Review Article

Review Article of Dissolution Test Method Development and Validation of Dosage Form by Using RP-HPLC

Prachi Barsagade *, Roshan Khetade, Kalyani Nirwan, Tikesh Agrawal, Santosh Gotafode, Upadesh Lade.
Chatrapati Shivaji College of Pharmacy, Deori, Gondia, Maharashtra, India.
***Corresponding author's E-mail:** prachibarsagade398@gmail.com

Received: 21-06-2021; **Revised:** 23-08-2021; **Accepted:** 28-08-2021; **Published on:** 15-09-2021.

ABSTRACT

Dissolution is precise test method used for evaluating drug release of solid and semisolid dosage forms. It is the process by which a solid solute enters a solution. For the analysis of dissolution test method development, the Reverse Phase High Performance Liquid Chromatography is used, and it is the modern of column chromatography. In the reverse phase partition HPLC the relative polarity of the stationary and mobile phase opposite to those in normal HPLC. The main applications of the dissolution testing include bio-pharmaceutical characterization of the drug product, as a tool to make sure consistent product quality and to predict in vivo drug bioavailability. The article represents the current updates in dissolution testing methods by using Reverse Phase High Performance Liquid Chromatography. The validation parameters explain for the analysis of sample is accuracy, precision, repeatability, detection of limit, quantitative limit, linearity, range and robustness. Analytical method validation includes all procedure and checks required to prove the reliability of a method for quantitative determination of concentration of an analyte or series of analyte in a given sample. The conventional and novel pharmaceutical dosage forms and gives an insight to possible alternatives in drug dissolution testing design. The aim of this review is to represent all the potential standardized test methods development and validation of pharmaceutical dosage form by using RP-HPLC which are analyzed the accuracy of the dissolution sample of the dosage form.

Keywords: RP-HPLC, Dissolution, Drug release, Dosage form, Validation.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v70i01.005

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v70i01.005

INTRODUCTION

Analytical Chemistry is a scientific discipline that develops and applies methods, instruments and strategies to obtain information on the composition and nature of matter. Pharmaceutical analysis is branch of pharmaceutical sciences which deals with analysis of drug and adjuvant used in manufacture of drug formulation assuring the quality of drugs and their formulation i.e. pharmaceuticals. Analytical chemist or quality control chemists have the responsibility to develop sensitive, reliable and accurate method for the estimation of the drug in a pharmaceutical dosage form and in biological fluid. Analytical chemistry has played the major role in the changes faced by the pharmaceutical industry today. The analytical department has become significant partner in the drug development process[1]. Drug absorption from a solid oral dosage depends on the release of the drug substance from the drug product (dissolution), the solubility and permeability across the gastrointestinal tract. The first aspect of the tables is determined by the manufacture of the product and the next two aspects are determined by the properties of the pharmaceutical. All solid oral dosage forms can be characterized according to these properties [2].

Dissolution

Dissolution is the process by which a solid solute enters a solution. In the pharmaceutical industry, dissolution is defined as amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition. Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage form and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence. Dissolution behavior of drug has a significant effect on their pharmacological activity.

The bioavailability and bioequivalence data obtained as a result of dissolution testing can be used to guide the development of new formulation and product development process toward product optimization, as well as to insure continuing product quality and performance of manufacturing process. In addition, dissolution is a requirement for regulatory approval for product marketing and is a vital component of the overall quality control program. The process involved in dissolution of solid dosage form[3].

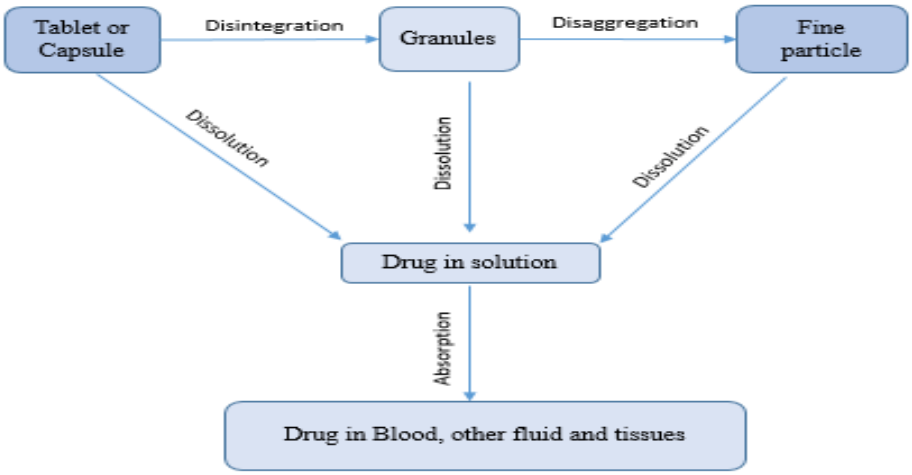

Figure 1: Schematic illustration of dissolution process

Importance (Need of Dissolution) [5]

