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Review Article

Method development and validation of some API in bulk and pharmaceutical dosage form using RP-HPLC

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High-performance liquid chromatography (HPLC) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. RP-HPLC is a very powerful technique for the analysis of peptides and proteins. RP-HPLC can cause the irreversible denaturation of protein samples thereby reducing the potential recovery of material in a biologically active form. An active ingredient (AI) is the ingredient in a pharmaceutical drug that is biologically active. The primary, active ingredient(s) of a final pharmaceutical product, produced in the first stage of pharmaceutical production and usually in bulk quantities. 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. Bulk drugs are the active chemical substances in powder form, the main ingredient in pharmaceuticals – chemicals having therapeutic value, used for the production of pharmaceutical formulations. Major bulk drugs include antibiotics, sulpha drugs, vitamins, steroids, and analgesics. Often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency.

Key Words: Reversed-phase high-performance liquid chromatography (RP-HPLC), Active Pharmaceutical Ingredient (API), Bulk drugs.

INTRODUCTION

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one.[1] Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias4. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, withdrawal from the market), development of patient resistance and introduction of better drugs by competitors.[2] Under these conditions, standards reports of new toxicities (resulting in their and analytical procedures for these drugs may not be available in the pharmacopoeias. Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. [3,4]

Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product5. These combination products can present daunting challenges to the analytical chemist responsible for development and validation of analytical methods

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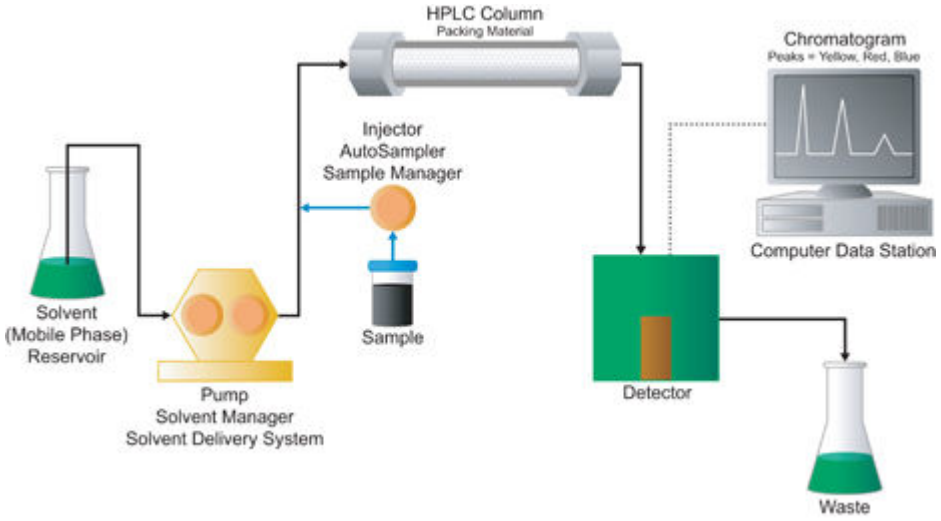


Fig.1: Schematic diagram of High Performance Liquid Chromatography (HPLC).

The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products[5,6,7].

