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A Literature Review on Analytical Method Development and Validation of Hydroxy Chloroquine

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ABSTRACT: The information in this review is compiled from previously published techniques for analyzing hydroxychloroquine, either by itself or in conjunction with other medications. Numerous spectroscopic techniques, including as derivative and chromogenic approaches, were employed for newly created and enhanced chromatographic procedures that were also made possible by the use of pharmaceutical formulations and biological fluids. A few LC-MS/MS and HPTLC techniques are also available in addition to these two approaches. In the current world of analytical research, an enhanced method for method validation is obtained through the application of quality by design or design of expert technique. An analyst can use this succinct review to help them select the optimal analytical technique for creation and verification approach.

Keywords: Chromogenic, LC-MS/MS, HPTLC, Hydroxy Chloroquine

I. INTRODUCTION

[1-2]The most common application of Hydroxychloroquine (HCQ), a derivative of chloroquine with antimalarial and anti-inflammatory properties, is as an antirheumatologic medication for rheumatoid arthritis and systemic lupus erythematosus (SLE). Hydroxychloroquine therapy is a very uncommon cause of acute liver injury that is clinically As an antirheumatologic drug for rheumatoid arthritis and Systemic Lupus Erythematosus (SLE), Hydroxychloroquine (HCQ), an analog of chloroquine with antimalarial and anti-inflammatory activities, is most frequently used. One extremely rare cause of acute liver damage that is clearly visible and unrelated to alterations in liver function is HCQ treatment.

[3-6]A diffuse connective tissue disorder, SLE is mediated by autoimmune and is primarily recognized by its clinical presentation of vasculitis. Its etiology is yet unknown, though. SLE manifests itself by recurring illness episodes and impacts various systems, including multiple kinds of autoantibodies being the primary sign. It can cause irreversible harm to the afflicted organs if left untreated, which would finally lead to patient death. A hydroxyl group is added to the side chain of chloroquine to create HCQ, a structural variant of the antimalarial medication that has relatively less side effects.

[7-16]Because of its enhanced safety profile, it was used to treat SLE in the 1950s after becoming well-known as an immune-modulating agent. HCQ is often employed for treating autoimmune illnesses because of its exceptional safety record. Its capacity to lower SLE disease activity, avoid flare-ups, lessen the requirement for long-term steroid therapy, and offer advantages during pregnancy has been validated by numerous research. Additionally, HCQ shows a variety of therapeutic benefits, such as UV protection, glycemic management, cholesterol level improvement, cardiovascular risk decline, and anti-thrombotic properties.

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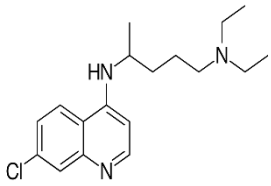
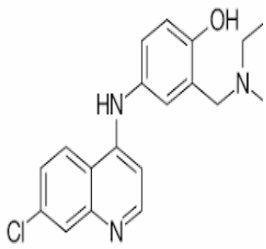
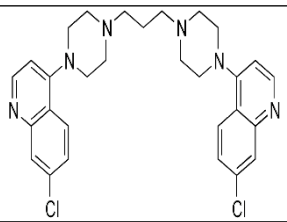
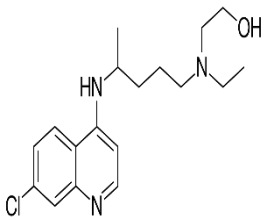
Table 1: Details of 4-Aminoquinoline derivatives

Drug	Structure	IUPAC Name	Molecular weight	Solubility
Chloroquine		7-chloro-4-((4-(diethylamino)-1-methylbutyl)amino)quinoline	319.9 g/mol	It is slightly soluble in water and more soluble in dilute acids, chloroform and ether. It is also soluble in dimethylformamide (DFM), ethanol, n-propanol & isopropanol.
Amodiaquine		4-[(7-chloroquinolin-4-yl)amino]-2-(diethylaminomethyl)phenol	335.9 g/mol	It is soluble in organic solvents like ethanol, DMSO, and dimethyl formamide (DFM), with solubility values of roughly 2, 5, and 2.5 mg/ml. It is also soluble in water with reported solubilities ranging from 16.47 to 24.9 mg/ml particularly with ultrasonic assistance.
Piperaquine		1,3bis[4-97-chloroquinolin-4-yl0piperazin-1-yl]propane	535.5 g/mol	It is poorly soluble in water but readily soluble in organic solvents like chloroform. It is also slightly soluble in ethanol.
Hydroxy chloroquine		2-[4-[(7-chloroquinoline-4-2-yl)amino]pentyl-ethylamino]ethanol	335.9 g/mol	Freely soluble in water, and also soluble in alcohol
Quinacrine		6-chloro-9-(4-diethylamino-1-methylbutylamino)-2-methoxyacridine	400.0 g/mol	It is soluble in organic solvents like ethanol and DMSO and in water (with