- Result from in vitro dissolution rate experiments can be used to explain the observed differences in in-vitro availability.
- Dissolution testing provides the means to evaluate critical parameters such as adequate bioavailability and provides information necessary to formulator in development of more efficacious and therapeutically optimal dosage forms. Most sensitive and reliable predictors of in-vivo availability.
- Dissolution analysis of pharmaceutical dosage forms has emerged as single most important test that will ensure quality of product. It can ensure bioavailability of product between batches that meet dissolution criteria.
- Ensure batch-to-batch quality equivalence both in-vitro and in-vivo, but also to screen formulations during product development to arrive at optimally effective products.
- Physicochemical properties of model can be understood needed to mimic in-vivo environment. Such models can be used to screen potential drug and their associated formulations for dissolution and absorption characteristics. Serve as quality control procedures, once the form of drug and its formulation have been finalized.
- Dissolution testing is widely used in the pharmaceutical industry for optimization of formulation and quality control. It is useful in the pharmaceutical and biotechnology industry to formulate drug dosage forms and to develop quality control specifications for its manufacturing process.
- To identify the critical manufacturing variable, like the binding agent effect, mixing effects, granulation procedure, coating parameters and comparative profile studies.
- The comparative profile studies are to comply with guideline set in the scale up and post approval changes (SUPAC) and ICH, select candidate formulation, simulate food effect on bio availability, support waiver for bio equivalence requirements, in the study of Bio waivers, surrogate for in-vivo studies and in the in-vitro in-vivo correlations.

Theories of Dissolution [4,6]

Many workers have reviewed the factors which can affect the dissolution of tablets and these include the stirring speed, temperature, viscosity, pH, composition of the dissolution medium and the presence and absence of wetting agents. These are the diffusion layer model, interfacial barrier model and Danckwert's model. In the diffusion layer model (Fig 2) assumes that a layer of liquid, 11 cm thick, adjacent to the solid surface remains stagnant as the bulk liquid passes over the surface with a certain velocity. The rate of dissolution is governed entirely by the diffusion of the solid molecules from the static liquid film to the bulk liquid according to Fick's first law. The theory predicts that if the concentration gradient is always constant i.e. C s — C t is constant because C s and gt; and gt; C t (sink condition usually mean Cs and gt; 10 Ct) then a uniform rate of dissolution is obtained. In the interfacial barrier model, the rate of diffusion in the static layer is relatively fast in comparison with the surmounting of the energy barrier, which therefore becomes rate limiting in the dissolution process and the Danckwert's model (Fig 3) assumes that macroscopic packets of solvent reach the solid/liquid interface by eddy diffusion in some random fashion. However, the earliest equation expressing dissolution rate in a quantitative manner was proposed by Noyes and Whitney 51.

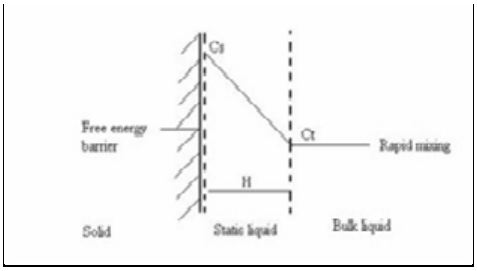

Figure 2: Diffusion Layer Model

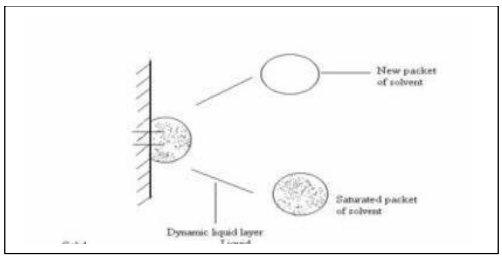

Figure 3: The Danckwert's Model

Dissolution and Solubility

Dissolution is the process by which a solid drug substance becomes dissolve in a solvent. Solubility is the mass of solute that dissolve in a specific mass or volume of solvent at a given temperature (Ex, 1g of NaCl dissolves in 2.786 mL off water at 25ºC). Solubility is a static property; whereas dissolution is a dynamic property. In biologic systems, drug dissolution in an aqueous medium is an important prior condition for systemic absorption. The rate at which drugs with poor aqueous solubility dissolve from an intact or disintegrated solid dosage form in the gastrointestinal tract often controls the rate of systemic absorption of the drug. Thus, dissolution tests may be used to predict bioavailability and may be used to discriminate formulation factors that affect drug bioavailability. The dissolution test is required for all U. S. Food and Drug Administration (FDA) approved solid oral drug products7. The overall rate of drug dissolution may be described by the Noyes-Whitneyequation.

$$dC/dt = DA/h\ (Cs - C)$$

Where, dC/dt= rate of drug dissolution at time t, D= diffusion rate constant, A= Surface area of the particle, Cs= concentration of drug (equal to solubility of drug) in the stagnant layer, the rate of dissolution [8].

