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Chapter

Drug Analysis

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Abstract

Instrumental methods are widely used for the analysis and stability studies of compounds in bulk and pharmaceutical forms. They vary in their sensitivity, techniques and reagents involved. This chapter will overview those different techniques and the application of the analytical methods. It will also describe how to design and develop simple, sensitive and accurate method for routine quality control of specified compound depending on its molecular structure. Quality control and assurance of the analytical process will be discussed. Furthermore, the chapter will describe a number of factors affecting the chemical and physical stability of Pharmaceutical formulations and how to develop stability-indicating methods to qualify and quantify the drug degradation.

Keywords: instrumental, development, quality control, validation, stability

1. Introduction

Drug discovery and development process can be divided into two major stages: drug discovery which involves isolation of the active constituent, purified, and standardized. The second major stage, drug development, starts with a solitary compound, which at that point progresses through different studies intended to support its endorsement as a new drug [1]. The new drug will then be formulated as an appropriate pharmaceutical dosage form.

Pharmaceutical product is medicine intended for human or veterinary use in cure, alleviation, prevention or diagnosis of disease. The use of ineffective, harmful or poor-quality drugs will cause health hazards and waste of funds. The problem is aggravated by adverse climatic conditions and weak drug supply system (including storage and transport). These lead to deterioration of drug quality, loss of activity and may be formation of harmful degradation products [2]. All this made it a must that any pharmaceutical product should be subjected to different analytical procedures in order to ensure its efficacy and safety. Therefore, an effective drug quality assurance and assessment system should be developed and maintained.

2. Pharmaceutical analysis

Broadly speaking, this is the application of a process in order to identify a drug (single or combined) in its bulk or pharmaceutical dosage form. Testing pharmaceutical product involves chemical, physical and sometimes microbiological analysis [3].

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Pharmaceutical analysis can be divided into two types of methods:

a. Qualitative methods: these methods usually are used to ascertain the presence or identity of a component and\or impurities (predicted or expected).

b. Quantitative methods: determine how much of known drugs are present in bulk form or in a formulation.

Since the judgment for quality of a drug depends on the method of analysis used, the validity or control of the method used is required. The methods used in pharmaceutical analysis should be capable of:

a. Correct identification of the drug in bulk form or as a formulated product.

b. Indicating the percentage of the stated content of a drug present in formulation within acceptable stated limits.

c. Indicating the stability of the drug in the formulation and hence the shelf life i.e. indicating the presence of a drug in its intact form and or the presence of any impurities (whether as drug precursors, decomposition products due to chemical or photochemical causes).

d. Application in the dissolution rate studies i.e. at what rate is the drug released from its formulation so that can be absorbed by the body (bioavailability studies).

e. Ensuring that the identity and purity of pure drug (bulk form) meet official standards or monograph.

f. Ensuring that the identity and purity of excipients used in formulation meet specifications set by official standards.

g. Indicating the concentration of the specified impurities in the pure drug substance (limit test application).

3. Drug stability

The stability of a pharmaceutical product is defined as the capability of the product, in a specific container, to retain its efficacy, properties and characteristics throughout its shelf-life [4]. The recommended shelf-life (expiry date) for a commercial pharmaceutical product is 3–5 years. During this time, the concentration of the drug should not be reduced more than 95% of its value when originally prepared [5].

There are five types of stability that concern the pharmacist in the manufacturing of drugs:

a. Chemical (including photochemical): the product retains its chemical integrity and potency.

b. Physical: the conformity of the pharmaceutical product (color, appearance, dissolution, etc.) does not change upon storage or handling.

c. Microbiological: sterilized products should remain sterile (no pyrogenicity).

d. Therapeutic: the therapeutic effect remains unchanged within the specified dosage regimen.

e. Toxicological: no significant increase in a predetermined toxicity effect is noted.

Stability types (therapeutic, microbiological, and toxicological) are basically dependent on the chemical and physical properties of the drug.

Knowledge of the chemical stability of a drug is of great concern for selecting suitable storage condition against the effects of light, temperature, humidity, etc. and for anticipation of drugs interaction with each other or with excipients [6, 7].

A stable drug is of great concern to the pharmacist (in view of marketing, storage and distribution); to the physician and patient (in view of safety and efficacy); and to the regulator and quality control analyst (in view of quality, strength, purity and identity).

3.1 Chemical reactions that cause drug degradation

Many drugs are derivatives of carboxylic acid or contain functional groups based on this moiety, for example esters, amides, lactones, lactams, imides or carbamates [5]. Accordingly, various chemical reactions can result in the degradation of the drug. These reactions include hydrolysis, oxidation, photochemical reactions, polymerization, isomerization, racemization and dehydration [4, 5, 8].

3.1.1 Hydrolysis (or solvolysis)

Hydrolysis forms the most common pathway by which drugs become degraded since many drugs contain hydrolysable functional groups. It can be defined as the process by which drug molecules interact with water to yield breakdown products of different chemical constitution. Hydrolysis occurs more readily in liquid state than in the solid state. It may occur in aqueous suspensions of sparingly soluble drugs. In tablets and other solid dosage forms, there may be sufficient water to allow hydrolysis of the drug [8].

Solvolysis is a term used for the reactions involving the decomposition of the active drugs with their solvent present (not water).

