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Review Article

Development and validation of stability indicating RP-HPLC method for estimation of some API in bulk and Pharmaceutical dosage form

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Analytical method development and validation that is phase-appropriate across a range of techniques supporting pharmaceutical product development. Stability studies are an integral part of the drug development program. The need for the stability studies on a drug arises from the fact that the chemical integrity of the drug substance should be maintained until the compound is delivered to the intended site of action. International Conference on Harmonization (ICH) has made mandatory requirement of stability-indicating assay method (SIAM) for every drug product. A SIAM is a validated qualitative analytical procedure that can detect the changes with time in the properties of the drug product and drug substance under defined storage condition. In this study, the drug candidate is exposed to a variety of stress condition like acidic, alkali, oxidative, thermal and photolytic. API is any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function of the body. Bulk Drug Substances are any substance that is represented for use in a drug and that, when used in the manufacturing, processing, or packaging of a drug, becomes an active ingredient or a finished dosage form of the drug, but the term does not include intermediates used in the synthesis of such substances.

Key Words: Bulk Drug Substances, Stability-Indicating Assay Method (SIAM), International Conference on Harmonization (ICH).

INTRODUCTION

Method development involves a series of sample steps; based on what is known about the sample, a column and detector are chosen; the sample is dissolved, extracted, purified and filtered as required; an eluent survey (isocratic or gradient) is run; the type of final separation (isocratic or gradient) is determined from the survey; preliminary conditions are determined for the final separation; retention efficiency and selectivity are optimized as required for the purpose of the separation (quantitative, qualitative or preparation); the method is validated using ICH guidelines. The validated method and data can then be documented.[1]

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. A process of evaluating method performance and demonstrating that it meets a particular requirement. In essence, it is knowing

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Table 1 : Pharmaceutical processing of Method Development and Validation[1,2,3]

TABLE 1	
Methods and Tools	Analysis Purpose
QbD Approach	Speed Up Method Development and Reduce Risk
Design of Experiments using Screening and Optimization Experiments	Method Development including Creation of Test Design Space
Gage Repeatability and Reproducibility Studies	Improve Measurement Quality
Method Robustness Studies	Create Methods Robust to Small Variations in How the Method is Used
Blind Control Sample	Continued Verification of Method Repeatability and Reproducibility Over Time
Process Variation Studies	Assess Process Variation to Determine the Relative Contributions of the manufacturing process, sampling Procedure and Test Method to the Observed Process Variation

what your method is capable of delivering, particularly at low concentrations.[2,3]

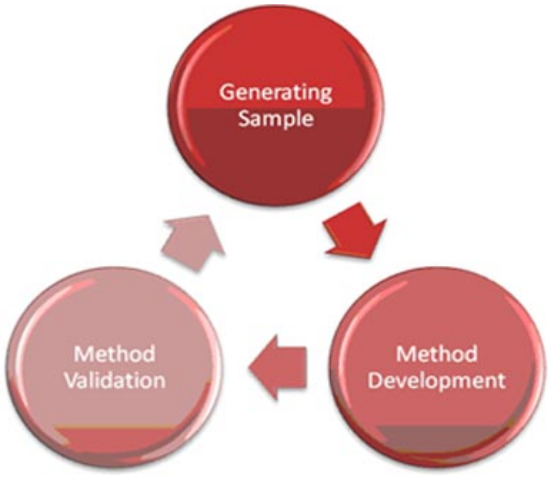

Fig.1: Process of Product Development[3]

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.[4]

Analytical procedures[5,6]

Validation of analytical procedures is directed to the four most common types of analytical procedures.

- Identification test.
- Quantitative test for impurities content.
- Limit test for the control of impurities.
- Quantitative test of the active moiety in samples of drug substance on drug product on other selected components in the drug product.