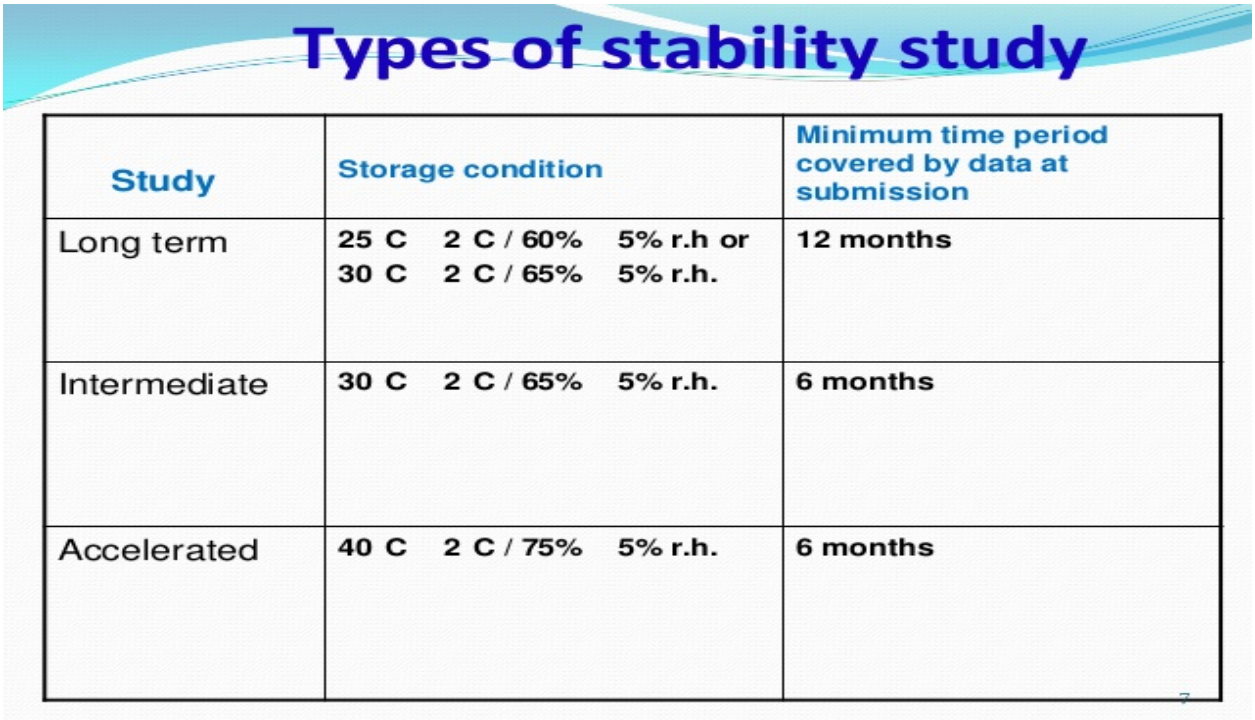
Basic criteria for new method development of drug analysis[8,9]:-

- The drug or drug combination may not be official in any pharmacopoeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Regulatory Status of Stability-Indicating Assays[10]

The ICH guidelines have been incorporated as law in the EU, Japan and in the US, but in reality, besides these other countries are also using them. As these guidelines reflect the current inspectional tendencies, they carry the de facto force of regulation[10]. The ICH guideline Q1A on Stability Testing of New Drug Substances and Products emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods. It is also mentioned that forced decomposition studies (stress testing) at temperatures in 10 °C increments above the accelerated temperatures, extremes of pH and under oxidative and photolytic conditions should be carried out on the drug substance so as to establish

Table 1 : Various types of Stability Studies.[10]



the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures. The ICH guideline Q3B entitled 'Impurities in New Drug Products' emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products. It is also required that analytical methods should be validated to demonstrate that impurities unique to the new drug substance do not interfere with or are separated from specified and unspecified degradation products in the drug product. The ICH guideline Q6A, which provides note for guidance on specifications, also mentions the requirement of stabilityindicating assays under Universal Tests/Criteria for both drug substances and drug products10. The same is also a requirement in the guideline Q5C on Stability Testing of Biotechnological/Biological Products. Since there is no single assay or parameter that profiles the stability characteristics of such products, the onus has been put on the manufacturer to propose a stabilityindicating profile that provides assurance on detection of changes in identity, purity and potency of the product[11].

Role of Degradant Profiling in Active Pharmaceutical Ingredients and Drug Products[12,13,14,15,16]

Regulatory Requirements

• Identification of possible degradants

• Degradation pathways and intrinsic stability of the drug molecule

• Validation of stability indicating analytical procedures.

Issues addressed in regulatory guidance include:

• Forced degradation studies are typically carried out using one batch of material.

• Degradation products that do not form in accelerated or longterm stability may not have to be isolated or have their structure determined.

• Mass balance should be considered.

Active Pharmaceutical Ingredient

The specified stress conditions should result in approximately 5-20% degradation of the API or represent a reasonable maximum condition achievable for the API. The specific conditions (intensity and duration) used will depend on the chemical characteristics of the API. The stressed sample should be compared to the unstressed sample (control) and the appropriate blank[12,13].

Acid study

For a force degradation acid study for a particular API, the API is exposed to acidic conditions. The API (at a known concentration) is usually prepared in the sample preparation solvent, which gives 0.1-1 M acid solution of either hydrochloric acid or sulphuric acid[12].

