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A concise review on method development and validation parameters

Shipra Thapar [1,*], Anshul Chawla [1] and Girish Kumar Gupta [2]

[1] *Department of Pharmaceutical Sciences, CT University, Place- Ludhiana, 142024, (Punjab) India.*
[2] *Department of Pharmaceutical chemistry, Sri Sai College of Pharmacy, Badhani, Place- Pathankot, 145001, (Punjab) India.*

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Abstract

Method development is a broad term. In both quantitative and qualitative analysis developing a new method either for estimation of quantity of substance or to check the presence of the required component is a necessitate step. Validation means to establish the characteristics parameters of the method. It also helps by revealing the limitations and their extent of the method. Both terms are essential to create and then establish for any drug component so that it can be used in any pharmaceutical industry for its benefit.

Generally, the guidelines are followed as laid by ICH and USP which are followed worldwide. In this review article, some parts have been explained in reference to the subject quoted above.

Keywords: ICH; USP; Method Development; Validation; Analysis

1. Introduction

Analytical chemistry dealing with both active pharmaceutical ingredients (Drug substances) and pharmaceutical formulations (Drug products), is traditionally defined as pharmaceutical analysis. Some other branches of analytical chemistry that are involved in academia and pharmaceutical industries are like Drug metabolism studies, analytical biotechnology, and bioanalytical chemistry. The pharmaceutical analysis involves identity testing of the bulk drug and pharmaceutical products. Identification testing is necessary to verify that the drug substance is what or that the pharmaceutical preparation contains the correct drug substance. Pharmacopoeia contains a set of identification tests i.e., optical rotation values, the melting point of the compound, UV spectral data such as maximum wavelength. However, IR spectroscopy is widely used in industries because sample preparation is not needed. For quality control of a drug substance, typical properties of the drug substances are measured i.e. pKa values, spectral information, solubility, and melting point [1].

Pharmaceutical analysis deals with the analysis of drugs in pharmaceutical and biopharmaceutical samples. It involves the development of new pharmacopeia methods, analysis of herbal drugs and formulations, impurity analysis, stability-indicating, and degradation studies [2].

Pharmaceutical analysis plays an important role in drug development because drug development requires robust, precise and accurate analytical methods from preclinical to clinical studies. Various analytical techniques are involved for drug development, drug assay, quality control, and quality assurance like Capillary electrophoresis, High-

*Corresponding author: Shipra Thapar
Department of Pharmaceutical Sciences, CT University, Place- Ludhiana, 142024, (Punjab) India CT College of Pharmacy, Shahpur, Place- Jalandhar, 144020, (Punjab) India.

performance liquid chromatography, vibrational spectroscopies (IR and Raman), hyperspectral imaging techniques, mass spectrometry, and X-ray diffractometry [3].

1.1. Need for analytical method development

Analytical method development and validation are necessary due to the maintenance of standards of products in high commercial and market value, ethical reasons, and international competition. Various international regulatory bodies have set the standards to fix protocol to match the reference for granting approval, authentication and registration of pharmaceutical products. Some of the organizations governing the quality standards are:

- United States of Food and Drug Administration (USFDA)
- Good Laboratory Practice (GLP) regulations
- World Health Organization (WHO)
- The Pharmaceutical Inspection Cooperation Scheme's (PIC/S)
- The International Conference of Harmonization (ICH)

A set of analytical techniques like ultraviolet-visible (UV-Vis) spectroscopy, High- performance liquid chromatography, Infrared (IR) spectroscopy, Nuclear magnetic resonance (NMR) spectroscopy is employed for analytical method development and validation. By using these techniques, a large amount of data can be collected in a short period of time [4, 5].

1.2. Need for analytical method validation

Method validation is necessary for the following reasons:

- For assuring the quality of the products.
- For achieving the acceptance of the products by the international agencies.
- It is a mandatory requirement for accreditation as per ISO 17025 guidelines.
- A mandatory requirement for registration of any pharmaceutical product or pesticide formulation.

Validated methods are only acceptable for undertaking proficiency testing. Validation not only improves the processes but also confirms that the process is properly developed [6, 7].