In Vitro- In Vivo Correlation [9,10]

The term correlation means interdependence between qualitative and quantitative data and relationship between measurable variable and ranks. For biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate in vitro release characteristics and in vivo bioavailability parameters. An in-vitro in vivo correlation (IVI VC) has been defined as the food and drug administration (FDA) as "a predictive mathematical model describing the relationship between an in vitro property of a dosage form and an in-vivo response" Generally the in vivo property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed. The United States Pharmacopoeia (USP) also defined IVI VC as "the establishment a relationship between a biological property, or a parameter derived from a biological property product from a dosage form and a physicochemical property of a same dosage form.

Levels of IVIVC

Five correlation levels have been defined in the IVIVC FDA guidance. There are three primary IVIVC categories, known as Levels A, B, C (there is also a Multiple Level C correlation) and D. The concept of correlation level is based upon the ability of correlation to reflect the complete plasma drug level time profile which will result from administration of the given dosage form.

(A) In the level A correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form.

(B) In level B correlation use all the in vivo and in vitro data it is not considered to be a point-to-point correlation, since there are a number of different in vivo curves that will produce similar mean residence time value. The level B correlation does not uniquely reflect the actual in vivo plasma level curves.

(C) In the level C correlation one dissolution time point (t50%, t90%, etc.) is compared to one mean pharmacokinetic parameter such as AUC, T max or C max. This is the weakest level of correlation as partial relationship between absorption and dissolution is established. And multiple level C correlation relates one or seven pharmacokinetic parameters of interest (C max, AUC or any other suitable parameters) to the amount of drug dissolved at several time points of the dissolution profile. A multiple level C correlation should be based on at least three dissolution time points covering the early, middle and late stages of the dissolution profile.
(D) Level D correlation is a rank order and qualitative analysis and is not considered useful for regulatory purposes. It is not a formal correlation but serves as an aid in the development of a formulation or processing procedure.

Biopharmaceutics Classification System (BCS)

Biopharmaceutics Classification System (BCS) is a regulatory mechanism through which drug developers and generic companies can obtain a waiver of clinical bioequivalence studies, also called a bio-waiver. According to the 2000 FDA BCS Guidance, compounds that are classified as Class I (highly soluble, highly permeable) are eligible for BCS bio-waivers. Biopharmaceutics is the science that examines this interrelationship of the physicochemical properties of the drugs, the dosage form in which the drug is given and the route of administration on the rate and extent of systemic drug absorption. Thus, Biopharmaceutics involve factors that influence: The

stability of the drug within the drug product, the release of the drug from the drug product, the rate of dissolution/release of the drug at the absorption site and the systemic absorption of the drug [11].

Biopharmaceutical Classification System is a scientific framework for classifying drug substance based on their aqueous solubility and integral permeability. When combine with the dissolution of drug product, the BCS take into account three major factors that govern the rate and extent of drug absorption from immediate release solid oral dosage forms: solubility, permeability, and dissolution.

BCS Divides Compounds into Four Categories:

Class I — High Solubility, High Permeability

Class II — Low Solubility, High Permeability

Class III — High Solubility, Low Permeability

Class IV — Low Solubility, Low Permeability

The BCS was developed on the theory that drug dissolution is controlled by solubility and drug surface area as defined by dose and drug particle size. In according to the BCS, it follows that there should be a theoretical rate of drug dissolution given the solubility, dose, particle size, dissolution volume and hydrodynamic conditions.

• A drug substance is considered Highly permeable when the extent of absorption in humans is determined to be > 90% of an administered dose, based on mass balance or in comparison to an intravenous reference dose [12].

Physicochemical Properties of Drug

Factors Affecting Dissolution Technique [13,14]

1. Physicochemical Properties of Drug

2. Drug Product Formulation Factors

3. Processing Factors

4. Factors Relating Dissolution Apparatus

5. Factors Relating Dissolution Test Parameters

Drug Solubility

Solubility of drug plays a prime role in controlling its dissolution from dosage form. The compounds with high solubility generally exhibit higher dissolution rates. The solubility of ionizable drugs, such as weak acid and bases, depends upon both the pH of the medium and the pKa of the compound. Aqueous solubility of drug is a major factor that determines its dissolution rate. Minimum aqueous solubility of 1% is required to avoid potential solubility limited absorption problems. Therefore, it is important to ascertain the aqueous solubility of the drug substance over the physiologically relevant pH range of 1-7.5 in order to predict the effect of solubility on dissolution.

Salt Formation

It is one of the common approaches used to increase drug solubility and dissolution rate. It has always been assumed that sodium salts dissolve faster than their corresponding insoluble acids. Ex. Sodium and Potassium salts of Penicillin G, sulfa drugs, phenytoin, barbiturates etc. The solubility enhancement of a drug substance by salt formation is related to several factors including the thermodynamically flavored aqueous salvation of cations or anions used to create the salt of the active moiety, the differing energies of the salt crystal lattice, and the ability of the salt to alter the resultant.