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	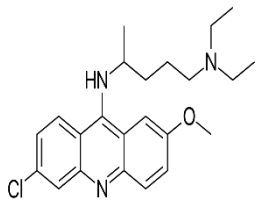			heat). It is also soluble in PBS (pH 7.2)

Hydroxy chloroquine

[17-20]Out of all these 4-Aminoquinoline compounds, HCQ is briefly covered in this publication. A well-tolerated disease modification anti-rheumatic medication (DMARD), HCQ has a greater prevalence than chloroquine and is frequently used to treat RA (rheumatoid arthritis) and DLE (discoid lupus erythematosus) or SLE. HCQ (C18H26ClN3O) is known by its IUPAC designation, 2-[4-[(7-chloroquinoline-4-2-yl)amino]pentyl-ethylamino] ethanol. In 1946, the antimalarial medication chloroquine (CQ) was produced by adding a hydroxyl group to its molecule. HCQ was determined to be approximately two to three times less hazardous than CQ after its discovery.

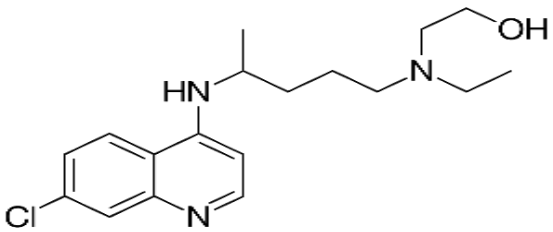

Fig 1: Chemical structure of HCQ

[21-24]Another name for HCQ is immunosuppressive medication. In contrast to CQ, which is similarly linked to acute liver lesions, HCQ medication rarely results in abnormalities. Tablet dosages of HCQ are commercially available. The daily dosage of HCQ is between 100 mg and 1.2 g, and it is rapidly absorbed over the course of two to four hours. About 74%±13% of it is absorbed. Immediately after absorption, the blood concentration of HCQ increases, but it quickly drops since it is quickly distributed throughout the body's organs. Antimalarial medications affect interior vesicles' pH, which is known to affect the immune system. As previously stated, HCQ and CQ deposit widely upon rapid absorption, especially in acidic cytoplasmic vesicles. The anti-arthritis impact of HCQ is due to its accumulation in cells from the immune system. Adaptation to greater doses for a greater amount of time is one of HCQ's strengths over its parent medication, CQ. By regulating the immune system and reducing inflammation, HCQ treats SLE. It mainly works by preventing Toll-like receptors (TLRs), which are essential for the innate immune response and immune-mediated diseases like SLE.

[25-29]Furthermore, by disrupting the digestion and delivery of peptides in cells that present antigens, HCQ impairs the delivery of antigen and suppresses the immune response contrary to the autoantigen.

IMPORTANCE OF ANALYTICAL ESTIMATION

[30-32]Analytical method development and validation (AMDV) are found critical processes in pharmaceutical industry & other scientific fields, ensuring that analytical techniques are found to be robust, reliable, and suitable for their intended purposes. This discussion highlights the importance

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of AMDV, its key components, and its implications for drug development and quality assurance.

Importance of Analytical Method Development

❖ **Establishing Methodology**

[33-34]Analytical method & development involves creating and refining techniques to accurately measure and analyze the components of a product. This can include both the development of advanced methods & the improvement of existing ones. The aim is to established that the methods used was appropriate for the specific characteristics of the substances being tested, such as their identity, purity, potency, and stability.

❖ **Regulatory Compliance**

[35]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.