For the manufacturer method validation is important in the following aspects:

- It decreases the risk of defect costs.
- It decreases the risk of regulatory noncompliance.
- A fully validated process may require fewer in-process controls and end-product testing.

1.2.1. Advantages of analytical method validation

- The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also for the user.
- Although the validation exercise may appear costly and time-consuming, it results in inexpensive, eliminates frustrating repetitions, and leads to better time management in the end [8].

2. Validation of developed analytical methods

The analytical method validation is adopted to confirm that the employed analytical procedure for specific tests meets the intended requirements. USP, ICH, FDA, etc., guidelines provide a framework for validations of pharmaceutical methods. Results from the method validation can be considered to judge its quality, reliability as well consistency about analytical results. In the pharmaceutical industry, the prominent reasons for validating assay are of two types. The first crucial reason is the validation of assay which is an integral part of the quality-control system and secondly regulation of genuine manufacturing practices inevitably needs assay validation [9].

The recent U.S. Food and Drug Administration (FDA) method validation guidance documents [10] as well as the United States pharmacopoeia (USP) both refer to ICH guidelines [11].

Analytical methods play an important role in many branches such as pharmaceutical analysis, natural product analysis, biomedical analysis, food production, etc. to reach reliable, repeatable, and accurate data validated analytical methods need to achieve these objectives [12].

Validation is a continuous process, and it should comprise at least four steps for an analytical method validation:

- Planning and performing the tests
- Statistical evaluation of the results
- Report on the validation parameters
- Application of all information gained during validation
- Full validation processes and their explanations [13].

Types of analytical procedures to be validated:

The discussion of the validation of analytical procedures is directed to the four most common types of analytical tests:

- Identification tests
- Quantitative tests for impurities content
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of a drug substance or drug product or another selected component(s) in the drug product

Table 1 Validation parameters according to ICH and USP guidelines

ICH	**USP**
Specificity	Specificity
Linearity	Linearity and range
Range	Accuracy
Accuracy	Precision
Precision	Limit of detection
Limit of detection	Limit of quantification
Limit of quantification	Ruggedness
---	Robustness

Validation is a very important factor in controlling the reliability of the method that is determined by the validation results, where accuracy, sensitivity, applicability, specificity, the limit of detection, and the limit of quantification are reported. Validated analytical methods play a key role in achieving the quality and safety of the final product, especially in the pharmaceutical industry. Analytical method validation should always be understood concerning the lifecycle of the analytical procedure [14].

2.1. Specificity

Specificity refers to the ability of the analytical method to differentiate and quantify the analyte in complex mixtures. An investigation of specificity is to be conducted during the determination of impurities and validation of identification tests. One of the significant features of HPLC is its ability to generate signals free from interference [15].

ICH guideline defines specificity as the ability to assess unequivocally the analyte in the presence of other compounds that may be likely to be present. Typically, these might be impurities, degradants, matrices, etc. The definition has the following implications

2.2. Identification test

Identification tests should be able to differentiate compounds of closely related structures which are expected to be present i.e., to assure the identity of an analyte.

2.3. Purity test

To ensure that the analytical procedure performed allows an accurate statement of the content of the impurity of an analyte i.e., related substances, residual solvents content, heavy metals, etc [16].

2.4. Assay

To arrive at an accurate result, this permits a correct report on the potency or content of the analyte in a sample [17].

2.5. Linearity and Range

The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line [18]. Linearity can be assessed by performing single measurements at several analyte concentrations. The data is then processed using linear least- squares regression. The resulting plot slope, intercept, and correlation coefficient provides the desired information on linearity.

2.6. Precision

The precision of an analytical procedure represents the closeness of agreement between a series of measurements got from multiple sampling of the same homogenous sample under similar analytical conditions and it is divided into 3 categories [19].

2.6.1. Repeatability

Precision under the same operating conditions, the same analyst over a short period.

2.6.2. Intermediate precision

Method is tested on multiple days, instruments, analysts, etc.