VALIDATION OF METHODOLOG[7,8,9]

- **Accuracy as recovery**

  The accuracy of the method was determined

as recovery by standard addition method. For this, pre-analyzed samples were spiked with standard

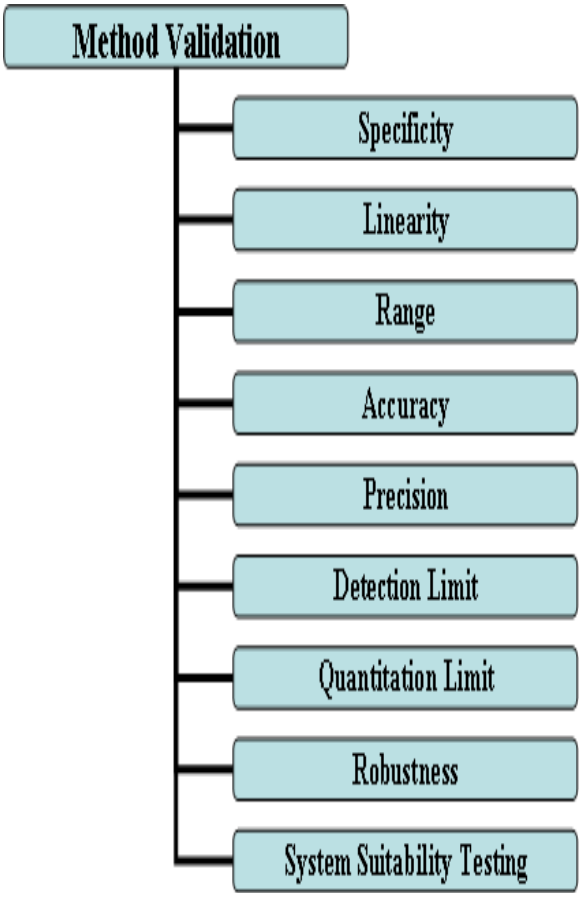

Fig.2: The USP and ICH Method Validation Parameters[5,6]

drug at three different concentration levels, i.e., 50, 100, and 150%, and the mixtures were reanalyzed by the proposed method.[7]

Precision

The precision of the method was carried out by doing repeatability and intermediate precision. In repeatability, six different injections of the same standard (three concentrations) were injected and calculated the assay. The %RSD of area and R_t were calculated. In intermediate precision, intra-day, inter-day, and inter-system precisions were carried out. Intra-day and inter-day precisions were done by preparing and applying three different concentrations of standard in triplicate six times a day and similarly on six different days, respectively. Inter-system precision was done by repeating the same procedure in different HPLC system. Assay for each analysis was calculated, and %RSD was determined.[7]

Robustness of the method

Robustness of the proposed method was determined at single concentration level (100 µg/mL) in three different ways, i.e., by changing the composition of mobile phase, making deliberate change in the flow rate, and changing the detecting wavelength. The %RSD of the experiment was calculated to assess the robustness of the method.[7]

Specificity

The specificity of the method was determined by doing stability studies by exposing the sample solution (100 µg/mL) in accelerated conditions like 0.1 M formic acid, 0.1 M NaOH, and dry heat. The resulting solutions were analyzed, and the analyte peak was evaluated both for peak purity and for resolution from the nearest eluting peak. All the samples were filtered before injecting to

HPLC system.[7]

Limit of quantification and limit of detection

The limit of quantification and limit of detection were determined based on the technique of signal-to-noise ratio. The concentration of sample giving a signal-to-noise ratio of three was fixed as the LOD. The concentration of the sample giving a signal-to-noise ratio of ten was fixed as LOQ. Once the LOD and LOQ were determined, six replicates of blank and the standard solution at the level of LOD and LOQ were applied and the %RSD calculated.[7]

Acid-induced degradation study

Freshly prepared solution of 6-gingerol (1,000 µg/mL) in 0.1 M methanolic formic acid solution was transferred to a 100-mL volumetric flask. Warmed the solution at 30°C for 5 min and allowed to stand for 1 h for the completion of degradation reaction at room temperature, neutralized the solution using dilute NaOH solution and made up the volume with methanol (100 µg/mL solution). The blank and sample solutions were injected, and the chromatograms were analyzed for peak purity and resolution between the peaks.[7]