3.1.2 Ester hydrolysis

Hydrolysis of drugs with an ester functional group (e.g. procaine, atropine, etc.) forms one of the most common types of drug instability. It is usually a bimolecular reaction involving acyl-oxygen cleavage. Ester hydrolysis is (H^+) or (OH^-) ion catalyzed and is dependent on the specific compound and the pH of the solution. Atropine hydrolysis is totally pH dependent and this was characterized by the slopes of −1 and +1. In some cases, the hydrolysis of the drug can show a pH-profile with three regions: a hydrogen ion (proton) catalyzed region, (slope = −1), an uncatalyzed region (solvent dependent, slope = 0) and a hydroxyl ion-catalyzed region (slope = +1) (**Figure 1**) [5].

3.1.3 Amide hydrolysis

Amides are generally more stable to hydrolysis than esters. In general the rate of hydroxyl ion-catalyzed reaction of amides is greater than the proton-catalyzed hydrolysis [6].

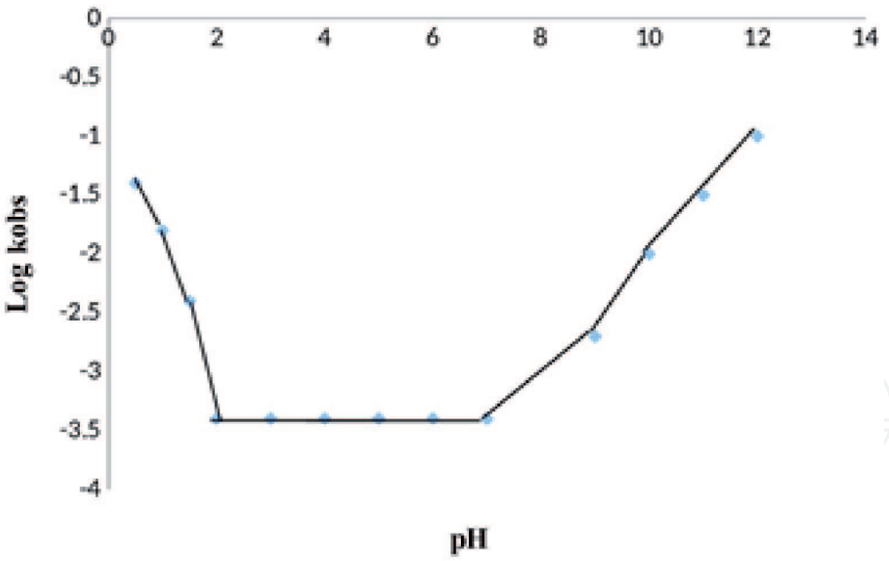

Figure 1.
Log rate-pH profile for the degradation of atropine at 60°C.

Penicillins and cephalosporins are amides in which the amide bond is part of the strained four membered ß-lactam rings. Their decomposition is catalyzed by hydrogen ion, hydroxyl ion and many buffers. Therefore, these compounds are too unstable to be formulated as solutions. Their pH profile is generally similar to the pH-profile shown in **Figure 1**.

In addition to acid-base catalyzed hydrolysis, enzyme-catalyzed hydrolysis may take place in drugs of natural origin; for example enzymes catalyzes the hydrolysis of cardiac glycosides in digitalis leaf [8].

3.1.4 Oxidation

Oxidation involves the removal of an electropositive atom, radical or electron, or the addition of an electronegative atom or radical. When a reaction involves molecular oxygen (O═O), it is commonly called autoxidation and this forms the most common pathway of oxidative decomposition of pharmaceuticals.

Oxidative degradation by autoxidation may involve chain processes consisting of three concurrent reactions—initiation, propagation and termination. Initiation can be via free radicals formed from organic compounds by the action of light, heat or transition metals such as copper and iron which are present in trace amounts in almost every buffer [5].

Many drugs are complex molecules and can be subjected to both hydrolysis and oxidation e.g. steroids, anti-inflammatory, polyene antibiotics (amphotericin B) etc.

Figure 2 show the oxidation of phenothiazines to the sulfoxide which involves two single-electron transfer reactions involving a radical cation intermediate. The sulfoxide is subsequently formed by reaction of the cation with water [5].

Oxidation in solution generally follows first or second order kinetics. Some oxidation reactions are redox reactions that involve the loss of electrons without the addition of oxygen e.g. oxidation of ascorbic acid, ferrous sulfate, adrenaline and riboflavin [8].

In addition to oxidation and hydrolysis, many other degradative reactions had been studied including addition, dehydration, polymerization, isomerization, acylation, transesterification, etc.