2.6.3. Reproducibility

Inter-laboratory studies

The ICH guidelines suggest that repeatability should be conformed duly utilizing at least 9 determinations with a specified range for the procedure (e.g., three concentrations / three replicates each) or a minimum of 6 determinations at 100 % of the test concentration [20].

2.7. Accuracy

The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose “true value” is known) is analyzed and the measured value is identical to the true value [21]. Typically, accuracy is represented and determined by recovery studies. There are three ways to determine accuracy:

- Comparison to a reference standard.
- Recovery of the analyte spiked into a blank matrix.
- Standard addition of the analyte.

It should be clear how the individual or total impurities are to be determined.

2.8. Limit of detection

LOD is determined by the analysis of samples with a known concentration of analyte and by establishing that minimum level at which the analyte can reliably detect, but not necessarily quantitated as precise value, under the stated experimental conditions. It is generally expressed in the concentration of an analyte (ppm).

Several approaches are recommended by the ICH for determining the detection limit of the sample, depending on the instrument used for analysis, the nature of the analyte, and the suitability of the method [21]. The acceptable approaches are:

- Visual evaluation
- Signal-to-noise ratio
- Standard deviation of the response
- Standard deviation of the slope of linearity plot The formula for calculating LOD is

$$LOD = 3.3\ \delta/S$$

Where δ = standard deviation of intercepts of calibration curves. S = the slope of the linearity plot.

2.9. Limit of quantitation

LOQ is the least concentration of drugs in a sample which is estimated with appropriate precision and accuracy under the affirmed experimental conditions.

Similar to LOD, ICH recommends the following four methods for the estimation of LOQ. The acceptable approaches are:

- Visual evaluation
- Signal-to-noise ratio
- Standard deviation of the response
- Standard deviation of the slope of linearity plot The formula for calculating LOQ is

$$LOQ = 10\ \delta/S$$

Where δ = standard deviation of response. S = Mean of slopes of the calibration curves (19).

2.10. Robustness

Robustness is defined by the measure of the capability of an analytical method to stay unchanged by small deliberate changes in method parameters. The variable method parameters in the HPLC technique may involve flow rate, column temperature, sample temperature, pH, and mobile phase composition.

2.11. System Suitability Test

Suitability testing was originally believed by the industry of pharmaceuticals to decide whether a chromatographic system is being routinely utilized day to day in pharmaceutical laboratories where the quality of results is most important which is suitable for a definite analysis [22].

2.12. Stability studies

The stability of drug substances or drug products is an essential quality attribute; so, stability studies play a very important role in the manufacturing of pharmaceutical products. Stability studies of pharmaceutical formulations are very important because pharmaceutical formulations are marketed in different strengths and various types of packages. There are a set of regulatory guidelines for the stability testing programme of pharmaceutical substances and products. Stability studies are the essential criterion for confirming the quality and approval of the manufactured preparations. So, stability studies may be an important element of manufacturing trade. Manufacturing industries depend upon the stability studies of manufactured products to make sure of the safety and potency of the drug to assign shelf life for the formulation. To make sure that good products are prepared, which may be potent enough to last till their stability period, Stability studies are essential.

The international council for harmonization of technical requirements for pharmaceuticals for human use provides regulatory guidelines on pharmaceutical substances and products. ICH topics are divided into four categories i.e., quality, safety, efficacy, and multidisciplinary topics. Quality topics include the guidelines for conducting stability studies, defining the threshold of impurities testing. Safety topics include the study of potential risks like nephrotoxicity, carcinogenicity, and genotoxicity. Efficacy topics include the design, conduct, safety, and reporting of clinical trials. Multidisciplinary topics include cross-cutting topics which do not fit into a quality, safety, and efficacy topics. This includes the development of electronic standards for the transfer of regulatory information [23].