Base study

For a force degradation acid study for a particular API, the API is exposed to acidic conditions. The API (at a known concentration) is usually prepared in the sample preparation solvent, which gives 0.1-1 M base solution of either sodium hydroxide or potassium hydroxide or lithium hydroxide[12].

Oxidation study

Oxidation can be carried out under an oxygen atmosphere or in the presence of peroxides. The

Table 2: A dosage form containing two or more active pharmaceutical ingredients in different physical forms[14,15,16]

First API	Second API
Fluoxetine hydrochloride	Olanzapine
Metformin hydrochloride	Pioglitazone hydrochloride
Metformin hydrochloride	Rosiglitazone maleate
Metformin hydrochloride	Sulphonylurea (glimepiride, glyburide, glipizide, etc)
Metformin hydrochloride	Pioglitazone hydrochloride & Sulphonylurea (as above)
Rosiglitazone maleate	Glimepiride
Dipyridamole	Aspirin
Hydralazine	Isosorbide dinitrate
Verapamil	Trandolapril
Naproxen sodium	Sumatriptan succinate
Naproxen	Lansoprazole
Galantamine hydrobromide IR	Galantamine hydrobromide ER
Acetaminophen	Tramadol hydrochloride
Levodopa	Carbidopa
Sartans (losartan, irbesartan, etc)	Hydrochlorothiazide

use of oxygen is a more realistic model. Free radical initiators may be used to accelerate oxidation[12].

Thermal/humidity study

If the forced degradation thermal/humidity conditions produce a phase change, it is recommended to also run thermal/humidity conditions below the critical thermal/ humidity that produce the phase change[12].

Photostability

Studies are performed in accordance with ICH photostability guidelines. According to the ICH guidelines, "the design of the forced degradation experiments is left to the applicant's discretion although the exposure levels should be justified[12].

Drug product

Drug product (DP) degradation cannot be predicted solely from the stability studies of the API in the solid state or solution. The non-active pharmaceutical ingredients can also react with the API or catalyze degradation reactions[12].

Stability-Indicating Method Development:-[12,13,14]

A stability-indicating method is defined as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. A method that accurately quantitates significant degradants may also be considered stability-indicating. A proactive approach to developing a stability indicating HPLC method should involve forced degradation at the early stages of development with the key degradation samples used in the method development process. Forced degradation should be the first step in method development. If forced degradation studies are performed early, method development and identification of primary degradation products and unknown impurities can be run in parallel. Using this process, a validated HPLC analytical assay, mechanisms of degradation, and the impurity/degradant information for filing can all be generated without delays in the project timeline.

Method Development[15,16]

Method development should be based on several considerations. It is preferable to have maximum sample information to make development fast and desired for intended analytical method application, physical and chemical properties are most preferable as primary information.

Steps in method development

1. Sample information, define separation goals
2. Sample pre-treatment, need of special HPLC procedure
3. Selection of detector and detector settings
4. Selection of LC method; preliminary run; estimate best separation conditions
5. Optimize separation conditions
6. Check for problems or requirement for special procedure
7. Method validation

Sample information

1. Number of compounds present
2. Chemical structure of compounds
3. Chemical nature
4. Molecular weight of compounds

5. pKa Value(s) of compounds
6. Sample solubility
7. Sample stability and storage
8. Concentration range of compounds in sample
9. UV spectra of compounds or properties for detection of compounds

Chromatographic detection

Before the first sample is injected during the HPLC/UPLC method development we must be reasonably sure that the detector selected will sense all sample components of interest. Normally variable wavelength UV detector is the first choice of the chromatographers, because of their convenience and applicability for most organic samples. UV spectra can be obtained by PDA detector. When the UV response of the sample is inadequate, other detector or derivative UPLC/HPLC method can be used.

List of solvent (based on polarity):

Toluene	Diethyl ether (ether)
Chloroform	Methylene chloride
Tetrahydrofuran (THF)	Acetone
Ethyl acetate	Dioxane
Acetic acid	Methanol
Acetonitrile	Water (most polar)

List of functional group (based on polarity):

Hydocarbons (least polar)	Ethers
Esters	Ketones
Aldehydes	Amides
Amines	Alcohols
	Water (most polar)

Stationary phases

Many types of stationary phases available commercially with different column material chemistry start from C-18 for reversed phase to silica for Normal phase chromatography Chromatographers may need to consider many aspects before selecting a column.