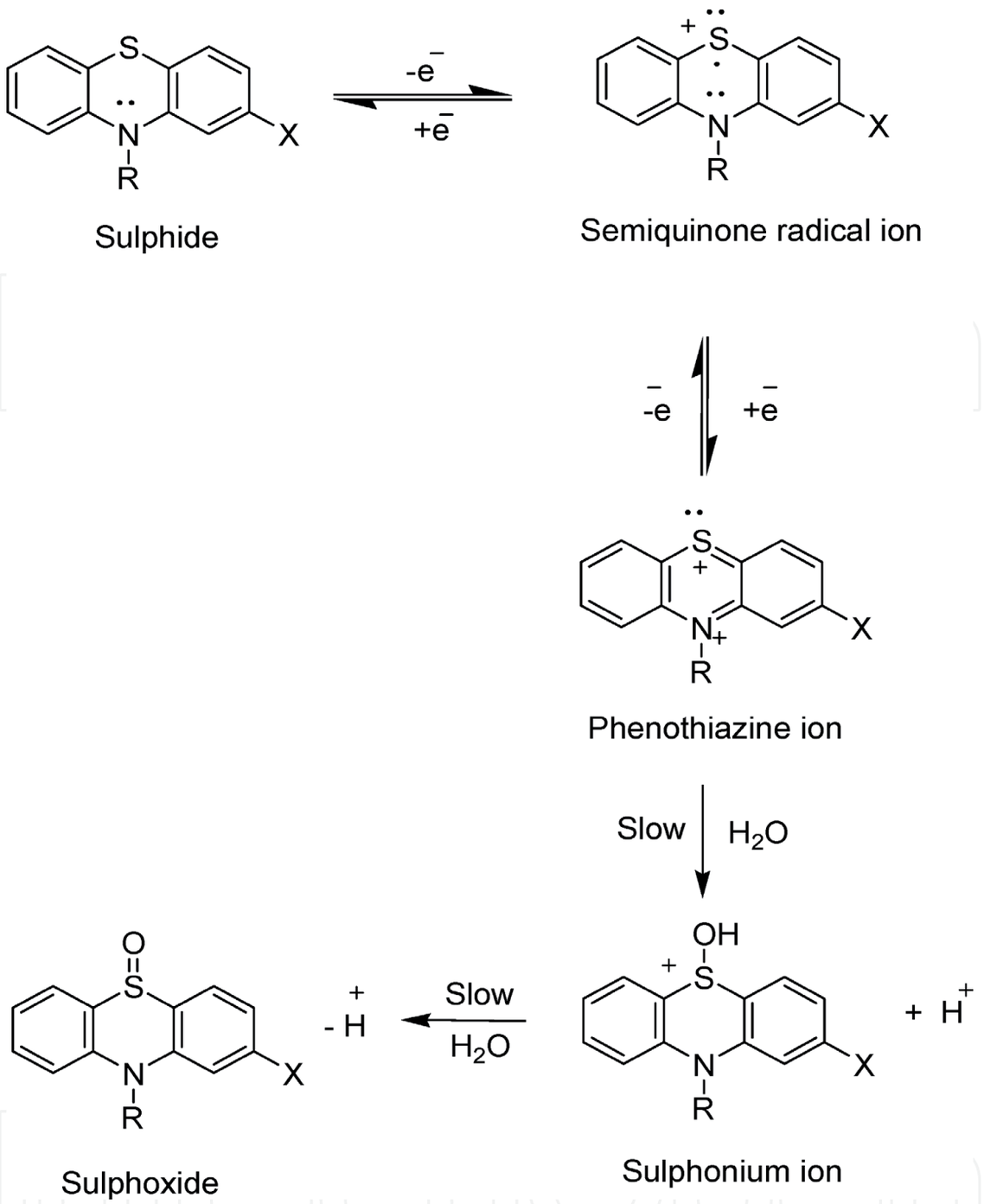

Figure 2.
Phenothiazine oxidation process.

3.1.5 Photochemical degradation

These are the reactions that take place by absorption of the visible or ultraviolet light. The reactant molecule absorbs photons of light (energy) and get excited. The excited molecule then produces the photodecomposition product.

In many photochemical reactions, the reactant molecule may not absorb the radiation directly but through a mediator which absorbs the incident radiation and subsequently transfers its energy to the reactant molecule that becomes activated. Such type of mediator is called photosensitizer.

At times, a molecule can act as a protector for the photolabile drug by preferentially absorbing the radiant energy and produce products. These compounds are referred to as screening agents [9].

3.1.5.1 Light sources for photodegradation studies

The majority of therapeutic substances are white in appearance, which means that they may absorb in the UV region depending on their chemical structure.

Grossweiner, 1989 [10], divided the ultraviolet radiation (UV-R) into three sub-bands:

1. UV-C: which ranges between 200 and 290 nm and is termed shortwave or far UV. Sunlight at the earth's surface is devoid of this band due to its absorption by molecular oxygen and ozone in the upper atmosphere. Artificial radiation sources such as discharge and germicidal lamps and welding arcs form the sources of UV-C which are used for forced drug photodegradation studies (stress-conditions). These also cause serious damage to the skin and cornea [11].

2. UV-B band: this covers the region 280–320 nm. It causes sunburn, skin cancer and other biological effects and it is responsible for the direct photoreaction of many chemicals in natural sunlight.

3. UV-A band: this is the long wavelength region from 320 to 400 nm, also called near-UV because it is near the visible region.

The most commonly sources for photostability studies include: day light, window-glass filtered day light and room light [12]. All these sources can be generated artificially. The artificial light source should have an output with spectral power distribution (SPD) as near as possible to the sunlight. This can be achieved by the use of arc lamps and fluorescent tubes.