Particle Size

There is direct relationship between surface area of drug and its dissolution rate. Since, surface area increases with decrease in particle size, higher dissolution rate may be achieved through reduction of particle size. It is increase in the “effective” surface area, or area exposed to dissolution medium and not the absolute surface area, that is directly proportional to dissolution.

Solid State Characteristics

Solid phase characteristics of drug, such as amorphicity, crystallinity, state of hydration and polymorphic structures have significant influence on dissolution rate. Anhydrous forms dissolve faster than hydrated form because they are thermodynamically more active than hydrates.

Co-Precipitation

Dissolution rate of sulfathiazole could be significantly increased by co-precipitating the drug with povidone.

Drug Product Formulation Factors

Dissolution rate of pure drug can be altered significantly when mix with various adjuncts during manufacturing process such as.

Diluents

Diluent in capsule and tablets influence the dissolution rate of drug. Different type of dissolution apparatus utilized affect ranking of different varieties. The dissolution rate is not only affected by nature of the diluent but also affected by excipient dilution (drug/excipient ratio).

Disintegrants

Disintegrating agent added before and after the granulation affects the dissolution rate. Disintegration and dissolution rate of disintegrant with moderate swelling capacity depend to a large extent on mixing time of drug/excipient blend, with lubricant.

Binders and Granulating Agents

The hydrophilic binders increase dissolution rate of poorly wettable drug. Large amount of binder increase hardness and decrease disintegration / dissolution rate of tablet. Non-aqueous binders such as ethyl cellulose also retard the drug dissolution.

Lubricants

Lubricants are hydrophobic in nature (metallic stearates) and prolong tablet disintegration time by forming water repellent coat around individual granules. This retarding effect is the most important factor in influencing rate of dissolution of solid dosage forms.

Surfactants

They enhance the dissolution rate of poorly soluble drug. This is due to lowering of interfacial tension, increasing effective surface area, which in turn results in faster dissolution rate. Surfactant improves the solubility of the drug substance by promoting drug wetting, by forming micelles and by decreasing the surface tension of hydrophobic drug particles with the dissolution medium.

Water-Soluble Dyes

The inhibiting effect was related to preferential adsorption of dye molecules on primary dissolution sources of crystal surfaces. They inhibit the micellar solubilization effect of bile salts on drug. Cationic dyes are more reactive in lower concentration than are anionic dyes.

Processing Factors

Method of Granulation

Granulation process in general enhances dissolution rate of poorly soluble drug. Wet granulation is traditionally considered superior. But exception is the dissolution profile of sodium salicylate tablets prepared by both wet granulation and direct compression where the dissolution was found more complete and rapid in latter case. A newer technology called as APOC "Agglomerative Phase of Comminution" was found to produce mechanically stronger tablets with higher dissolution rates than those made by wet granulation.

Compression Force

The compression process influence density, porosity, hardness, disintegration time & dissolution of tablet. First condition, higher compression force increase the density and hardness of tablet, decrease porosity and hence penetrability of solvent into the tablet retard the wet ability by forming a fermer and more effective sealing layer by the lubricant and in many cases tighter bonding between the particle so decrease dissolution rate of tablet.

Drug Excipient Interaction

These interactions occur during any unit operation such as mixing, milling, blending, drying, and/or granulating result change in dissolution. Similar as increase in mixing time of formulation containing 97 to 99% microcrystalline cellulose or another slightly swelling disintegrant result in enhance dissolution rate.

Storage Conditions

For tablets granulated with PVP there was no change at elevated temperature but slight decrease at room temperature. Tablets with starch gave no change in dissolution rate either at room temperature or at elevated temperature.

Factors Relating Dissolution Apparatus

Agitation

Relationship between intensity of agitation and rate of dissolution varies considerably according to type of agitation used, the degree of laminar and turbulent flow in system, the shape and design of stirrer and physicochemical properties of solid. Speed of agitation generates a flow that continuously changes the liquid/solid interface between solvent and drug. In order to prevent turbulence and sustain a reproducible laminar flow, which is essential for obtaining reliable results, agitation should be maintained at a relatively low rate. Thus, in general relatively low agitation should be applied.

I. BASKET METHOD- 100 rpm II**.** PADDLE METHOD- 50-75 rpm

Stirring Element Alignment

The USP/ NF XV states that the axis of the stirring element must not deviate more than 0.2 mm from the axis of the dissolution vessel which defines cantering of stirring shaft to within ±2 mm.

Sampling Probe Position & Filter

Sampling probe can affect the hydrodynamic of the system & so that change in dissolution rate. For position of sampling, USP/ NF states that sample should be removed at approximately half the distance from the basket or paddle to the dissolution medium and not closer than 1 cm to the side of the flask.

Factors Relating Dissolution Test Parameters

Temperature

Drug solubility is temperature dependent, therefore careful temperature control during dissolution process is extremely important. Generally, a temp of 37º ± 0.5 is maintained during dissolution determination of oral dosage forms and suppositories.

Dissolution Medium

It is very imp factor affecting dissolution and is itself affected by number of factors such as:

A. Effect of pH

Weak acids, dissolution rate increases with increase in pH whereas for weak bases, increase with decrease in pH.