Method Validation[15,16]

Once an analytical method is developed for its intended use, it must be validated. The extent of validation evolves with the drug development phase. Usually, a limited validation is carried out to support an Investigational New Drug (IND) application and a more extensive validation for New Drug Application (NDA) and Marketing Authorization Application (MAA). Method validation is vast area which includes many validation parameters with different approaches for different level of requirement based on intended use of analytical method, criticality and regulatory requirements. Validated method also can give the unpredicted or unknown problem during the course of routine usage, because validated method has also limited level of confidence, as method was validated for known or predicted variable parameters or every method can fail sooner or later. But still after method development it needs to be validated as per requirement which gives certain level of confidence for its intended use.

Specificity

Specificity is the ability of the method to measure the analyte in the presence of other relevant components those are expected to be present in a sample. The relevant components might include impurities, degradants, matrix, etc. Specificity can

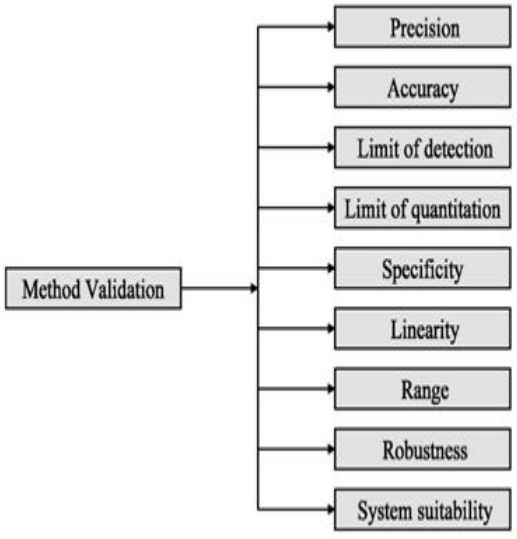


Fig. 2: Various Process of Method Validation.[15,16]

also be demonstrated by verification of the result with an independent analytical procedure. In the case of chromatographic separation, resolution factors should be obtained for critical separation15.

Force degradation studies:

Force degradation of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used15. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample. Precision may be considered at two levels: repeatability and intermediate precision[15]. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability: Repeatability study is performed by preparing a minimum of 6 determinations at 100% of the test concentration and analyzed as per the respective methodology.

Intermediate Precision: The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. Here, intermediate precision of the method is checked by carrying out six independent assays of test sample preparation on the different day by another person under the same experimental condition and calculated the % RSD of assays[15].

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value

found. The evaluation of accuracy has got very prime importance as it deliberately force the method to extract the drug and impurities at higher and lower level.

Solution stability

Drug stability in pharmaceutical formulations/active pharmaceutical ingredients is a function of storage conditions and chemical properties of the drug, preservative and its impurities. Condition used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis.

Limit of detection

The limit of detection (LOD) for an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected[16].

Limit of quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The limit of quantitation (LOQ) is a parameter of quantitative assays for low levels of compounds in sample matrices. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified[16].

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The factors chosen for all the drugs under investigation were the flow rate, mobile phase composition, pH of a mobile phase and using different lot of LC column. The observation shall be summarized and critical parameters shall be listed out in the validation report[16].

Advantages of analytical method validation[15,16]

The advantages of the analytical method validation are as follow:

- The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.
- Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.
- Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

CONCLUSION

Pharmaceutical analytical chemistry is an important part in monitoring the quality of pharmaceutical products for safety and efficacy. With the advancement in synthetic organic chemistry and other branches of chemistry including bioanalytical sciences and biotechnology, the scope of analytical chemistry has enhanced to much higher levels. Pharmacopoeias rely more on instrumental techniques rather than the classical wet chemistry method. In the present research work a modest attempt has been made to develop validated analytical methods for the determination of single or combined dosage form. Estimation of degradants generated during formulation and storage of finished products using a UPLC technique. The UPLC method development for the separation, identification and quantification of APIs/ impurities/excipients present in the pharmaceutical formulations and its validation as per guidelines is furnished. Related components are the impurities in pharmaceuticals which are unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during stability testing, or develop during formulation or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Various analytical methodologies were employed for the determination of assay and related components in pharmaceuticals drug substance and drug product. There is a scope for development of new analytical methods for quality evaluation of new emerging drugs.

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