Table 2 ICH Quality guidelines

ICH CODE	Guideline title
Q1 A-Q1F	Stability
Q2	Analytical validation
Q3A-Q3 D	Impurities
Q4A-Q4 B	Pharmacopoeias
Q5A– Q5 E	Quality of biotechnological products
Q6A– Q6 B	Specifications
Q7	Good manufacturing practice
Q8	Pharmaceutical development
Q9	Quality risk management
Q10	Pharmaceutical quality system
Q11	Development and manufacture of drug substances
Q12	Life cycle management
Q13	Continuous manufacturing of drug substances and drug products
Q14	Analytical procedure development

Table 3 ICH stability guidelines

ICH CODE	Guideline title
Q1A	Stability testing of New Drug Substances and Products(Second Revision)
Q1A(R2)[2]	Stability testing of new drug substances andproducts[2]
Q1B	Stability testing: Photo stability Testing of New Drug Substances and Products
Q1C	Stability testing of New Dosage Forms
Q1D	Bracketing and Matrixing Designs for stability testing of Drug Substances and Products
Q1E	Evaluation of stability data
Q1F	Stability data package for Registration Applications in Climatic Zones III and IV
Q5C	Stability testing of Biotechnological/Biological Products

When the degradation behaviour of products is not known, forced degradation or stress testing conditions are used for stability-indicating studies. These studies help to provide information about the degradation pathways of the degradation products. Forced degradation studies help to facilitate the formulation development, manufacturing, and packaging of pharmaceutical products in which the chemical behaviour of products can be used to improve the drug product stability.

Table 4 General protocol for forced degradation studies (stress testing) of drug substances and drug products

Condition	Drug substance		Drug product	
	Solid	Solution/ Suspension	Solid (Tablets, Capsules, Blends)	Solution(IV, Oral Suspension)
Acid/base		✓		X
Oxidative	X	✓	✓	✓
Photo stability	✓	X	✓	✓
Thermal	✓		✓	✓
Thermal/humidity	✓		✓	

*✓=Recommended; X= Optional, suggested for some compounds

3. Need for forced degradation studies of drugs

Forced degradation studies are carried out for the following reasons:

- Development and validation of stability-indicating methodology.
- Determination of degradation pathways of drug substances and drug products.
- Discernment of degradation products in formulations that are related to drug substances versus those that are related to non–drug substances (e.g., excipients).
- Structure elucidation of degradation products.
- Determination of the intrinsic stability of a drug substance molecule [24].

Forced degradation studies are helpful for the determination of the degradation pathways and structural elucidation of the degradants produced. Stress studies are also used to select the storage conditions and improve the manufacturing process of pharmaceutical formulations. Some regulatory agencies have set the limits of degradation products in their guidelines. For validation of chromatographic assays, 5–20% degradation is accepted. The stability limit should be > 90% in the case of small molecules and about 10% degradation is sufficient.

3.1. Forced degradation studies

3.1.1. Oxidation

Most of the drug substances are found to be auto-oxidizers. For initiation of oxidation reaction, free radical initiators are required which are Hydrogen peroxide, impurities, and metal ions. Hydrogen peroxide (0.1-3 %) is generally used as an initiator of an oxidation reaction in forced degradation studies. Forced degradation oxidation studies should be conducted at 40°C for 1– 7 days. If the drug is degraded more than 20 %, then it should be considered abnormal.

3.1.2. Thermal conditions

Some drugs are found to be thermo-labile. By increasing the temperature, the rate of reaction increases which leads to the production of degradation products. Forced degradation studies under thermal conditions should be conducted at 40–80°C. Thermal stress studies last for 1-2 months and are conducted at 70°C and high humidity. Solid drug molecules are subjected to both dry and wet heat conditions, while liquids are exposed to dry heat for a shorter period.

3.1.3. Hydrolysis

In forced degradation hydrolysis studies, the drug is subjected to both acidic and basic conditions at different pH. In general, the drug substances are treated with 0.1N HCl or H2SO4 or 0.1N NaOH at 50–60°C. The degradation of the molecule depends on the strength of acid or alkali used. The strength of acid or alkali should be maintained in the range between 0.1 N to 1 N solutions. The duration of the study should not exceed 7 days.

3.1.4. Photolytic conditions

Drug substances are exposed to UV or fluorescent conditions in photolytic degradation studies. The commonly used radiation range is about 300–800 nm for degradation studies.