3.1.5.2 Drug molecules labile to photodecomposition

A number of medicinal products have been studied for their photostability. Carbonyl, nitroaromatic and *N*-oxide functions, aryl halides, alkenes, polyenes and sulfides are certain chemical functions that are expected to introduce photoreactivity [13].

Photodegradation of a drug is considered of practical significance if the compound absorbs light >300 nm and the photodegradation becomes evident in a short period.

Factors that govern photochemical reaction rate include aerobic (most reactions proceed in presence of oxygen) and anaerobic (N_2) conditions, solvents (H_2O, organic solvents), buffers, temperature, metals, intensity of radiation and spectral distribution of light, drug concentration and volume of the sample [14].

Thus, in formulations that contain low drug concentrations, the primary photochemical reaction follows first-order kinetics; the kinetics is more complicated at higher concentrations and in the solid state because most of the light is then absorbed near the surface of the product.

The mechanisms of photodegradation are of such complexity as to have been fully elucidated in only a few cases. For example, the phenothiazine chlorpromazine (CLP) is rapidly decomposed under the action of ultraviolet light, the decomposition being accompanied by discoloration of the solutions (**Figure 3**). Chlorpromazine behaves differently towards ultraviolet irradiation under anaerobic conditions.

A polymerization process has been proposed which involves the liberation of HCl in its initial stages [5].

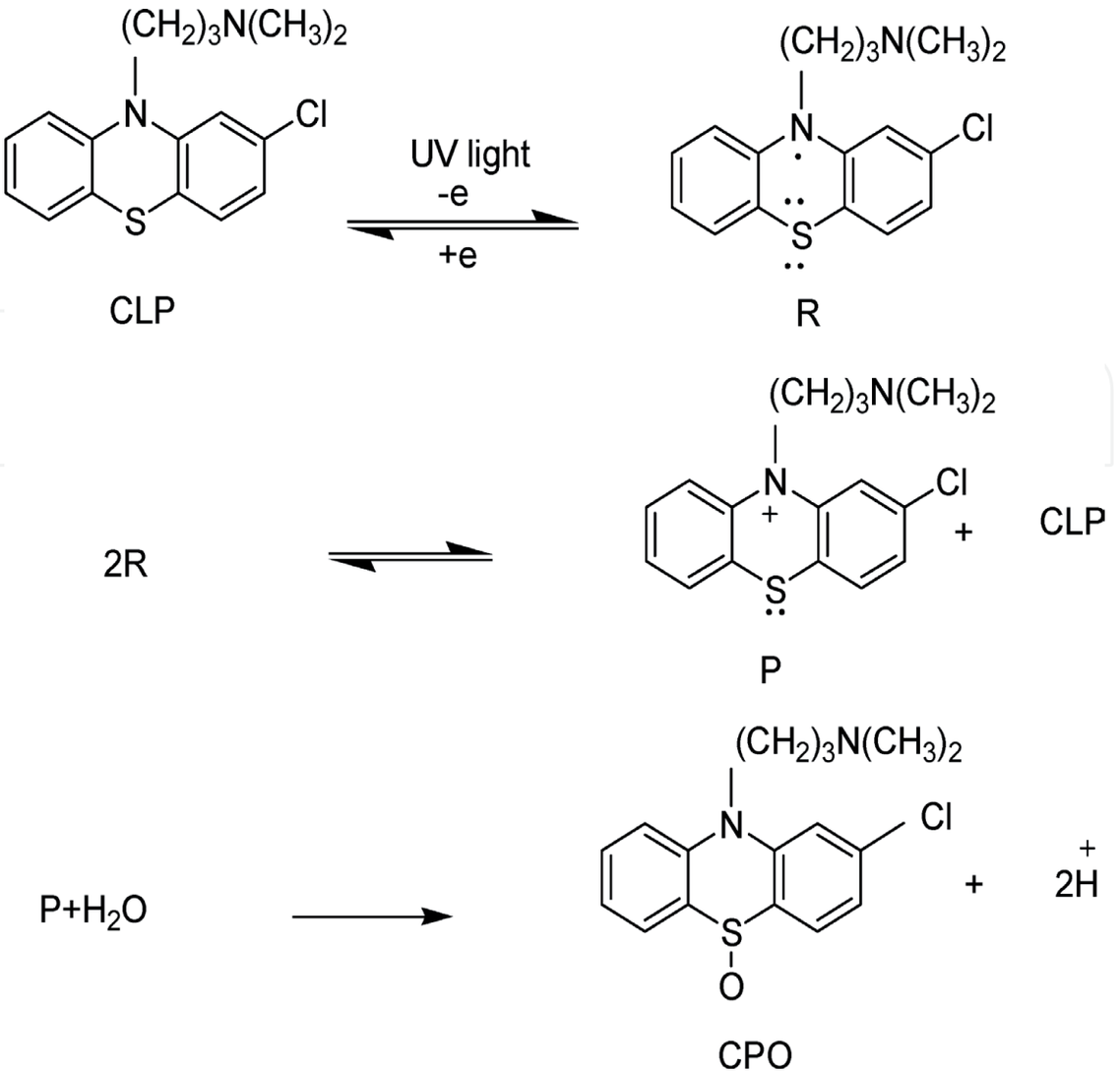

Figure 3.
The effect of ultraviolet light on chlorpromazine (CLP).