❖ **Quality Assurance**

[36-39]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.

Key Components of Analytical Method Validation

Analytical method validation involves several critical parameters:

- ✓ **Accuracy:** The closeness of the calculated quality to the observed value.
- ✓ **Precision:** The degree to which repeated measurements identical findings are obtained from under unchanged conditions shows the same results.
- ✓ **Limit of Detection; LOD & Limit of Quantification; LOQ:** The lowest analyte concentration that can be accurately measured or detected.
- ✓ **System Suitability Testing:** Ensuring that the analytical system is operating correctly before analysis.
- ✓ **Specificity:** The method's capacity to measure the analyte while additional analytes are present.
- ✓ **Robustness:** The method's capacity to remain functional independently of small variations in method parameters.

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

Role in Pharmaceutical Industry

[40]In order to guarantee that medications are high-quality, safe, and effective, the pharmaceutical industry relies heavily on the development &validation of sophisticated analytical techniques. These processes are integral to the overall drug development lifecycle, from initial research through to clinical trials and manufacturing. An overview of their significance, processes involved, and regulatory considerations are narrated below.

Regulatory Guidelines

The validation of analytical methods and development is associated with guidelines established by various regulatory bodies. These include:

- ❖ **ICH Q2(R1):** Provides guidelines for the validation of analytical procedures.
- ❖ **FDA Guideline for Industry:** Outlines expectations for Validation of analytical techniques and processes for pharmaceuticals and biologics.

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

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ANALYTICAL TECHNIQUES FOR ESTIMATION

Pharmaceutical Analysis Techniques in figure 2:

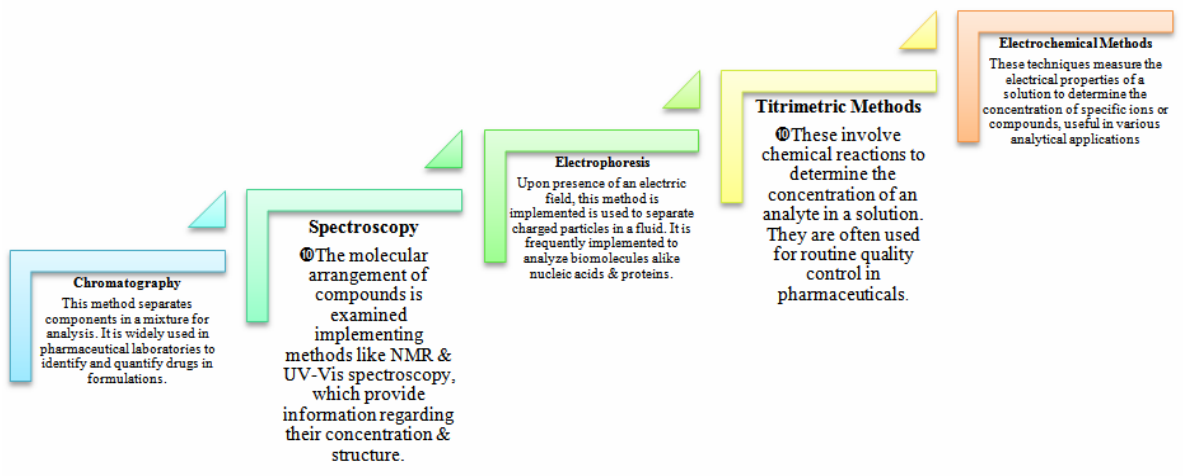

Fig 2. Different techniques for Analysis

[41-42]The choice of analytical technique for estimation depends on the specific requirements of the project or analysis being conducted. Every approach has advantages and disadvantages, and frequently a mix of both of techniques is employed to achieve the most accurate and reliable results. Understanding these techniques is crucial for effective project management, data analysis, and pharmaceutical research In Table 2 and 3.