3.2. Factors affecting degradation

3.2.1. Moisture

- Moisture plays an important role in the degradation of drug substances and products. In the presence of moisture, water-soluble substances may get dissolved which leads to physical and chemical changes within the molecule.

3.2.2. Excipients

- Some excipients may contain high content of water which affects the stability of the drug. In some cases, Chemical interactions that occur between the excipients and the drug substances often result in decreased stability of the drug.

3.2.3. Temperature

- The rate of drug hydrolysis is increased by an increase in temperature.

3.2.4. pH

- pH shows an important role in the degradation rate of drugs by hydrolysis.

3.2.5. Oxygen

- Oxidation of drugs is increased in the presence of oxygen.

3.2.6. Light

- Some drugs are decomposed when exposed to light because of their photolabile nature [25].

3.3. Types of stability studies on drug substances

The pharmacopoeial protocol prescribes the criteria for acceptable levels of microbiological, toxicological, chemical, physical, and therapeutic stability studies.

3.3.1. Microbiological stability

The microbiological stability of the drugs is the tendency to resistant to sterility and microbial growth. The antimicrobial agents used in the preparation retain the effectiveness within specified limits. This microbiological instability could be hazardous to the sterile drug product.

3.3.2. Chemical stability

It is the tendency to resist its change or decomposition due to the reactions that occur due to air, atmosphere, temperature, etc.

3.3.3. Physical stability

The original physical properties such as appearance, colour, dissolution, palatability, suspend ability are retained. The physical stability may affect the uniformity and release rate; hence it is important for the efficacy and safety of the product [25].

3.3.4. Therapeutic stability

The therapeutic effect (Drug Action) remains unchanged.

3.3.5. Toxicological stability

Toxicological stability has no significant increase in the toxicity that occurs.

Table 5 Types of Stability Studies

Types of Stability Studies	Storage condition	Minimum period (Months)
Long term	25±2°Cand60±5%RH or 30±2°Cand65±5 %RH	12
Intermediate	30±2°Cand65±5 %RH	6
Accelerated	40±2°Cand75±5 %RH	6

3.4. Stability testing procedures

Based on the aim and steps followed, stability testing procedures have been categorized as:

3.5. Real-time stability testing

Real-time stability testing is generally performed for a longer duration to allow significant product under recommended storage conditions. The duration of the studies depends upon the stability of the product that should indicate clearly that no measurable degradation occurs.

3.6. Accelerated stability testing

In accelerated stability testing, the products are subjected to the conditions that accelerate their degradation. In this study, the products are stressed at high temperatures (warmer than ambient), and the heat required to cause product degradation is measured. Results of accelerated stability give information about the shelf life of the products. In addition to temperature, other stress conditions are applied during accelerated stability testing i.e., gravity, agitation, moisture, package, light, and pH [26].

3.7. Retained sample stability testing

In the first introduction of the product in the market, samples from every batch are taken for stability studies. When the number of batches marketed exceeds 50, then stability samples from two batches are required to be taken. The stability samples are tested at predetermined intervals i.e., if a product has a shelf life of 5 years, it is conventional to test samples at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. This study involves the stability testing of products already available in the market place.

3.8. Cyclic temperature stress testing

In this study, the period of the cycle generally considered is 24 hours because the diurnal rhythm on earth is 24 hours, which the marketed pharmaceuticals are most likely to experience during storage. Based on the product to product, the minimum and maximum temperature should be selected for stress conditions. The recommended cycles for the test should have 20 cycles [27].

Table 6 Test Schedule for stability testing of new products

Environment	Sampling Time Points(Months)	Method & Climaticzone
25°C/60%RH	3, 6, 9, 12, 18, 24,36	Long term for zones I and IV
30°C/35%RH	3, 6, 9, 12, 18, 24,36	Long term for zones III
30°C/65%RH	3, 6, 9, 12, 18, 24,36	Long term for zone IVa, or intermediate condition for zones I and II
30°C/75%RH	3, 6, 9, 12, 18, 24,36	Long term for zone IVa, or intermediate condition for zones I and II
40°C/75%RH	3, 6	Accelerated condition for all zones

3.9. Techniques employed for stability-indicating analytical methods

These methods explain the step-by-step stability and analytical method development for the degradation of drug substances and products [28-31].