The photodegradation of ketoprofen can involve decarboxylation to form an intermediate which then undergoes reduction, or dimerization of the ketoprofen itself.

4. Analytical methods

4.1 Method development

Prior the development of any method for the analysis of certain compound or formulation, there are many factors must be considered before developing the method and applying it to the intended use. The first step include collecting information about the analyte itself (the analyte structure and its physicochemical properties). The mode of detection should be selected (e.g. UV detection). Sample preparation which may include centrifugation, sonication and filtration. The type of the diluent also plays an integral role in the analysis as it should be transparent and does not interfere in the analysis. The stability of the prepared solution, the mobile phase; stationary phase and mode of elution in case of chromatographic elution. All these factors and much more should be considered, optimized and the developed method is then validated and applied for the analysis.

4.2 Spectrophotometric methods

4.2.1 UV/VIS spectrophotometry

Absorption spectrophotometry is the measurement of an interaction between electromagnetic radiation and the molecules, or atoms, of a chemical substance [15]. Techniques frequently employed in pharmaceutical analysis include UV, visible, IR and atomic absorption. Spectrophotometric measurement in the visible region was referred to as colorimetry.

The procedure of UV-unmistakable spectrophotometry includes the estimation of the measure of bright (190–380 nm) or noticeable (380–800 nm) radiation consumed by a substance in arrangement. Retention of light in both the UV and unmistakable area of the electromagnetic range happens when the vitality of the light matches the vitality required to instigate an electronic change and it is related with vibration and rotational progress in the atom. There are two systems of utilizing spectroscopic estimations in medication examination, the total and the similar strategies for measure, and the one utilized relies upon which side of the Atlantic Ocean you complete the investigation. In the UK and Europe the Beer-Lambert condition will in general be utilized in what is known as the outright technique for examine. In this strategy the absorbance is estimated tentatively and the Beer-Lambert condition is comprehended for c, the medication fixation. Hence, the British Pharmacopeia and European Pharmacopeia quote A1% 1 cm qualities in medication monographs. In the US Pharmacopeia, the near strategy for test is liked. In this sort of examine a standard arrangement of the medication to be investigated is readied, the absorbance of the example and the standard are estimated under indistinguishable conditions, and the centralization of the example is determined from the relationship:

$$\frac{\text{Atest}}{[\text{test}]} = \frac{\text{Astd}}{[\text{std}]} \tag{1}$$

Where [test] is the centralization of the example and [std] is the convergence of the readied standard. The relative strategy for test has the bit of leeway that it very well may be utilized regardless of whether the medication experiences a substance response during the measure (for example development of a shaded subsidiary to permit estimation in the obvious district of the range), yet experiences the hindrance that a credible example of the medication being referred to must be accessible for examination. When doing medication examines by spectroscopy it is frequently important to set up a scope of groupings of a standard example of the analyte and measure the absorbance of every arrangement. At the point when these information are plotted, a straight line of positive incline ought to be acquired that goes through the inception. Developing diagrams of this sort not just confirms that the Beer-Lambert law applies to the test at the wavelength of estimation yet additionally enables the chart to be utilized for alignment purposes. An answer of obscure fixation is set up in the very same manner as the benchmarks and its absorbance is estimated at a similar wavelength as the principles. This absorbance is then perused off the alignment chart and the fixation is determined. Standard arrangements arranged independently from the example along these lines are known as outer models. An increasingly thorough system includes the utilization of inside models. An inside standard is an exacerbate that is comparative in compound structure and physical properties to the example being investigated. The inner standard ought to be added to the example being referred to before extraction or measure initiates and is then present in the example framework all through the consequent test. In the measure of complex examples, some example pre-treatment is normally required and the recuperation of the example from the

extraction procedure may not be 100%. On the off chance that an inner standard is utilized, misfortunes in test will be reflected by comparative misfortunes in the standard and the proportion of test to standard ought to stay consistent. Inner measures are especially utilized in chromatographic examination (particularly gas chromatography and elite fluid chromatography), where fluctuations in instrumental parameters (for example flow rate of versatile stage) influence precision. In certain spectroscopic examinations a comparable way to deal with the utilization of inner benchmarks is utilized. This is the strategy of standard augmentations and includes expansion of expanding volumes of a standard arrangement of the analyte to a fixed volume of the example and development of an alignment diagram. The diagram in a standard expansion examine is of positive incline however converges they-pivot at a positive estimation of absorbance. The measure of medication in the example is found by extrapolation of the alignment chart back to the point where the line crosses the x-pivot (for example at the point when y 0 in the condition of the line). The strategy for standard increments is generally utilized in nuclear spectroscopy (for example assurance of Ca^{2+} particles in serum by nuclear emanation spectrophotometry) and, since a few aliquots of test are examined to create the alignment chart, should expand the exactness and accuracy of the measure. The chief advantage of colorimetric and spectrophotometric methods is that they provide a simple means for determining minute quantities of substances [16, 17]. Although spectral interference (degradation products, excipients, etc.) can often occur, the selectivity and sensitivity of these methods can be improved by employing an instrumental technique such as derivative spectrophotometry.

4.2.2 Derivative spectrophotometry

In derivative spectrophotometry the absorbance (A) of a sample is differentiated with respect to wavelength (λ) to generate the first, second or higher order derivative [18].