B. Volume of dissolution medium and sink conditions

Volume generally 500, 900 or 1,000 ml. Simulated gastric fluid(SGF) — pH 1.2 Simulated intestinal fluid (SIF) pH 6.8 (not exceed pH 8.0). If drug is poorly soluble, a relatively large amount of fluid should be used if complete dissolution is to be expected.

C. Deaeration of dissolution medium

Dissolved air in distilled water could significantly lower its pH and consequently affect the dissolution rate of drugs that are sensitive to pH changes, weak acids. Another effect is to be released from the medium in form of tiny air bubbles. These bubbles collect at the surface of the dosage forms, thereby acting as a hydrophobic barrier between solvent and solid surface. This inhibits wetting and reduction of surface area and lower dissolution rate.

Compendium Methods Of Dissolution [15,16]

The USP-NF provides several official methods for carrying out dissolution tests of tablets, capsule and other special products.

Table 1: Dissolution Apparatus

Apparatus	Name	Drug Product
Apparatus 1	Rotating Basket	Tablets
Apparatus 2	Paddle	Tablets, capsules, modified drug products, suspensions
Apparatus 3	Reciprocating cylinder	Extended-release drug products
Apparatus 4	Flow cell	Drug product containing low-water soluble drugs
Apparatus 5	Paddle over disk	Transdermal drug products
Apparatus 6	Cylinder	Transdermal drug products
Apparatus 7	Reciprocating disk	Extended-release drug products
Rotating bottle	(Non-USP-NF)	Extended-release drug products (beads)
Diffusion Cell (Franz)	(Non-USP-NF)	Ointments, creams, transdermal drug products

USP Apparatus 1(Basket Apparatus)

The basket method was first described in 1968 by Pernarowski and his co-workers [17]. The most commonly used methods for evaluating dissolution first appeared in the 13th edition of the U.S. Pharmacopeia in early 1970. These methods are known as the USP basket (method I) and paddle (method II) methods and are referred to as "closed-system" methods because a fixed volume of dissolution medium is used [18].

In practice a rotating basket method provides a steady stirring motion in a large vessel with 500 to 1000 mL of fluid that is immersed in a temperature-controlled water bath. Basket method is very simple, robust, and easily standardized. The USP basket method is the method of choice for dissolution testing of immediate- release or also lidos age forms [19]. This apparatus is useful for tablets, capsules, beads and floaters. Solids (mostly floating), mono disperse (tablets) and poly disperse (encapsulated beads) drug products are commonly tested using USP Apparatus1

USP Apparatus 2 (Paddle Apparatus)

An apparatus described by Levy and Hayes [20], may be considered the forerunner of the beaker method. It consisted of a 400 ml beaker and a three-blade, centrally placed polyethylene stirrer (5 cm diameter) rotated at 59 rpm in 250 ml of dissolution fluid (0.1NHCl). The tablet was placed down the side of the beaker and samples were removed periodically. In the Apparatus 2, (the paddle apparatus method) a paddle replaces the basket as the source of agitation. As with the basket apparatus, the shaft should position no more than 2 mm at any point from the vertical axis of the vessel and rotate without significant wobble [21]. The apparatus is useful for tablets, capsules and suspensions. Like USP Apparatus 2 solids (mostly floating), monodisperse (tablets) and poly disperse (encapsulated beads) drug products are commonly tested using USP Apparatus 2. But floating dosage forms require sinker which could be considered as a disadvantage of the apparatus. More over cone formation and positioning of tablet during the test is sometimes hard to maintain [22].

Chromatography [23]

Chromatography is a group of techniques for the separation of the compounds of mixture by their continuous distribution between two phases, one of which is moving past the other. This is feature they distinguish chromatography from most other physical and chemical methods of separation in that is two mutually immiscible phases brought into contact, one phase is stationary and other phase is mobile phase. The mobile phase can be gas or liquid, whereas the stationary phase can be liquid or a solid. When the separation involves a simple partitioning between two immiscible liquid phases, the process is called liquid-liquid chromatography. When physical surface is mainly involved in retentive of the stationary phase, the process is denoted as liquid solid chromatography.

High Performance Liquid Chromatography [24]

The high-performance liquid chromatography is the modern of column chromatography. This type of chromatography works on the principal of partitioning, a drug or a solute get partitioned in the mobile phase depending upon partition coefficient, and finally it travelled to the detector where it is detected, and we get the peaks of it in the chromatogram not its turn of mathematics the areas under the curve gets integrated, and we get the peak area similarly the peak height, retention time, peak start time, peak end time can also be

recorded automatically to get the complete description of the peak. The elution in HPLC depends upon number of theoretical plates and height equivalent to theoretical plates.

Advantages of HPLC are high resolving power, speed of separation, continuous monitoring of column effluent, accurate quantitative measurement, repetitive and reproducible results can be obtained and automation of analytical procedure and data handling. Amenable to diverse sample it offers advantages over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermolabile as well as non-volatile substances.