Previously only titrimetric, spectrophotometric, and chromatographic methods are used in the analysis of the stability of the samples but nowadays hyphenated techniques are also used titrimetric and spectrophotometric methods are inexpensive and simple but because of their less sensitivity chromatographic methods are widely used to provide good, precise, sensitive and accurate results and also can detect the degraded amount of product even in a small amount can also detect the degraded amount of product even in small amount and can easily trace. Therefore, the HPLC method has been readily utilized because of high sensitivity high resolution and high specificity.

3.10. Different techniques employed for stability studies

- Electrometric Methods: Titrimetric and polarographic methods
- Spectrophotometric Techniques: UV-visible spectroscopy, Fluorescence spectroscopy, and nuclear magnetic resonance.
- Chromatographic techniques: Thin-layer chromatography (TLC), High-performance thin-layer chromatography (HPTLC), Gas chromatography, High-performance liquid chromatography (HPLC),
- Hyphenated techniques: Gas chromatography-mass spectrometry (GC-MS), Liquid chromatography-Mass Spectrometry (LC-MS), LC-MS-MS, Liquid chromatography- nuclear magnetic resonance (LC-NMR), and Capillary electrophoresis-mass spectrometry (CE-MS).

3.11. Decision trees for respective stress conditions

This approach is based on the labile behavior of the drug. The decision tree provides information to stop the stress conditions at a particular time interval. From the decision tree, a decision can be taken on whether to increase or decrease the stress conditions. The stress conditions are accepted wherever sufficient degradation is achieved. The generally recommended range for degradation varies between 5-20 %. This range covers the generally permissible 10 % degradation for small molecule pharmaceutical drug products, for which the stability limit is 90 %-110 % of the label claim.

3.12. Hydrolytic degradation (under acidic and basic conditions)

Initially, hydrolytic degradation can start by refluxing the drug in 0.1N HCl/NaOH for 8 hours. If sufficient degradation is not achieved, then the drug should be kept in 1N, 2N, 5N HCl/NaOH for 12- and 24-hours periods. In the conditions where the drug is completely degraded, the drug is kept in 0.01N HCl/NaOH for 8 hours at 40°C and 2 hours at 25°C.

3.13. Oxidative degradation

For the determination of degradation of the drug in oxidation, initially, the drug substance is kept in 3% H_2O_2 for 6 hours at room temperature, if there is no degradation, the reaction period should be increased to 24 hours in the same conditions. If there is no degradation, then the drug is kept in 10 % H_2O_2 for 24 hours. When the drug is not oxidized in these conditions then extreme conditions of 30 % H_2O_2 for 24 hours may be tried. When the drug is completely degraded in 3 % H_2O_2 then the strength of the H_2O_2 is decreased to 1%.

3.14. Photolytic degradation

In photo degradation studies, the drug substance should be exposed to illumination up to 1.2 × 106 lux hours. The exposure may be increased by 5 times in case there is negligible degradation of the drug substance. The drug may be declared photos table if the increase in exposure to 6.0 × 106 lux hours does not affect the stability of the drug.

3.15. Thermal degradation

Thermal stress studies are conducted at elevated temperatures for a short duration of time. Solid-state samples of drug substances and drug products should be exposed to both dry and wet heat. For liquid drug products only, dry heat is used for stress studies. Based on the nature of the product thermal degradation temperature is selected. The temperature for thermal degradation should be in 10 °C increments above that for routine accelerated testing, and humidity at 75 % relative humidity or greater. Initially, thermal degradation started at 50°C, if sufficient degradation is not achieved the temperature is increased by 10°C at every time [32].

4. Conclusion

Method development and validation are interconnected with each other. Both are important for continuing any assay procedure, drug estimation, and degradation study and so on. Any new analytical method which is developed should undergo validation before being used by any laboratory. Validation parameters are utmost important as per the guidelines by ICH and USP.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declares that there's no conflict of interest and that we all agreed that the paper be published.

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