In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order or ^{0}D spectrum.

$A = f(\lambda)$	$dA/d\lambda = f(\lambda)$	$d^2A/\lambda^2 = f\,\Delta(\lambda)$, etc.
Zero order	first order	second derivative

The first derivative (^{1}D) spectrum is a plot of the rate of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength. The second derivative (^{2}D) spectrum is a plot of the curvature of the ^{0}D spectrum against wavelength.

The first order derivative spectrum of an absorption band is characterized by a maximum, a minimum and a crossover point at λ_{max} of the absorption band. This bipolar function is characteristic of all odd-order derivatives.

The second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ_{max} of the fundamental band.

A derivative spectrum is therefore gives better resolution of overlapping bands than the corresponding fundamental spectrum and may permit the accurate determination of the λ_{max} of the individual bands. Secondly, it discriminates in favor of substances of narrow spectral band width against those with broad bandwidth. And consequently, substances with narrow spectral bandwidth display larger derivative amplitude than those with broad bandwidth [15].

These advantages of enhanced resolution and band width discrimination found in derivative spectrophotometry permit the selective determination of certain

absorbing substances in samples in which non-specific interference may limit the application of simple spectrophotometric methods. Ephedrine hydrochloride in ephedrine hydrochloride elixir is assayed by second derivative spectrophotometry, which eliminates the broad band absorption of the excipient.

Derivative spectrophotometry has found significant application in clinical, forensic and biomedical analysis. It has been widely applied in the analysis of different pharmaceutical dosage forms. It solves the problem of analysis associated with drug combination, stability studies of drug and degradation products, drug impurities and interference of excipient in drugs [19, 20]. It also solves the problem of analysis of drugs in biological fluids.

4.2.3 Difference spectrophotometry

Both selectivity and accuracy of spectrophotometric analysis of samples, which contain absorbing interferons, may be greatly improved by the technique of difference spectrophotometry. In difference spectrophotometry assays the measured value is the difference in absorbance (ΔA) between two equimolar solutions of the analyte, in different chemical forms which exhibit different spectral characteristics. It is sometimes referred to as differential spectrophotometry.

Certain criteria are required for applying difference spectrophotometry for the analysis of a substance in the presence of other absorbing substances:

1. Reproducible changes are induced in the spectrum of the analyte by the addition of one or more reagents.

2. The absorbance of the interfering substances is not altered by the addition of such reagents.

The simplest and most commonly used techniques for altering the spectral properties of the analyte is the adjustment of the pH of the solution by means of aqueous solution of acids, alkali or buffers [21]. The measured value (ΔA) in a quantitative difference spectrophotometric assay can be proportional to the concentration of the analyte and so it obeys Beer's law. A modified equation may be derived.

$$\Delta A = \Delta abc \qquad (2)$$

Where Δa is the difference absorptivity of the substance at the wavelength of measurement.

The accuracy and selectivity of the method was found to be increased by conversion of normal zero-order or differential UV spectra into higher order [21, 22]. Therefore, the application of difference spectrophotometry is expected to have the totality of advantages of both derivative spectrophotometry (first, second, etc.) combined with delta spectrophotometry [23].

On the other hand, the stability-indicating property, coupled with the selectivity and simplicity of application, of the derivative spectrophotometry (first, second, etc.) and ΔD_1 make these methods more preferable to use for drug analysis than the costly HPLC methods, especially in developing countries.

4.2.4 Colorimetric method

Colorimetric methods, although are generally dependent on functional group in the drug molecule (NH_2, OH, SH), are sometimes utilized as stability-indicating

methods. This can be achieved by selectively transforming a drug, its degradation product or its impurity into a derivative so that the spectrum of the derivative is shifted to the visible region.

There are several parameters, which require careful and critical consideration in colorimetry. Firstly, the color reagent should be selective for the drug molecule itself, discriminating degradation products which might be present. Secondly, the effect of any parameters which can affect the development and stability of the color should be established. Moreover, the time required to establish the chromophore should be carefully monitored and assessed.

4.3 Chromatographic methods (HPLC and TLC)

Chromatography is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases. One of the two phases is the fixed (stationary) phase, which can be solid or a liquid supported on a solid. The other phase is a moving (mobile) phase which can be gas or a liquid that flows continuously around the stationary phase.

According to the nature of the mobile phase, chromatography is subdivided into liquid chromatography (LC) where the mobile phase is a liquid, and gas chromatography (GC) where the mobile phase is a gas [15].

4.3.1 Liquid chromatography

There are four classifications for liquid chromatography:

a. Adsorption chromatography: liquid solid chromatography (LSC).

b. Partition chromatography: liquid-liquid chromatography (LLC).

c. Ion exchange chromatography: an ionic liquid mobile phase and a solid polymeric stationary phase containing replaceable ions.

d. Size-exclusion chromatography.

The adsorption and partition chromatography (most widely used in pharmaceutical analysis) are subdivided to:

a. Column chromatography and thin-layer chromatography (TLC): solid stationary phase and liquid mobile phase.

b. Column, paper and thin-layer chromatography: liquid stationary phase and liquid mobile phase.

High performance liquid chromatography (HPLC) belongs to the category of column chromatography and it covers four classes of chromatography: adsorption (LSC), partition (LLC), ion exchange and size-exclusion.

