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Review Article

A Review on Method Development and Validation

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ABSTRACT

Developing analytical methods and then validating them is a crucial step in the drug discovery process. The absence of an approved analytical technique will prevent the medicine from reaching the market, even though it has good potency. As a result, the quality and safety of the medication. In order to assess the stability of the drugs in the presence of excipients and other stress conditions encountered during their shelf life period, this review's primary goal is to provide an overview of the traditional and innovative methods available for the analysis of drugs in their raw material and formulated forms. LC-MS, LC-MS-MS, LC NMR-MS, GC-MS, and LC-MS are some of the hyphenated techniques for drug analysis and impurity profiling that are clarified by the review study.

INTRODUCTION

The pharmaceutical industry's primary domains for material analysis, encompassing raw materials, intermediate products, APIS, and final products, are quality control and quality assurance. Occasionally, new methods are being created everywhere in the world. Consequently, instrumental methods and then hyphenated technology replaced classical approaches. Every technique is discovered to be better than the one before it. To get to this point in the analysis, the scientists have worked really hard. Numerous techniques are created to help a product or raw

material ultimately achieve its desired quantity and quality. Finding the best approach that is providing the best results is now essential. The precise outcome. This is where validation plays a part. Method validation is the process of repeatedly analyzing the materials using the developed method in order to verify the precision and accuracy of the findings. Once verified, the technique might be used to regular drug analysis. This review's main goal is to shed light on the several hyphenated methodologies that could be used for pharmaceutical estimate, validation, and degradation research.

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USING DIFFERENT METHODS

A. Stability Indicating Method

Important topics pertaining to SIAM development are also covered, including mass balance establishment, formulation stress testing, separation of all degradation products, construction of SIAMs for combination products, etc. The test for stability indication is a approach that the pharmaceutical industry uses to analyse stability samples. The guidelines specifically mandate that forced decomposition experiments be conducted under a range of conditions, including pH, light, oxidation, dry heat, etc., and that the medicine be separated from degradation products. Regretfully, no stability signalling mechanism is precisely defined in any of the ICH guidelines. Guidelines are offered, nevertheless, in the 1998 FDA draft guideline and the 1987 ICH stability guideline issued by the US Food and Drug Administration (US-FDA).¹⁻² There have also been reports of drugs decomposing when exposed to a combination of oxidation, photolysis, thermal stress, and acidic, neutral, or alkaline hydrolysis. In certain instances, stress investigations have been conducted on the formulation rather than the medication component to determine its stability-indicating behaviour.³⁻⁴ Although GC indicates stability, it lacks versatility because the medicinal ingredient may be thermally unstable or non-volatile. Furthermore, deterioration or racemization may result from any attempt to raise the temperature in order to increase the volatility of the medicine and its constituents. As a result, there aren't many reports on GC that are used to develop SIAMs. In contrast, HPLC has been used extensively. Because of its great sensitivity, specificity, and resolution capacity, it has become more and more common in stability investigations. This method can also be used to analyse polar/ionic, thermally unstable, or non-volatile substances. As a result, HPLC has been used to establish the majority of SIAMs. Proton nuclear

magnetic resonance (NMR) spectroscopy has also been used in a few research to create SIAMs.³⁻⁶

B. Impurity Studies Method

Through their ability to treat illnesses, drugs are essential to the advancement of human civilization. Nowadays, the vast majority of medications are synthetic in nature. The purpose of these mass-produced their therapeutic benefits in formulations of pharmaceuticals. These chemical compounds are physiologically active and are typically prepared into convenient dosage forms as injectables, pills, capsules, solutions, and ointments. The pharmacological material is delivered via these formulations in a stable, non-toxic, and palatable form, guaranteeing both its therapeutic activity and bioavailability. Two key concerns of significance in medication therapy are pharmaceutical safety and efficacy. The pharmacological toxicological profile of a medication, along with the side effects brought on by contaminants in bulk and dose forms, are what determine its safety. Drug impurities frequently have undesirable pharmacological or toxicological effects that could exceed any advantages of using the drug. A drug's quality and safety are often guaranteed by keeping an eye on and managing the impurities efficiently. Controlling impurities is very crucial. The production and sale of drug goods were unregulated during the start of the 20th century. The United States Pharmacopoeia, the International Conference on the Harmonization of the technical standards for registration of medicines for human applications, and regulatory documents of the Food and Drug Administration all specify the contaminants that must be taken into account for new drugs. The acknowledged benchmarks for the potency and purity of novel medications are the USP and National Formulary (NF). New recommendations about contaminants in new drug products were announced by the ICH, which was held in Yokohama, Japan in 1995. Both the business and the regulators benefit greatly



