reference
1	**Atazanavir**	**UV**	**Range of linearity: 4-20 µg/mL Wavelength: 283 nm %RSD: <2 Accuracy: 98-102% Detection limit: 0.228 µg/mL Quantification limit: 0.692 µg/mL**	**32**
	Cobicistat		**Range of linearity: 2-10 µg/mL Wavelength: 310 nm %RSD: <2 Accuracy: 98-102% Detection limit: 0.0518 µg/mL Quantification limit: 0.157 µg/mL**	
2	**Atazanavir**	**UV**	**Range of linearity: 15-75 µg/ml Wavelength: 292.5 nm %RSD: <2 %Recovery: 100.79 Assay: 100.46% Limit of Detection: 2.651 Quantification limit: 5.750**	**33**

	Cobicistat		**Range of linearity: 7.5-37.5 µg/mL Wavelength: 239.3 nm %RSD: <2 %Recovery: 100.84 Assay: 99.61% Limit of Detection: 0.795 Quantification limit: 1.7525**	

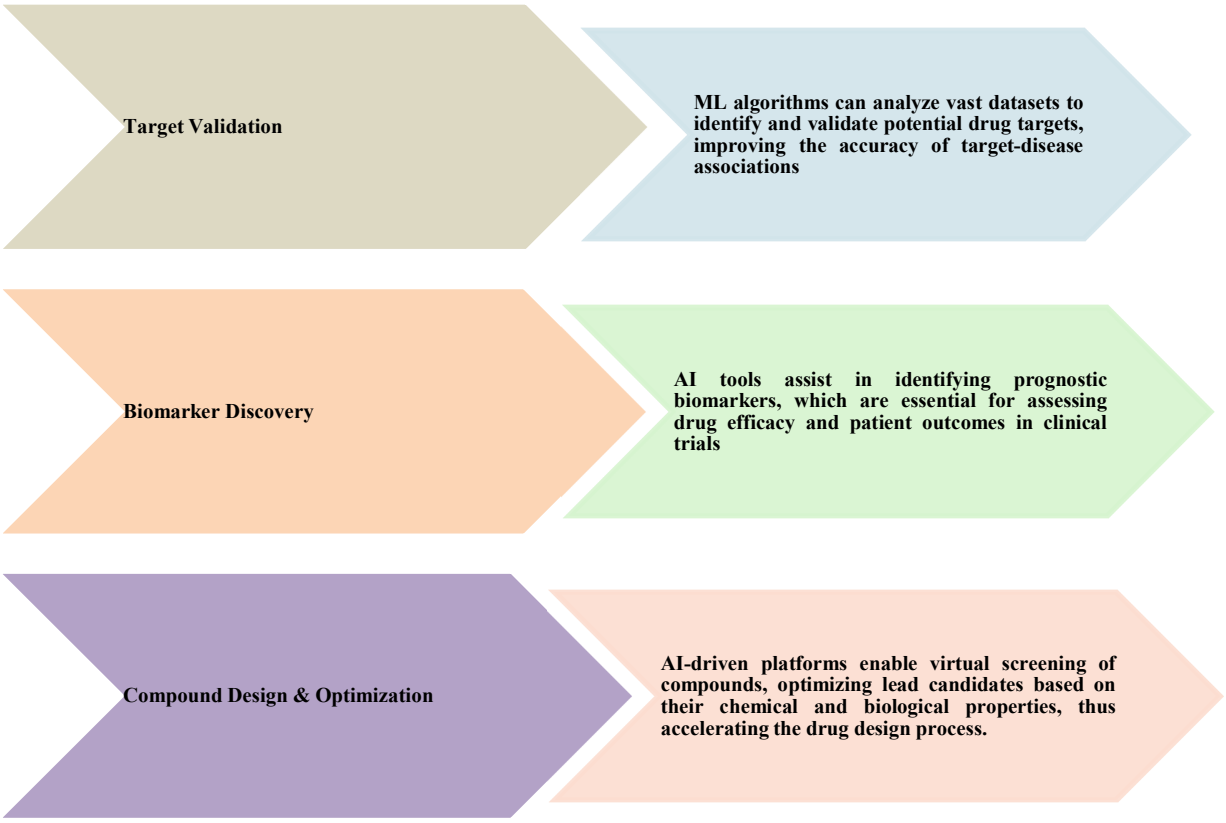
4. APPLICATIONS OF ANALYTICAL TOOLS IN DRUG DEVELOPMENT

Analytical tools play a crucial role in drug development, enhancing various stages from discovery to clinical trials. This overview highlights key applications of analytical methods and technologies in the pharmaceutical industry [34].