4.3.2 Thin-layer chromatography (TLC)

Thin-layer chromatography has developed into a very sophisticated technique for identification of compounds and for determination of the presence of trace

impurities. Separation in TLC occurs by either adsorption or partition. For adsorption, the stationary phase consists of a thin layer of sorbent (e.g. silica) which is activated by heating at 105°C to evaporate water and the mobile phase is devoid of water (usually a mixture of organic solvents).

The term retention time used in TLC is referred to as R_f value which is the distance traveled by the compound from the origin (where the compound is spotted on the plate) divided by the distance traveled by the solvent. Although TLC is widely used for qualitative analysis, it does not in general provide quantitative information of high precision and accuracy. Changes in the practice of TLC have resulted in improved performance of separation and quantitative measurement. These developments are referred to as high-performance thin-layer chromatography (HPTLC) [24].

TLC is widely used in pharmaceutical analysis for:

a. Identification of the components of a mixture by comparing their Rf values with those of reference standard.

b. Detection of any impurities (synthetic route, stability during manufacturing process or storage).

c. Separation of a mixture of compounds and recovery by elution technique.

d. Following synthetic reactions for their completion.

e. Forensic application in drug poisoning or addiction.

4.3.3 High performance liquid chromatography (HPLC)

HPLC is the most commonly used technique for the quantification of drugs in formulations. The principal advantages of HPLC compared to column chromatography are improved resolution of the separated substances, faster separation time and the increased accuracy, precision and sensitivity.

HPLC is based on the same separation modes of column chromatography i.e. adsorption and partition. Unmodified silica (silanol group) is the most widely used in adsorption HPLC. Partition HPLC is divided into two categories, normal-phase and reverse-phase, based on the relative polarities of the stationary and mobile phases.

In order to develop HPLC method and to select the appropriate column type, the analyst needs to know:

a. Suitable solvent for the drug.

b. Molecular structure.

c. Nature of analysis: whether for quantification analysis or stability-indicating method.

The following diagram (**Figure 4**) gives a general guide to the selection of a chromatographic method for separation of compounds of molecular weight < 2000; for samples of higher molecular weight the method of choice would be size-exclusion [25, 26].

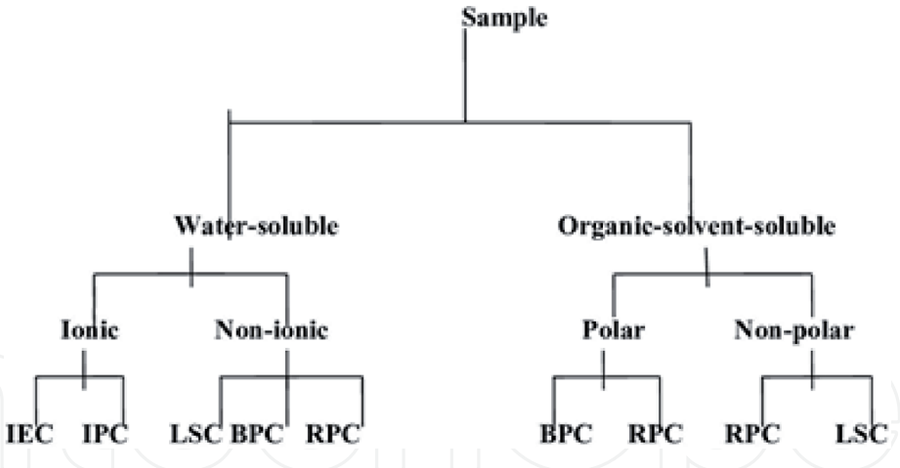

Figure 4.
General guide for selection of chromatographic method.

5. Quality assurance and quality control

In the pharmaceutical Industry, quality management is defined as the aspect of management function that determines and implements the quality policy.

The major elements of quality management are:

a. A quality system describing the organizational structure, procedures, processes and resources.

b. A systematic action or actions necessary to ensure adequate confidence that a product (or service) will satisfy given requirement for quality.

The concepts of quality assurance, GMP and quality control are interrelated aspects of quality management. They are inter-related and have fundamental importance to the production and control of pharmaceutical products.

In fact quality assurance covers quality control in exactly the same manner as it covers other functions such as manufacturing and ware-housing. It approves methods and standards and sees to it that good laboratory practices are operative.

Each manufacturing unit must have a quality control department independent from the production and other departments and under the control of a qualified and experienced personnel and has one or several quality control laboratories at his or her disposal.

Quality control is integral part to all modern industrial processes and the pharmaceutical industry is not an exception. Testing a pharmaceutical product involves physical, chemical and sometimes microbiological analysis. It is a critical function of any business offering a product or service to consumers. In the field of pharmaceutical chemistry, quality control is vital to the successful development, manufacturing, and use of drugs meant to save lives. It determines the quality and stability of drug products via pharmaceutical analysis; it includes areas such as method validation, handling raw materials and finished products, documentations, inspections that impact the development of pharmaceutical products that are governed by specified rules.

Pharmaceutical products are developed and produced according to GMP requirements and other associated codes e.g. good laboratory practices (GLP), and good clinical laboratory practices (GcLP), ... etc.