from these recommendations. The most important component of The identification and qualification process was the formulation of the guidelines. The approach should be optimized to separate and quantify the contaminants in the dosage forms, and analytical procedures should be able to separate all of the impurities from one another. Such techniques must be verified by proving their linearity, accuracy, precision, specificity, limit of detection, ranges, interferences, and quantification. The requirements have been standardized in two guidelines by the ICH. The first one provides a summary and definition of the validation qualities required for different kinds of test methods, while the second one expands on the first text by adding the necessary experimental data and some statistical analysis.⁷⁻⁹

Source of impurities

Drug impurities can come from a variety of origins and stages of the manufacturing process including the creation of medicinal dosage forms. A notable distinction between the procedure. It is never possible to find associated impurities and degradation products. Most of the contaminants are specific to the production process' synthetic approach. A medicine can be synthesized in a variety of ways, which means that the same product from several sources could produce distinct contaminants.

Types of Impurity¹⁰⁻¹¹

Impurities are categorized by the US Pharmacopoeia (USP) in a number of sections:

1. Organic impurities
2. Inorganic Impurities
3. Residual solvents

ICH limits for Impurities

The ICH recommendations on impurities in new drug products state that, unless probable impurities are anticipated, it is not necessary to identify impurities below the 0.1% limit to be exceptionally poisonous or powerful. The ICH states that a daily dose qualification criterion of

<2g/day 0.1% or 1 mg/day consumption (whichever is lower) >2g/day 0.05% must be taken into consideration.

Development of Analytical Methods: At different phases of the research process, new drugs must generate useful and trustworthy analytical data.¹²⁻¹³

- a) Selection of sample sets for the development of analytical methods
- b) Chromatographic conditions and phases are screened, usually with the linear solvent strong gradient elution model.
- c) Method optimization to fine-tune robustness and ruggedness-related characteristics

The following techniques are primarily used to identify the impurities: 1. Make use of the conventional procedure 2. Spectroscopic technique 3. The method of separation 4. Characterization and isolation methods

Reference Standard Methods

The reference standard is the benchmark for determining if a drug is safe for patients to consume and is the basis for evaluating the performance of both processes and products. These requirements apply not only to the active substances in dosage forms but also to excipients, starting materials, degradation products, contaminants, and process intermediates.

Spectroscopic Methods

Impurity characterization frequently involves the use of the UV, IR, MS, NMR, and Raman spectroscopy techniques.

Separation Methods

Impurity and degradation product separation is frequently accomplished using capillary electrophoresis (CE), chiral separations, gas chromatography (GC), supercritical fluid chromatography (SFC), TLC, HPTLC, and HPLC.

Isolation Methods

Isolation of contaminants is frequently required. However, since instrumental approaches characterize impurities directly, they prevent



impurity isolation. Techniques that can be applied the following techniques are used to isolate impurities: capillary electrophoresis (CE), column chromatography, flash chromatography, TLC, GC, HPLC, HPTLC, liquid-liquid extraction, solid-phase extraction, accelerated solvent extraction, and supercritical fluid chromatography (SFC). For a aprepitant prodrug and fosaprepitant Di meglumine, high performance chromatography and the chromatographic reactor method with solution phase hydrolysis kinetics can be employed. Ofloratidine was identified as the impurity in loratidine; celecoxib and amikacin are more examples.¹⁴⁻¹⁷

Characterization Methods

Highly advanced instruments, like an HPLC or MS connected to a GC, are essential for identifying trace amounts of substances (drugs, contaminants, degradation products, in different matrices (metabolites). Several methods are employed to characterize contaminants, including the following:

NMR Spectroscopy

NMR is a potent analytical tool because it can reveal details about the precise bonding structure and stereochemistry of compounds of medicinal interest. tool for clarifying structures. A standard combination of real materials comprising both monomers and dimers³¹ was used to confirm that NMR-based diffusion coefficient determination could differentiate between monomeric and dimeric compounds. Regretfully, in comparison to other analytical techniques, NMR has historically been employed as a less sensitive method. Unlike MS, which requires less than 1 mg, conventional NMR sample requirements are around 10 mg.¹⁸

Mass Spectroscopy

Over the past few decades, its influence on the pharmaceutical development process has grown significantly. improvements in interface efficiency and design that directly new possibilities for the monitoring, characterization, and quantification of

drug-related compounds in active pharmaceutical ingredients and pharmaceutical formulations have been made possible by the integration of separation techniques with mass spectrometers. Orthogonal coupling of chromatographic techniques, such as HPLC-TLC and HPLC-CE, or coupling of chromatographic separations with information-rich spectroscopic methods, such as HPLC-MS or HPLC-NMR, may need to be considered if a single method is unable to provide the required selectivity. Ideally, this will only be done as a development tool rather than as a tool for routine QC use.