Chromatographic Techniques

Table 2: Finding of CFP Quantitatively by HPLC Methods

Sl. no.	Stationary Phase(Column)	Mobile phase (with ratio)	Wavelength	Flow rate	Reference
01	C18 column	mixture of glycine buffer/sodium chloride (pH 9.7, 100 mM) and methanol (46:54; v/v)	320nm	1.2 ml/min	43
02	C18 (250mm, × 4.6mm D., 5µm) cyber-sil	OPA: Methanol: Acetonitrile (30:35:35 v/v/v)	266 nm	1.5ml/min	44
03	C-18 Inertsil ODS-3 (250 × 6 mm, 5 µm)	Water (inorganic): organic (acetonitrile: methanol, 50:50 v/v) — 75:25 (v/v)	343 nm	2.0 mL/min	45
04	Chiralpak AD-H column (4.6 × 150 mm, 5 µm)	n-hexane (0.5% diethylamine (DEA)): isopropanol, 93:7 (v/v)	343 nm	0.8 mL/min	46
05	C8 columns	Methanol:Water (80:20)	343 nm	0.5-2.0 mL/min	47
06	C18 column	Carboxymethyl-β-cyclodextrin (CM-β-CD) & triethylamine			48
7	Phenyl column, 250 × 4.6 mm, 5 µm (Waters	Gycine buffer / sodium chloride (100 mM, pH 9.7): Methanol in a 46:54 (v/v)	380 nm	1.2 mL/min	49

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8	X-terra phenyl column (250 × 4.6 mm, 5 µm)	Methanol:Water (80:20)	220 nm	1.5 mL/min	50
9	C18 (250mm, × 4.6mm D., 5µm) cyber-sil	Acetonitrile:methanol	268nm	0.8mL/min	51

Spectroscopic Techniques

Table 3. Determination of stability methods

Sl. No	Drug	Method	Description	Reference
1	Method Development and Validation of UV spectroscopicestimation of hydroxychloroquine sulphate in bulk and pharmaceutical dose forms (tablets) orm	Spectroscopic Method	Detection wavelength: 329.4 Linearity range: 5- 35 µg/ml Co-relation Coefficient: 0.9992 %Recovery:100.12% %RSD: 0.744 (≤2%) LOQ: 0.84 g/ml LOD: 0.24 g/ml	52
2	UV Spectrophotometric Analysis and Validation of hydroxychloroquine sulphate and Nitazoxanide in synthetic mixture	Spectroscopic Method	**Hydroxychloroquine sulphate** Detection wavelength: 220 nm Linearity range: 2-10 µg/ml Co-relation Coefficient: 0.999 %Recovery:100.51-100.88% %RSD: 1.6 –1.7% (≤2%) LOQ: 0.152µg/ml LOD: 0.0501µg/ml **Nitazoxanide** Detection wavelength: 345 nm Linearity range: 5- 35 µg/ml Co-relation Coefficient: 0.999 %Recovery:99.97-101.33% %RSD: 1.7- 1.8% (≤2%) LOQ: 0.1014µg/ml LOD: 0.0334µg/ml	53

APPLICATIONS OF ANALYTICAL TOOLS IN DRUG DEVELOPMENT

[54]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.

Applications of Analytical Tools in Drug Development

A. ML; Machine Learning &AI; Artificial Intelligence

[55]The implementation of artificial intelligence (AI, and machine learning ML algorithms in drug discovery & development procedures is expanding. These technologies facilitate in figure 3:

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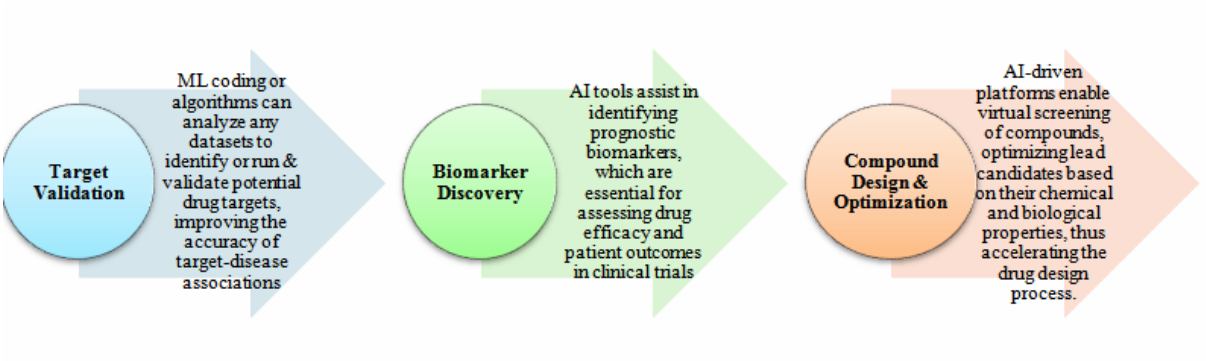