Production and control operations are clearly specified in a written form i.e. standard operating procedures (SOP's) and GMP requirements are adopted.

Control procedures on starting materials, intermediate products and finished products and other in process controls should be carried out according to written and validated procedures.

The finished products should be correctly processed, checked, packed according to defined procedures.

Finished pharmaceutical products should not be sold or supplied unless they are released by an authorized person.

6. Method validation and statistical interpretation of the analytical method

The function of the analyst is to obtain a result as near to the true value as possible by the correct application of the analytical procedure employed. Quantitative analysis is not simply a case of taking sample, carrying out a single determination and then claim that the value obtained is irrefutable. It also requires knowledge of the chemistry involvements and the possibilities of the interferences from other ions, elements and compounds as well as of the statistical distribution of values [27].

Different errors may occur during the analysis process which should be well noticed and overcome. There are three types of errors:

a. Gross errors: easily recognized as it leads to definite unreliable results: could be due to contaminated reagents, defective instruments, accidental loss of crucial sample etc. It is defined as a serious error so that there is no way to correct the experiment.

b. Random error (in determinate error): the average of the results are very close to the true value, so there is no evidence of bias i.e. some results could be high and some results could be low. Arises from sources that cannot be corrected i.e. the degree of sensitivity of the balance: fourth decimal, fifth decimal. Types of burettes or pipettes (A, B, C types) etc.

c. Systematic (determinant errors): this causes all the results to be in error in the same sense (constant error). May be due to (1) faults in analytical procedure or (2) the equipment used. Observed result could be too low or too high i.e. inaccuracy should be constant (all answers are 10% too high or too low). Example: True value for three samples were 25, 20 and 30% assay result was 35, 30 and 40% respectively i.e. 10% too high. It makes the assay precise but inaccurate sometimes the inaccuracy may be proportional to the true answer, giving rise to proportional error such as 10% of the answer i.e. for the above example for 25% result is 27.5%, 22% for 20% and 33% for30%.

Validation of methods for the quantitative analysis of drugs involves determining as a minimum, their selectivity, and limit of detection, limit of quantification, linearity, working range, accuracy and precision [28].

6.1 Accuracy

Accuracy is a measure of how closely the result of an experiment agrees with the expected result. The difference between the obtained result and the expected

result is usually divided by the expected result and reported as a percent relative error [29].

6.2 Precision

Precision is a measure of how close a set of results are to each other [30]. It is often measured under repeatable (same analyst, same day, same instruments and same materials) and reproducible conditions. Precision always accompanies accuracy, but a high degree of precision does not imply accuracy.

6.3 Linearity

For any developed analytical method, standard curve is constructed to verify the linear relationship between the concentration and a characteristic parameter for a component such as peak area, peak height or peak ratio in chromatographic analysis or UV-absorption in spectrophotometry.

Most analytical methods are based on processes where the method produces a response that is linear and which increases or decreases linearly with analyte concentration. In other words, it is the ability of the method to elicit test results that directly proportional to the concentration of analyte within a given range.

Statistical application is important in evaluating calibration graphs in instrumental analysis. The equation of a straight line takes the form:

$$y = a + bx \tag{3}$$

Where a is the intercept of the straight line with the y axis and b is the slope of the line.

The statistical measure of the goodness of the fit of the line through the data is the correlation coefficient "r". It falls in the range $-1 \leq r \geq +1$. Negative r-values indicate negative slope and vice-versa. It is important to note that calculated r-values can be sometimes misleading and a calibration curve must be physically plotted to ensure the shape of the plot. From the calculated regression line data, the concentration of the analyte can be estimated by interpolation. Each value of y is subjected to random error and likely an error in the slope and intercept values can occur. This can be resolved by calculating standard deviations of the slope (S_b) and intercept (S_a). S_b and S_a are obtained from a calculated statistic value $S_{y/x}$ [29]. The values of S_b and S_a are used to calculate the confidence limits for the slope and intercept using a *t*-value at a desired confidence level, normally 95% level. These limits are important to indicate if there is a significant difference between these values and certain true values, which reflects the effect of random or systemic errors.

6.4 Limit of detection

The limit of detection is the lowest content of analyte that can be distinguished from background noise and measured with reasonable statistical certainty. It can be calculated by the reduced formula:

$$3SB/b \tag{4}$$

Where SB = Sy/x (calculated from the regression analysis data), b is slope [29].

6.5 Limit of quantification

The lower limit of quantification is the amount equal to or greater than the lowest concentration point on the calibration curve that can be measured with an acceptable level of accuracy and precision [29].

It can be calculated by the equation:

$$10SB/b \quad (5)$$

Where SB = Sy/x (calculated from the regression analysis data), b is slope.

6.6 Method comparison

The comparison of two methods should be carried out using a suitable statistical procedure to test if there are significant differences between them. The *t*-test provides a simple check on accuracy and the *F*-test on precision. These tests require the knowledge of what is known as the number of degrees of freedom.

7. Conclusion

Pharmaceutical products must be analyzed regularly to ensure their safety and effectiveness. This chapter described the quality assurance and quality control of materials and finished products. The requirements to develop a suitable method and its validation. Also different analytical methods and their application in the field of pharmaceutical analysis was also discussed.

Conflict of interest

The author declares that there is no conflict of interest.

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