- a. HPLC-DAD-NMR-MS
- b. HPLC-DAD-MS
- c. GC-MS
- d. LC-MS

The atmospheric pressure ionization with electrospray source (API ESI) is one of two different soft ionization procedures used in gradient elution for reverse-phase LC-MS analysis. A shared objective for research on process and product degradation-related contaminants is to ascertain which of the numerous possible impurities actually arise during the manufacturing process and which take place under certain storage circumstances.

Applications

Many applications have been pursued in the fields of drug design and the quality, stability, and safety monitoring of pharmaceutical substances, whether they are synthetically manufactured or not derived from natural products or created using recombination techniques. Antibacterial, anticonvulsant, antidepressant, tranquilizers, antineoplastic drugs, macromolecules, steroids, analgesics, alkaloids, amines, amino acids, and other substances are among the applications.

Validation Parameters

The primary goal of method validation is to provide evidence that the approach will perform as intended, precisely, and dependably and reliably.¹⁹



The following describes the validation parameters in accordance with ICH guidelines:

1. Accuracy

The degree of agreement between the values discovered and those that are already known is known as accuracy. It may also be described as the degree to which the seen value and the true value are similar. Sometimes referred to as trueness, it can be ascertained by employing a minimum of nine determinations across three concentrations within the designated range.²⁰

2. Precision

The degree of agreement (or scatter) between a set of measurements derived from various samplings of a sample is expressed as the exactness of an analytical method. consistent sample under the specified circumstances.²¹ There are three levels at which precision can be considered.

3. Repeatability

It also goes by the name of intra-assay precision and describes the accuracy over a short period of time below a comparable operating state. Test preparation with at least six duplicates of an identical or reliable sample available at the 100% check.²²

4. Intermediate precision

It conveys the precision of research labs on different days, with different analysts, using different tools and equipment. Two distinct analysts. Following the prescribed procedure, each person prepares six sample solutions.²³

5. Reproducibility:

It speaks to the level of accuracy across various analytical labs. In accordance with the analytical technique, each research center set up an aggregate of six sample solutions.²²

6. Specificity:

The analytical method must exhibit specificity for each developmental stage. The method should be able to determine the target analyte with certainty. In contrast to when all anticipated components are

present, which may include sample blank peaks, excipients/sample matrix, and degradants.²⁴

7. Limit of detection (LOD):

The chromatographical separation may detect the lowest amount of an analyte, however this amount does not always have to quantify as a precise value. We must compute the peak-to-peak quantitative noise relationship using blank chromatograms after injecting blank resolution. After that, determine the concentration at the signal to quantitative noise ratio, which is roughly 3:1.²⁵⁻²⁶

8. Limit of Quantitation (LOQ):

It is distinguished by the smallest amount of an analyte that can be precisely and accurately quantified.²⁵

9. Linearity:

The ability of an analytical method to yield results that are directly correlated with an analyte's concentration in the sample is known as linearity.²⁷

10. Range:

It can be defined as the difference between the sample's higher and lower analyte concentrations. A minimum of 80% to 120% of the test sample for the assay must fall within the designated range examination.²⁸

11. Ruggedness:

The degree of consistency in many conditions, including in different labs, with different analysts, with different machines, in different environments, with different operators, etc., is known as ruggedness.²⁹

12. Robustness:

It is defined by an analytical technique's degree of capacity to maintain similarity after deliberate, small changes in technique parameters. The many method parameters that the pH, drift rate, column temperature, and mobile phase composition are all modifiable in high-performance liquid chromatography.³⁰

CONCLUSION



The data needed for a particular analytical problem, sensitivity, accuracy, range of analysis, and precision that is, the minimal needs, which are effectively the method's specifications for the intended purpose are provided to an analyst via analytical methodology. Analyse the target analyte in various matrices with confidence and assurance. Before being used on a regular basis, analytical techniques must be validated; any modifications to the method that go outside of its initial scope occur when the conditions for which the method has been validated change. Although many medications have stability indication assays designed for them, the majority of them do not satisfy the current regulatory standards for the analysis and separation of particular degradation products.

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