Fig 3. An analytical tool for a technology facility

B. Analytical Method Development

Analytical methods are vital for ensuring drug quality and safety. Key techniques include in figure 4:

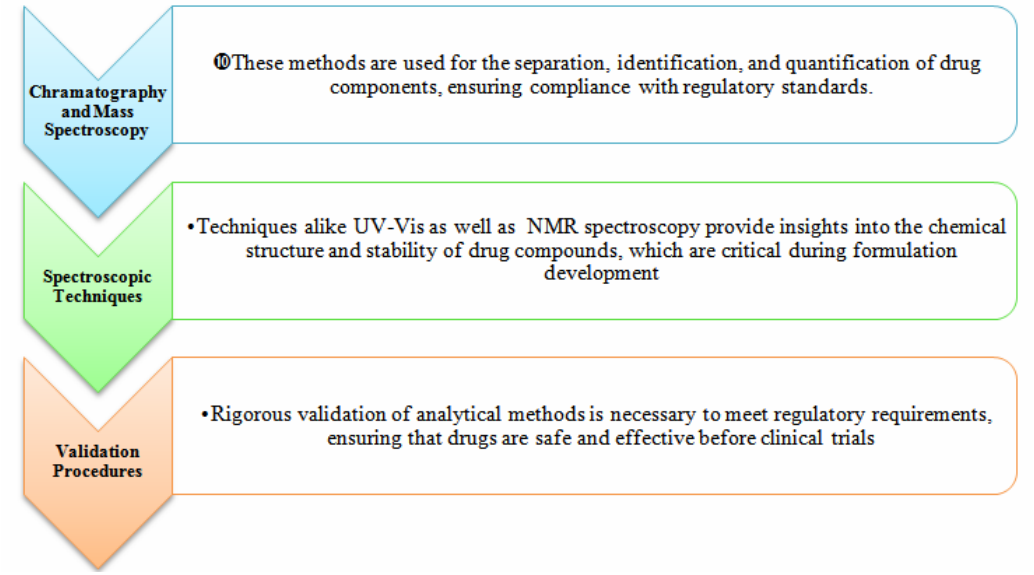

Fig 4. Key techniques for analytical methods are vital for ensuring drug quality and safety.

C. Process Analytical Technology (PAT)

PAT is a methodology that enhances the effectiveness and quality of pharmaceutical production. It involves in figure 5:

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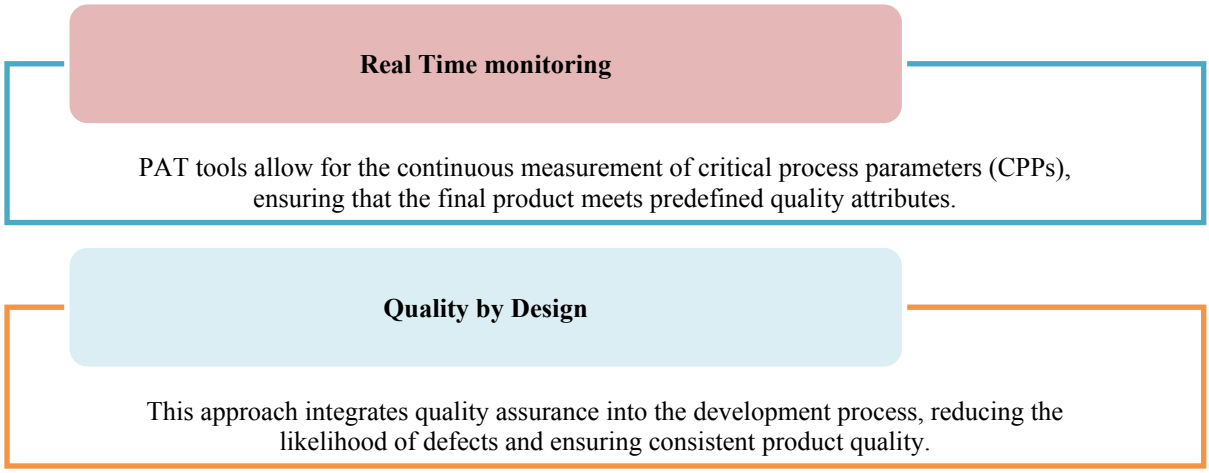