Mode of separation in HPLC

1) Reverse phase and normal phase- analysis of small (<2000Da) organic molecule

2) Ion chromatography-analysis of ions

3) Size exclusion chromatography- for separation of polymers

4) Chiral HPLC- Determination of enantiomeric impurities

Reverse Phase Chromatography

Method of Development in HPLC [26,27]

In the reverse phase partition HPLC the relative polarity of the stationary and mobile phase opposite to those in normal HPLC. It involves the use of a non-polar stationary phase and polar mobile phase. As a results decrease in the polarity of the mobile phase results in a decrease in solute retention. The most common stationary phase in RP-HPLC are those in which functional group in chemically attached to a silica support. The most popular bonded phase is the alkyl group such as –CH3, -C4H9, C8H17 and C18H37, phenyl group (C6H5), cyano-group {(CH2)3CN} and amino group {(CH2)3NH2}.

Components of HPLC [25]

The essential features of a modern liquid chromatography are mobile reservoirs and solvent treatment system, pumping system, column, sample injector system, detector and data collection device. Method development and optimization in liquid chromatography is an attractive field of research and researchers and attracts also a lot of interest from practical analysis. “Best column, the best mobile phase, the best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assure faster delivery of desired results and a validated method of separation.”

Role of HPLC in Dissolution

The continual evaluation of objectives during the drug product life cycle may require different detection method in order to have effective and efficient dissolution tests. In general, analytical method used for quantifying drug release in dissolution test can be classified into four categories: spectrophotometry, chromatographic, mass spectrometry and Potentiometry. Direct methods are simple, fast and direct can provide data in real time. With these features, they are well suited for online monitoring of the dissolution process. The HPLC separation process involves the partition of analyte between a stationary phase (packed in a column) and a mobile phase. The difference in mobility results in the separation of different components as they travel through the stationary phase, which is identified by retention time on the chromatogram. A large value for theoretical plates (*N*) indicates an efficient column, which allow sample molecule to migrate in a narrowband (small W and W1/2) with minimal dispersion. Therefor the theoretical plate(*N*) also indicate a sharpness of the peak. The chromatographic peak area or peak height is used to quantitative analysis. The UV detector is the most commonly used in HPLC methods for dissolution testing. However, other detector choices allow pharmaceutical sciences to use HPLC for dissolution testing for various drug products that otherwise could not be done with UV detection. An electrochemical detector coupled with LC is the fastest growing technique for biomedical analysis and is also used in a dissolution testing of pharmaceutical dosage forms.

Strategy for method development in HPLC [28]

Selection of suitable chromatography method first reverse phase should be tried, and it is not successful than normal phase should be taken into consideration. Before making experimentation with ion exchange or ion pair chromatography, ion suppression for pH controls and Reverse-Phase chromatography should be tried. For ion forming organic compound’s ion pair chromatography should be preferred to ion-exchange chromatography.

Reverse phase HPLC

Reverse phase chromatography is usually a method of first choice because of convenience, wide applicability and good understanding of operating principles. In the reverse phase partition HPLC i.e. the stationary phase is less polar than the mobile phase and consequently the solute are eluted in order of their decreasing polarities.

Eluent choice in reverse phase chromatography

In Reverse Phase Chromatography methanol is a preferred organic solvent because of low viscosity and high UV transparency (if pure); disadvantage being poisonous, expensive. Aqueous eluent preferred are water: for neutral compound, 10 mM H3PO4, PH5: for weak to medium acids (ion suppression), 10 mM phosphate buffer, pH 5: for weak to medium acids (partly ion suppression), 5 mM phosphate buffer, pH 7.2: for weak to medium bases or acid in ionization form, unknown sample can be analyzed first with water, then with an acid and a natural buffer: acid and basic compounds can be recognized by change of retention time.

Eluent choice

According to eluotropic sequence (the UV transparency must be taken into consideration) in Normal Phase Chromatography N- hexane/ dioxane can be used nearly universally. E.g. Amide, Sulfonamide, nitro compounds, hetero cycles, carbonates, urea and alcohols can be eluted in hexane system. Several aspects of the separation, as summarized in table: 2

Peak shape is often a problem, especially for basic compounds analyzed by reversed phase HPLC. To minimize any potential problems always use a high purity silica phase such as Wakosil II. This modern phase is very highly deactivated, so secondary interactions with the support are minimal. Buffers can be used effectively to give sharp peaks. If peak shape remains a problem, use as organic modifier such as triethylamine.

Table 2: experimental conditions for the initial HPLC separation

	Separation Variable	Preferred initial Choice
Column	Dimensions(Length × ID)	15 × 0.46 cm
	Particle size	5 µm
	Stationary Phase	C18 or C8
Mobile Phase	Solvent A and B	Buffer-methanol
	Buffer (compound, pH, concentration)	10-25Mm Phosphate Buffer 2.0<pH<3.0
	Additives (e.g., amine, modifiers, ion-pair reagents)	Do not use initially
	Flowrate	1-2.0 mL/min
	Temperature	25 -45ºC
Sample size	Volume	10 µL- 20µL

Where, w0.05= width of peak at 5% height and d= half of peak width at 5% peak height, Ideally the T value should be ≤ 2. The basic System Suitability Parameters and Recommendations are shown in Table.