Base-induced degradation study

Freshly prepared solution of 6-gingerol (1,000 µg/mL) in 0.1 M methanolic NaOH was transferred to a 100-mL volumetric flask, warmed at 30°C for 5 min, and allowed to stand for 1 h for the completion of degradation reaction at room temperature, neutralized using dilute HCl solution, and make up the volume with methanol (100 µg/mL solution). The blank and sample solutions were injected, and the chromatogram was analyzed for peak purity and resolution between the peaks.[7]

Heat degradation study

The standard 6-gingerol (10 mg) was stored under dry heat condition in hot air oven at 60°C for 1 h. The sample was taken out after 1 h, transferred to a 100-mL volumetric flask, and diluted up to the volume with methanol to get a known concentration of 100 µg/mL. The solution was injected in HPLC along with the blank solution, and the chromatogram was analyzed for peak purity and resolution between the peaks.[7]

BENEFITS OF VALIDATION[8]

- Produces quality products
- Helps in process improvement technology transfer, related product validation, failure investigation, and increased employee awareness.
- Cost reduction by increasing efficacy, few reject, longer equipment life, production of cost effective products
- Helps in optimization of process or method.
- Regulatory affairs-produces approved

products and increased ability to export.

STABILITY STUDIES[9]

Stability studies are an essential component of pharmaceutical development, allowing evaluation of active pharmaceutical ingredient (API) stability or drug product stability under the influence of a variety of environmental factors such as temperature, humidity and light. Data from these studies enable recommended storage conditions, retest intervals and shelf lives to be established.[9]

It is important that you select an experienced stability study outsourcing partner who offers efficient study management, flexible storage conditions and testing capabilities which satisfy all regulatory criteria for your real time, accelerated or forced-degradation study requirements. Stability testing can present significant analytical hurdles, with specialised knowledge required to develop and validate stability indicating methods and perform analysis of leachable substances which migrate from pharmaceutical packaging into the product.[9]

With a network of ICH stability storage facilities in the UK, US and Australia, we offer an extensive capacity and a range of conditions including climatic walk in chambers, cabinets and refrigerated as well as freezer storage which are fully controlled and monitored with back up chambers at each site. All sites have 24 hour alarm systems with dedicated on call teams to react to the excursions from storage conditions. Our stability teams provide professionally managed Good Manufacturing Practice (cGMP) stability programs for even the most complex of dosage forms, APIs or product types including orally inhaled and nasal drug products (OINDP), biopharmaceuticals, consumer healthcare, medical devices or vaccines. We also offer a responsive and bespoke stability contingency and disaster recovery storage service to help you mitigate the risks associated with costly stability trials.[9]

GMP Stability Services:[9]

- cGMP registration stability programs
- Design, storage and management
- Development and validation of stability indicating methods
- Stability testing for APIs, Clinical Trial Materials, formulated products
- Tailored reporting (timepoint and final reports)
- All ICH conditions storage
- Photostability (ICH Q1B Options 1 & 2)
- Temperature cycling, freeze-thaw and shipping studies
- Bespoke or specialised conditions
- Stability contingency and disaster recovery storage

Our analytical laboratory network provides development and validation of stability indicating

methods through state-of-the-art technology to identify and quantify degradation products. Routine time point testing includes the usual tests such as assay and impurity analysis, dissolution, moisture, hardness, friability and disintegration. Intertek's scientists have specialist knowledge for OINDP stability testing including measurement of particle or droplet size, providing data critical to understanding the size distribution of the delivered formulation and the delivery of the drug from the device. With unrivalled know-how in extractables and leachables studies, we can ensure that the complete product and packaging system demonstrates sufficient stability and protection over the relevant lifecycle of your product.[8,9]

With over 20 years' experience in stability studies integrated with a comprehensive understanding of the latest developments in regional, country and ICH stability study guidelines we offer a truly flexible stability outsourcing partnership with integrated storage and testing capability which allows you to focus on your core business objectives.[9]