Fig 5. Enhances the quality and efficiency of pharmaceutical manufacturing

D. Data Analytics

Pharmaceutical companies leverage data analytics to inform various aspects of drug development in figure 6:

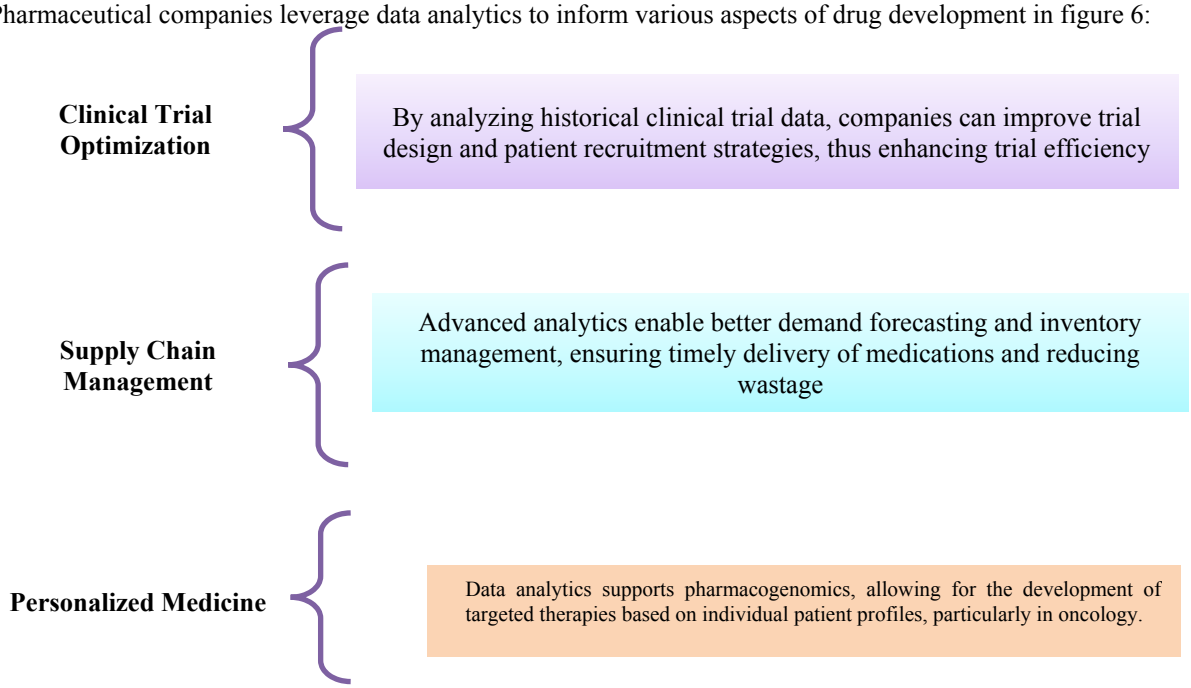

Fig 6. Leverage data analytics to inform various aspects of drug development

E. Quality Control and Compliance

Ensuring compliance with stringent regulatory standards is paramount in drug development. Analytical tools help in figure 7:

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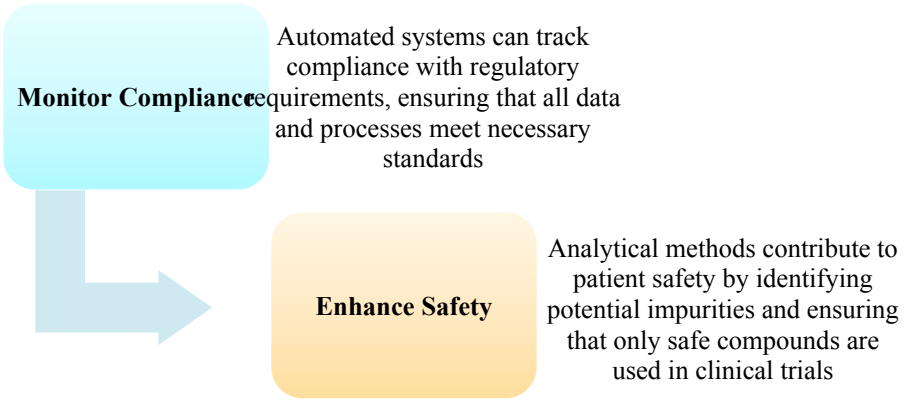