When separating acids and bases a buffered mobile phase is recommended to maintain consistent retention and selectivity. For basics or cationic samples, "less acidic" reverse phase columns are recommended and amine additives for the mobile phase may be beneficial. Optimum buffering capacity occurs at a pH equal to the pKa of the buffer. Beyond that, buffering capacity will be inadequate. The buffer salts reduce peak telling for basic compounds by effectively masking silanols. They also reduce potential ion exchange interactions with un-protonated silanols. To be most effective, a buffer concentration range of 10 – 50 Mm is recommended for most basic compounds.

Method Validation [29,30]

The validation is defined as "a documented program, which provides a high degree of assurance that a specific process will consistently produce, a product meeting its pre-determine specifications and quality attributes." Validation of analytical method is not only an integral part of quality control system but CGMP does require assay validation. In the absence of validated measurement system, there is no way of judging whether or not process has does what it purported to do.

The major advantages of validation are.

1. Reduction of quality costs

2. Process optimization

3. Assurance of quality

4. Safety

A method should be validated when it is necessary to verify that, the performance parameters are adequate for use for particular analytical problem, for examples

• When the established method needs improvement.

• Development of new method for particular problem.

• Established method used in a different laboratory or with different analysis or different instrumentation

The table lists those validation characteristics regarded as a most important for the validation of different type of analytical procedures

Table 3: Validation Characteristics

Type of analytical procedures characteristics	Identification	Testing of impurities		Assay-Dissolution -Content/Potency	
		Quantitation	Limit test		
Accuracy	-	+		-	+
Precision	-	+		-	+
Repeatability	-	+		-	+
Intermediate Precision	-	+(1)		-	+(1)
Specificity(2)	+	+		+	+
Detection limit	-	-(3)		+	-
Quantitation limit	-	+		-	-
Linearity	-	+		-	+
Range	-	+		-	+

There are four common type of analytical methods, each with its own set of validation requirements. The level of stringency is proportional to the criticality of the method of testing drug product. The four most common types of analytical procedure are:

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

Analytical method validation includes all procedure and checks required to prove the reliability of a method for quantitative determination of concentration of an analyte or series of analyte in a given sample. For analytical method validation, US-FDA has given some guidelines in USP and are referred as "Eight steps of analytical method validation". The term also referred as "analytical performance parameter".

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 which is the difference between the mean of a set of measured value and a true mean value. Accuracy is usually presented as a percent of nominal, although absolute bias is also acceptable. Accuracy claims should be made with acceptable precision. ICH guidelines suggest testing three replicates at a minimum of three concentrations. If the same data from the linearity experiment are used, then there would be five levels. The average percentage recovery should be 95-105%.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtain from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic sample. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. In standard deviation or coefficient of variation of a series of measurements. In case of HPLC, the precision result of all samples should not be more than 2% RSD.

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed as intra-assay precision.

Intermediate precision

Intermediate precision express within-laboratories variations: different day, different analyst and different equipment, etc.

Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the samples under a variety of conditions such as different laboratories, different analyst, different instruments, different lots of reagents, different elapsed assay times, different assay temperature, different days, etc. Ruggedness of the methods should be evaluated by running the method with multiple analyst on multiple system.

Detection Limits

The detection limit as an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value.

$$\text{Detection Limit (DL)} = 3.3\sigma S$$

Quantitation limits

The Quantitation limit of an individual analytical procedure is the lowest amount of analyst in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantitation limit is a parameter of quantitative assays for low level of compounds is sample matrices and is used particularly for the determination of impurities and/or degradation products.

$$\text{Quantitation limit (QL)} = 10\sigma S$$

Where, = the standard deviation of response S = the slope of calibration curve

The ICH guidelines suggest three different methods for determining the detection and quantification limits. These are; visual determination, signal-to-noise determination, standard deviation and slope method. Each method will give different results. The signal to noise method is the most logical, because it is based on comparing low level of the analyte to a blank or background sample. A signal-to-noise ratio between 3:1 and 2:1 is generally considered acceptable for estimating the detection limit.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration (amount) of analyte in the sample. It should be established across the range of analytical procedure. Linearity is generally represented as the correlation coefficient, the slope of regression line, etc.

Range

The range of the analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Robustness testing should evaluate the effect of varying media pH, media volume or flow rate, rotation speed; apparatus sample position, sinkers (if applicable), media deaeration, temperature and filters.