ACTIVE INGREDIENT [10,11]

An active ingredient (AI) is the ingredient in a pharmaceutical drug that is biologically active. The similar terms active pharmaceutical ingredient (API) and bulk active are also used in medicine, and the term active substance may be used for natural products. Some medication products may contain more than one active ingredient. The traditional word for the API is pharmacon or pharmakon (from Greek: φάρμακον, adapted from pharmacos) which originally denoted a magical substance or drug.[10]

The term **active constituent** is often chosen when referring to the active substance of interest in a plant (such as salicylic acid in willow bark or arecoline in areca nuts), because the word ingredient in many minds connotes a sense of human agency (that is, something that a person combines with other substances), whereas the natural products present in plants were not added by any human agency but rather occurred naturally ("a plant doesn't have ingredients").

In contrast with the active ingredients, the inactive ingredients are usually called excipients in pharmaceutical contexts. The main excipient that serves as a medium for conveying the active ingredient is usually called the vehicle. Petrolatum and mineral oil are common vehicles.

The dosage form for a pharmaceutical contains the active pharmaceutical ingredient (API), which is the drug itself, and excipients, which are the substances of the tablet, or the liquid the API is suspended in, or other material that is pharmaceutically inert. Drugs are chosen

primarily for their active ingredients.

Patients often have difficulty identifying the active ingredients in their medication, and are often unaware of the notion of an active ingredient. When patients are on multiple medications, active ingredients can interfere with each other, often resulting in severe or life-threatening complications. There now exist online services which can identify the active ingredient of most medications, such as the Medicines database providing information on medications available in Australia. [10]

Components of Medications

All drugs are made up of two core components: the API, which is the central ingredient, and the excipient, the substance inside the drug that helps deliver the medication to your system. Excipients are inactive substances, such as mineral oil, and not chemically active.

For instance, if you have a headache, acetaminophen is the active ingredient, and the liquid in the capsule is the excipient. [11]

Strength of APIs

Manufacturers use certain standards to determine how strong the API is in each drug. However, the standard can vary widely from one brand to another. One brand might use one test, another a different one.

Regulations

The quality of APIs has a significant effect on the efficacy and safety of medication. Poorly manufactured or compromised APIs have been connected to serious issues, such as illnesses and even death.

Even in the case of outsourcing, APIs are subject to stringent regulations and oversight from the country they are shipped to. For example, any APIs produced overseas for use in drugs produced in America still goes through inspection by the U.S. Food & Drug Administration.

As evidenced by the creation of APIs, the pharmaceutical industry is rapidly changing. Companies no longer handle every step of the drug-making process, from creating the API to building the capsule. In order to cut down on expenses and increase profits, companies have begun outsourcing the creation of APIs to foreign manufacturers based in Asia. While this has helped their bottom line, there is continued concern about the quality of these APIs produced overseas. In response, governing bodies responsible for patient safety, such as the FDA, have instituted intense screenings to ensure medication quality and prevent defects.[11]

Violating any of these established standards can result in fines or very expensive recalls for the pharmaceutical companies behind these manufacturers. [11]

Bulk Powders are multi dose preparations consisting of solid, loose, dry particles of

varying degrees offineness. Contain one or more active ingredients, with or without excipient sand, if necessary, coloring matter and flavoring substances. Usually contain non-potent medicaments such as antacids since the patient measures a dose by volume using a 5 ml medicine spoon. The powder is the nusually dispersed in water or, in the case of effervescent powders, dissolved be fore taking.

CONCLUSION

Analytic method development and validation are continuous and interconnected activities conducted throughout the drug development process. The practice of validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Although apparently contradictory, validated methods produce results within known uncertainties. These results are crucial to continuing drug development, as they define the emerging knowledge base supporting the product.

The time and effort that are put into developing scientifically-sound, robust, and transferrable analytic methods should be aligned with the drug development stage. The resources that are expended on method validation must be constantly balanced with regulatory requirements and the probability for product commercialization.

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