Fig 7. Stringent regulatory standards are paramount in drug development

Formulation Development

[56-63]Importances of analytical method validation in formulation development are illustrated in figure 8:

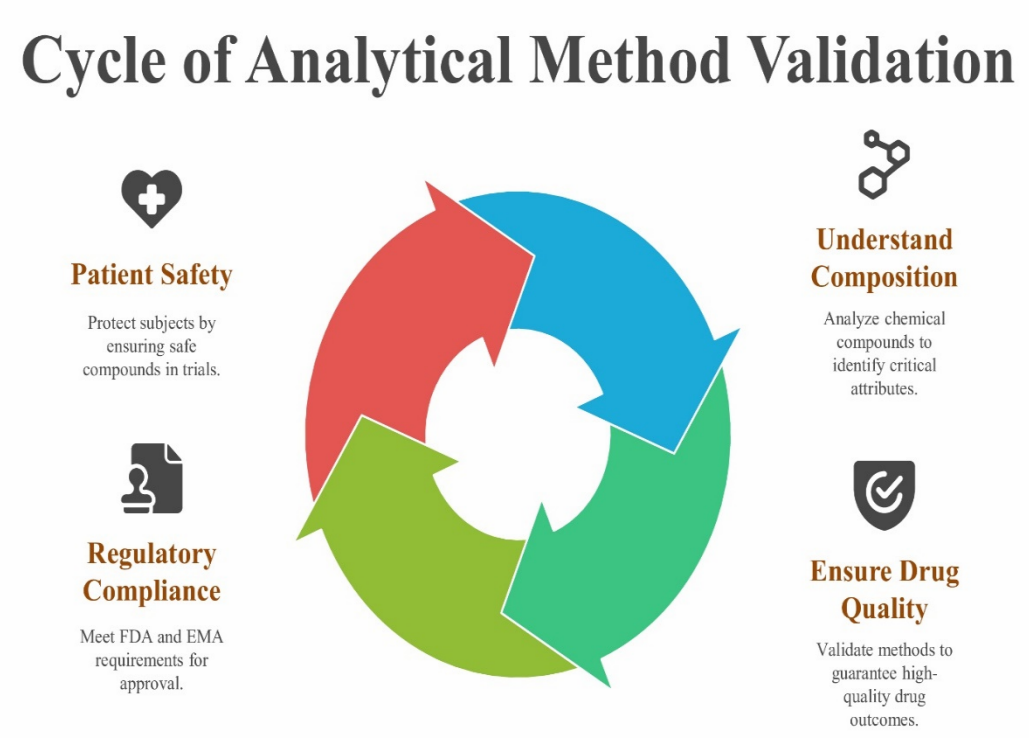

Fig 8. Step by step Analytical Method Validation

Steps in Analytical Method Validation

The validation procedure for analytical methods typically involves several key steps in figure 9:

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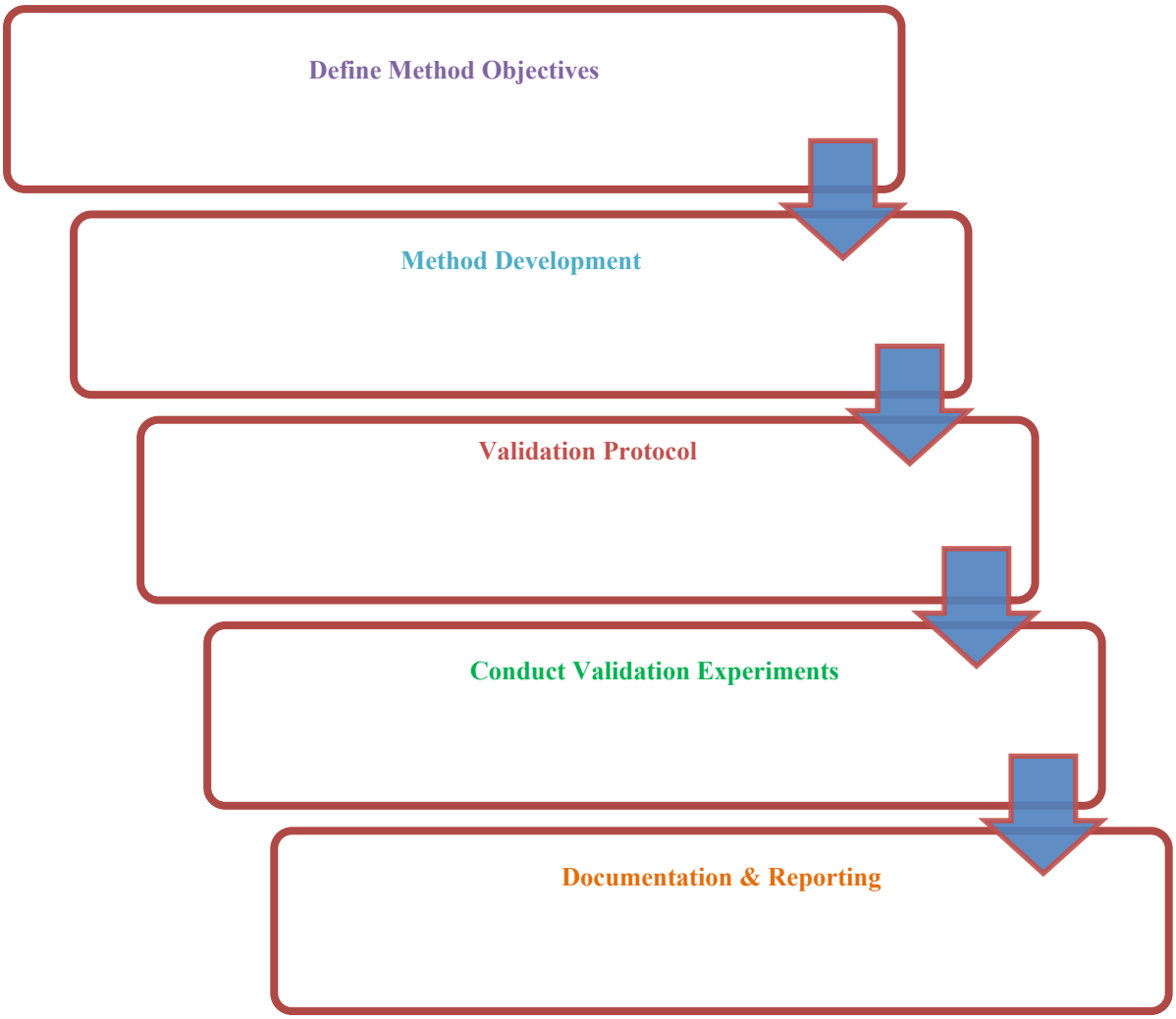

Fig 9. Validation procedure for analytical methods

CHALLENGES AND FUTURE PERSPECTIVES

A. Challenges

Complexity of Drug Formulations:

[64-66]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 HCQ.

Regulatory Compliance:

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

Method Transfer and Validation:

[67]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. This requires thorough validation processes that can be time-consuming and costly.

Technological Limitations:

[68]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 matrice. 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.

B. Future Perspectives

Integration of Advanced Technologies:

Future developments in analytical methods may increasingly rely on the integration of futuristic technologies such as AI and ML. These technologies can optimize method development processes, enhance data analysis, and improve the predictability of method performance.

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.

II. CONCLUSION

The challenges of biological matrices & CFP instability under specific circumstances make the development and validation of analytical techniques for the drug extremely difficult. Promising developments in the future have the potential to greatly increase these processes' accuracy, efficiency, and regulatory compliance. CFP measurement in clinical and pharmacokinetic research should become more sensitive and robust due to ongoing different techniques in LC / MS technology for better sample preparation methods. Researchers, physicians, & regulatory agencies must work together to overcome current obstacles and guarantee the accurate therapeutic monitoring and quality control of this vital broad-spectrum antibiotic

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