Applications of Analytical Tools in Drug Development

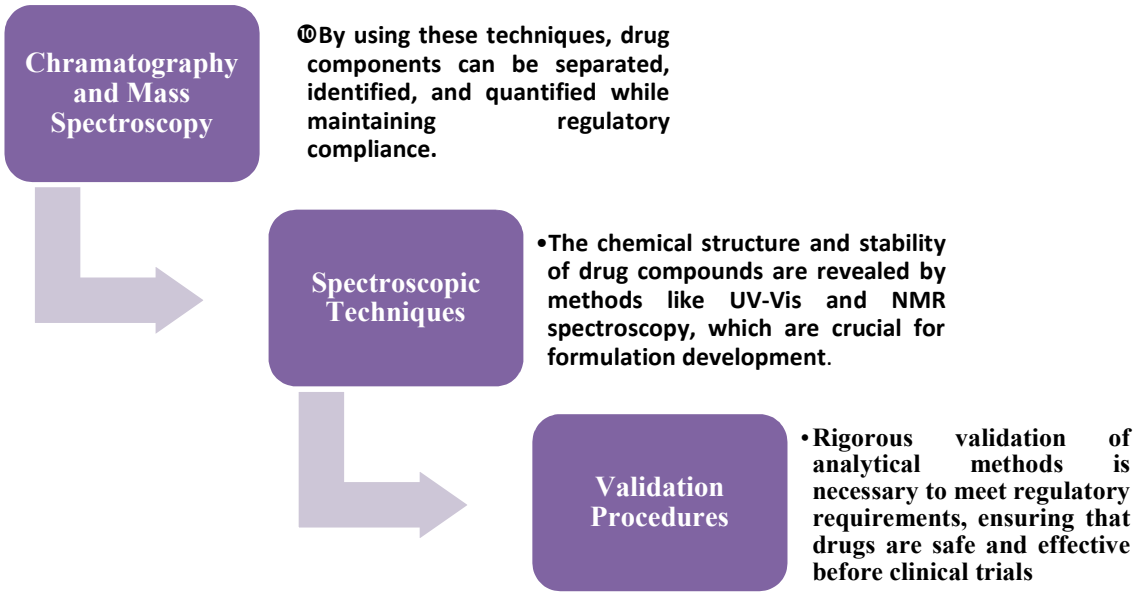
A. Human Intelligence and Machine Learning

Drug development and discovery procedures are increasingly incorporating artificial intelligence (AI) and machine learning (ML) [35]. These technologies facilitate:



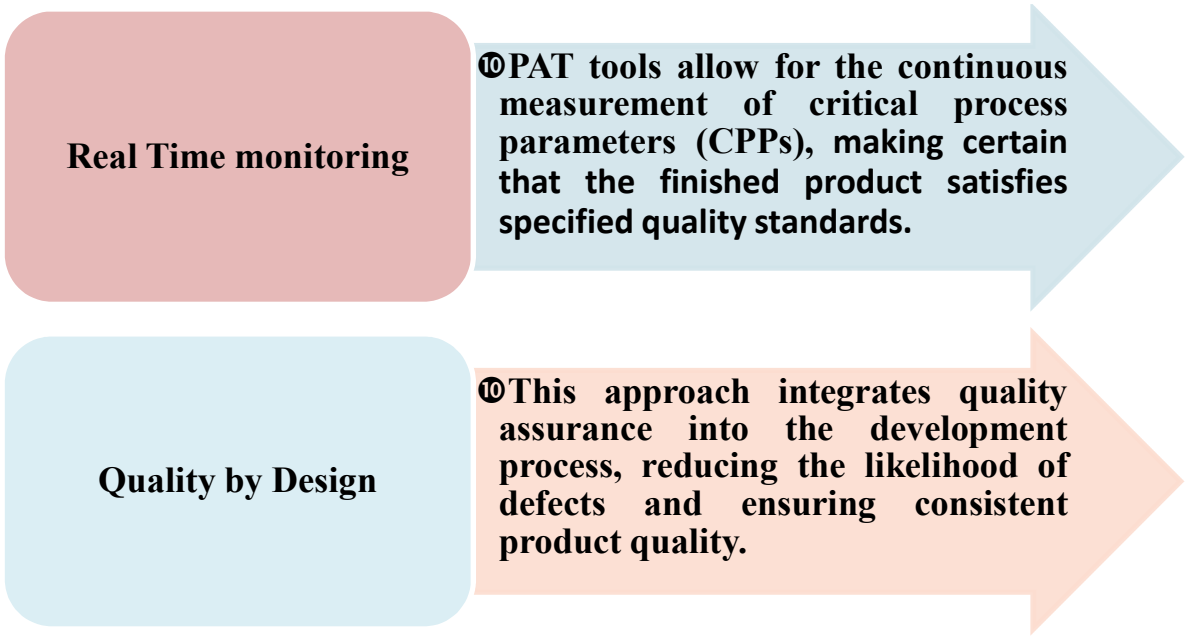
B. Analytical Method Development

Analytical methods are vital for ensuring drug quality and safety. Key techniques include:



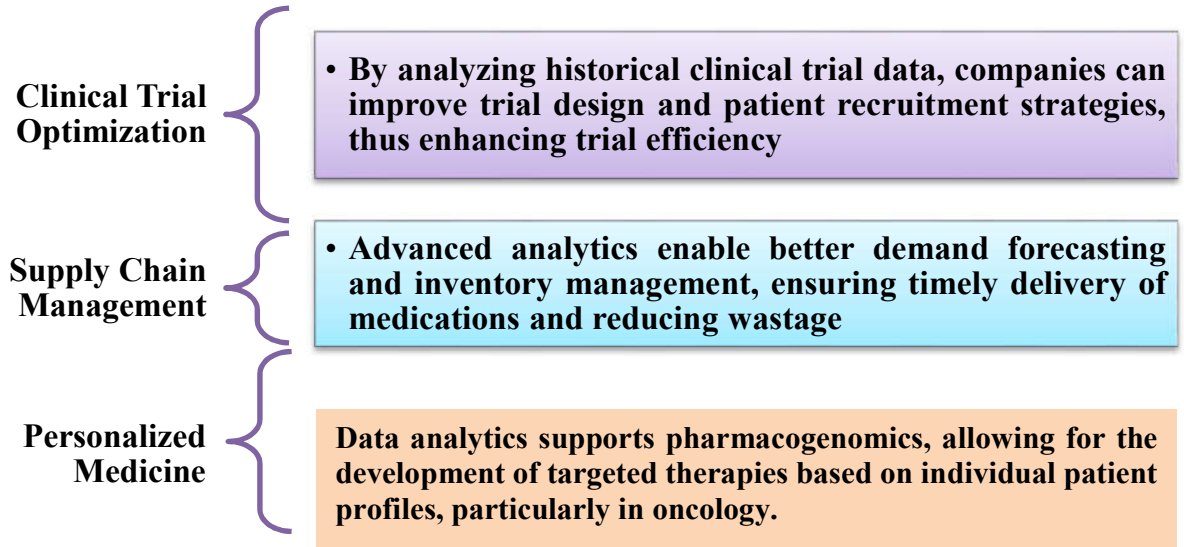
C. Process Analytical Technology (PAT)

PAT is a methodology that enhances the quality and efficiency of pharmaceutical manufacturing. It involves:



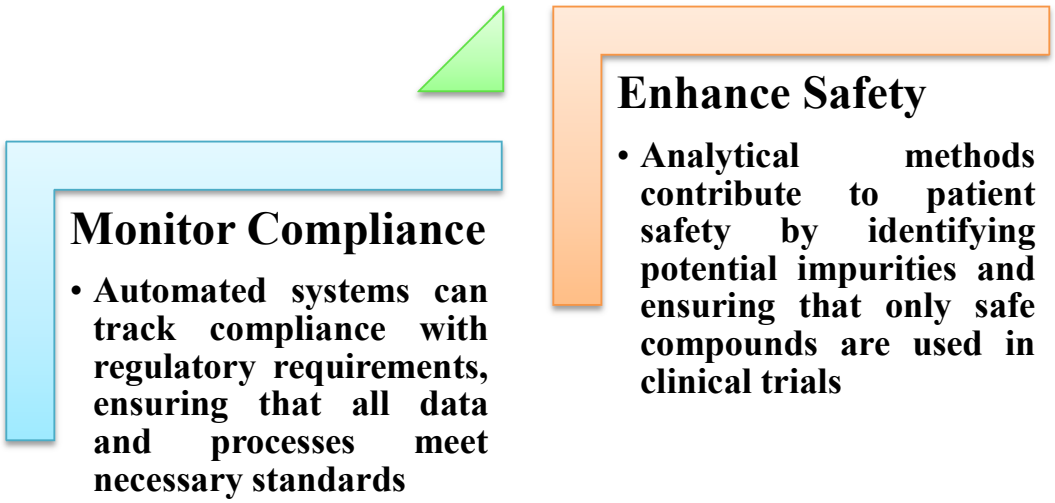
D. Data Analytics

Pharmaceutical companies leverage data analytics to inform various aspects of drug development:



E. Quality Control and Compliance

Ensuring compliance with stringent regulatory standards is paramount in drug development. Analytical tools help:



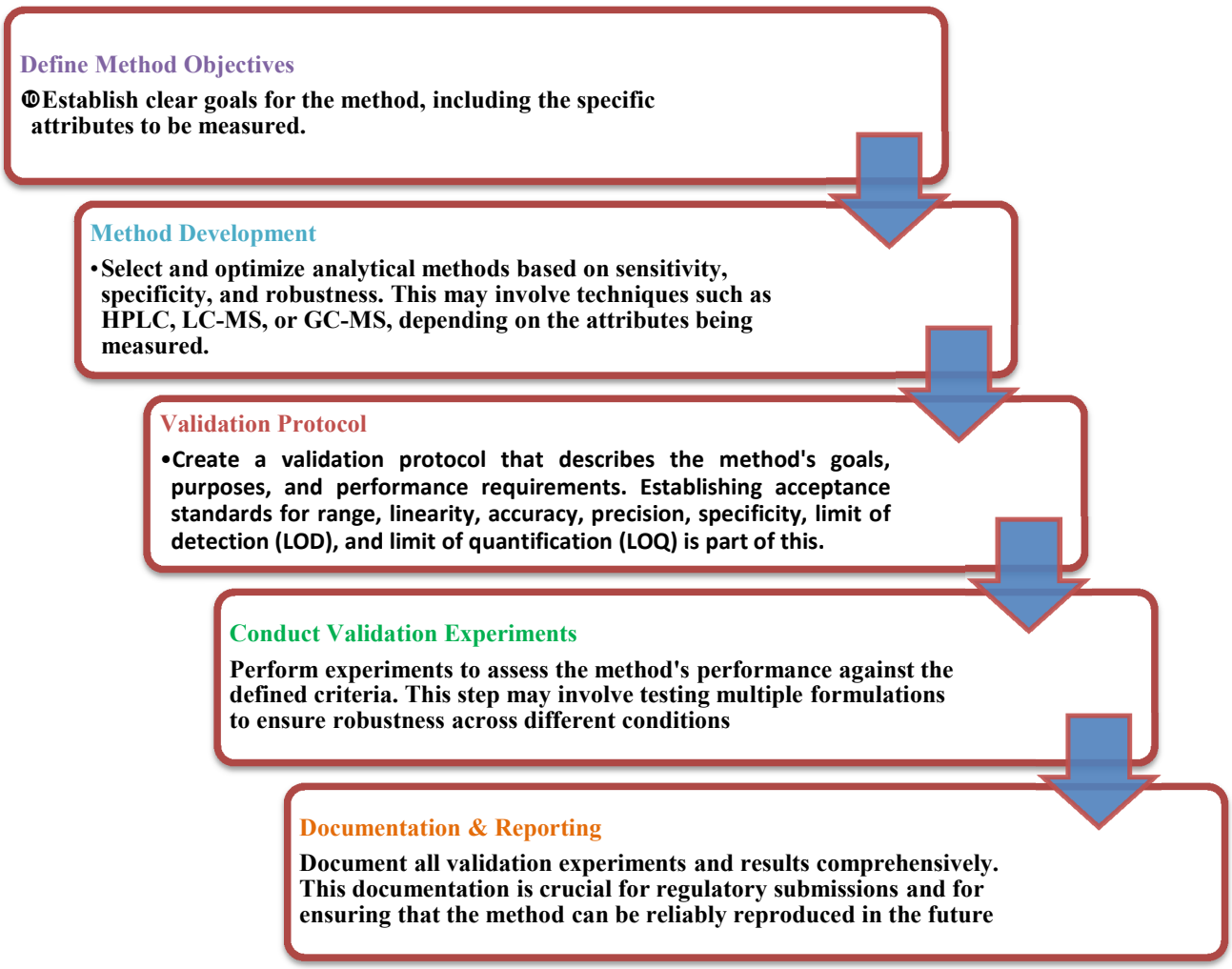
4.1. FORMULATION DEVELOPMENT

Importance of Analytical Method Validation in Formulation Development

- **Ensuring Drug Quality:** The creation of high-quality products is the main objective of any pharmaceutical development program. Analytical method validation helps in understanding the composition of chemical compounds, which is essential for creating new medications. [36-37]. This process allows for the identification of critical attributes that affect the drug's efficacy and safety.
- **Regulatory Compliance:** Regulatory authorities, such as the FDA and EMA, require that analytical methods be validated before they can be used in clinical trials or marketed. A poorly documented chemistry, manufacturing, and controls (CMC) section can hinder the approval process for clinical trials [38-40]. Thus, having validated analytical methods simplifies regulatory compliance and provides clear information about the drug's quality.
- **Patient Safety:** Patient safety is paramount in drug development. Analytical methods ensure that only safe compounds are used in clinical trials, which is essential for protecting human subjects during early-phase studies [41-43]. The validation of these methods helps guarantee that the drugs administered are of the highest quality and efficacy.

Steps in Analytical Method Validation

The process of analytical method validation typically involves several key steps:



5. CHALLENGES AND FUTURE PERSPECTIVES

A. Challenges

Complexity of Drug Formulations:

The intricate nature of pharmaceutical formulations, which often include multiple active ingredients and excipients, complicates the analytical method development process. This complexity can lead to issues with specificity and sensitivity, making it difficult to accurately quantify the active pharmaceutical ingredients (APIs) like Atazanavir and Cobicistat [36-38].

Regulatory Compliance:

Adhering to stringent regulatory requirements set by organizations such as the FDA and ICH is a significant challenge. The evolving nature of these regulations necessitates continuous updates to analytical methods to ensure compliance, which can be resource-intensive.

Method Transfer and Validation:

The transfer of methods from research and development (R&D) to quality control (QC) labs can introduce variability. Ensuring that analytical methods maintain their performance across different settings is crucial but often problematic [44]. This requires thorough validation processes that can be time-consuming and costly.

Technological Limitations:

While advancements in analytical instrumentation (e.g., UHPLC, MS) have improved the capabilities of analytical methods, there are still limitations regarding the sensitivity and specificity of these techniques when applied to complex biological matrices [45]. This can hinder the detection of low-level impurities or degradation products in formulations.

Sample Preparation Challenges:

The need for efficient sample preparation techniques that minimize matrix effects while maximizing recovery of the analytes is paramount. Inadequate sample preparation can lead to inaccurate results, necessitating further method optimization [46-47].

B. Future Perspectives

Integration of Advanced Technologies:

The incorporation of cutting-edge technologies like artificial intelligence and machine learning may become more and more important in future advancements in analytical techniques. The predictability of method performance, data analysis, and method development processes can all be improved by these technologies[48].

Focus on Robustness and Flexibility:

There is a growing emphasis on developing robust analytical methods that can withstand variations in sample composition and analytical conditions. This includes adopting systematic approaches to evaluate method robustness, such as design of experiments (DoE) methodologies, which can provide insights into how method parameters affect performance [49-50].

Regulatory Evolution:

As regulatory frameworks evolve, there will be a need for continuous education and adaptation of analytical methods to meet new guidelines. This includes a focus on the validation of methods for biopharmaceuticals, which may require different considerations compared to traditional small-molecule drugs [51-52].

Collaboration Across Disciplines:

Enhanced collaboration between analytical chemists, formulation scientists, and regulatory affairs professionals will be essential. This interdisciplinary approach can facilitate the development of more effective and compliant analytical methods that are aligned with the overall drug development strategy [53-55].

Sustainability Considerations:

The future of analytical method development will likely include a focus on sustainability, with an emphasis on reducing waste and energy consumption in analytical processes. This aligns with broader industry trends toward environmentally responsible practices in pharmaceutical development [56].

6. CONCLUSION

In conclusion, while the challenges in developing and validating analytical methods for Atazanavir and Cobicistat are significant, the future holds promising advancements that can enhance the efficiency, accuracy, and compliance of these processes. Continuous innovation and collaboration will be key to overcoming existing hurdles and meeting the demands of the pharmaceutical industry.

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