In case of liquid chromatography, examples of typical variations are:

• Influence of variations of pH in a mobile phase

• Influence of variations in mobile phase composition

• Different columns (different lots and/or supplier)

• Temperature

• Flow rate

REFERENCES

1. Sethi PD. HPLC in: Quantitative Analysis of Pharmaceutical Formulations, CBS Publication. 1st ed., New Delhi, 2001: 433.
2. Brahamankar D.M. and Jaiswal Sunil B., Biopharmaceutics and Pharmacokinetics, a treatise, Vallabh Prakashan, 2ndedn. Delhi. 2009; 331-345.
3. Subrahmanyam C. V. S, A Textbook of Physical Pharmaceutics, Vallabh Prakashan, 2ndedn. Delhi, 2000.
4. Wurster D. E and Taylor P. W, Dissolution rates, J, Pharm. Sci., 1986; 54: 169-175.
5. Remington, The Science & Practice of Pharmacy., Published by Pharmaceutical Press, 1: 654.
6. Higuchi W.I., Diffusional models useful in biopharm drug release rate process, J. Pharm. Sci., 56: 315-324.
7. Bai, G., Wang, Y., Armenante, P. M., "Velocity profiles and shear strain rate variability in the USP Dissolution Testing Apparatus 2 at Different Impeller Agitation Speeds" International Journal of Pharmaceutics, 2011; 403 (1,2): 1-14.
8. Pillay V and Fassihi R., Evaluation and Comparison of Dissolution data derived from different modified release dosage forms: an alternative method., J. Controlled 1998; 55(1): 45-55.
9. Invitro-in vivo correlations: From theory to applications, Journal of Pharmaceutical Science 2006; 9(2): 169-189.
10. Demrturk E, Oner L., Invitro-in vivo correlations, Fabad J. Pharm. Sci 2003; 28: 215-224.
11. Cardot J.M., Beyssac M., and Alric M., Invitro-in vivo correlation: importance of dissolution in IVIVC, Dissolution Technologies 2007: 1-5.
12. FIP guidelines for dissolution testing of solid oral products, Pharm Ind 1981; 43: 334-343.
13. Guidance for industry, Dissolution Testing for Immediate Release Solid Oral Dosage Forms, U.S. Department Of Health And Human Services, FDA And Center For Drug Evaluation And Research 1997; 4-14.
14. Importance, Objectives and Factor Affecting Dissolution Rate, Theories of Dissolution and Official Dissolution Tests (Equipments of Dissolution Study), Remington; (20): 654.
15. Prior A., Frutos P, Correa C.P., Current guidelines on Comparision of Dissolution Profiles, Docencia; 507-509.
16. Dressman J. and Kramer J., Pharmaceutical Dissolution Testing, published by Taylor and Francis group 6000 Broken Sound Parkway NW, Suite 300., 2005: 16-18.
17. Pernarowski M, Woo W, Searl RO. Continuous flow apparatus for the determination of the dissolution characteristics of tablets and capsules. J PharmaSci.1968; 57: 1419-1421. .
18. Sinko PJ. Martin's physical Pharmacy and Pharmaceutical Sciences. 6th edition. USA, Lippincott, Williams & Wilkins, 2010; pp351-353.
19. Vaghela B, Kayastha R, Bhatt N, Pathak N, Rathod D. Development and validation of dissolution procedures. Journal of Applied Pharmaceutical Science. 2011; 01(03): 50-56.
20. Levy G, Hayes BA. Physicochemical basis of the buffered acetylsalicylic acid controversy. New Eng lJMed.1960; 262: 1053–1058.
21. Dyas AM, Shah UU. Dissolution and dissolution testing. In: Encyclopedia of Pharmaceutical Technology. Swarbrick J (ed). USA Healthcare USA Inc., 2007, 908-928.
22. Abdou HM. Dissolution, Bioavailability and Bioequivalence. Easton, PA, Mack Publishing, 1989; 11–36.
23. Backett AH and Stenlake JB. Practical Pharmaceutical Chemistry, part-II. 4th ed., CBS Publishers and Distributers, New Delhi, 1997; 85-100.
24. Kasture AH Wadodkar SG, Mahadik KR and More HN. A Textbook of Pharmaceutical Analysis, Vol. II 17th ed., Nirali Prakashan, Pune, 2007; 40-48.
25. Snyder LR and Stadalius MA. High Performance Liquid Chromatography: Advanced and Perspectives, 1st ed., Vol. IV, Academic Press, a Diego, 1986; 294-295.
26. Snyder LR, Kirkland JJ and Glajch JL. Practical HPLC Method Development, 2-nd ed., Wiley-Inter science, New York, 1997; 2.

27. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Controller of Publication, New Delhi 2007; 1380.

28. Hobart HW, Lynee LM, John AD and Frank AS. Instrumental Method of Analysis. 7th ed., CBS Publisher and Distributer Pvt. Ltd. New Delhi, 1997; 580-608.

29. www.Chwm.Aligent.com/Product&Services/RRLC1200Series literature.

30. Analytical Methods: A Statistical Perspective on the ICH Q2A and Q2B Guidelines for Validation of Analytical methods, Bio-pharmaceutics International, 2006; 1-6.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.