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- A REVIEW

SPECTROPHOTOMETRIC METHODS IN THE ANALYSIS OF DRUGS IN PURE AND DOSAGE FORMS

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ABSTRACT

The assaying and stability testing in pharmaceutical analysis occupies an important role to meet the requirement of statutory certification of drugs and their formulations by the industry. The analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. Most widely used methods are based on HPLC/ UPLC, GC. Capillary electrophoresis and super critical fluid chromatography are slowly gaining ground in recent years. The selectivity and sensitivity of the visible spectrophotometric method depends only on the nature of chemical reactions involved in color development and not on the sophistication of the equipment. The colorimetric procedure must give reproducible results under specific experimental conditions. HPLC method validation studies include system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification, stability of samples. The variable method parameters adapted to carryout robustness in HPLC technique may involves the studies on the flow rate, column temperature, sample temperature, pH and mobile phase composition.

Key words: Drug delivery, Spectrophotometry, HPLC, pH and Linearity.

INTRODUCTION

Formulations containing various drugs and combinations of drugs for potentiating or complementing one another in therapy are available in market. Pharmaceutical equivalents containing identical amounts of the same active ingredient (s) in the same dosage form and targeted to give in the same route of administration are called as generics drugs. For a generic drug to be approved it must be shown to be pharmaceutically equivalent and bioequivalent to the Reference Listed Drug (RLD). They must also meet all relevant standards of strength, quality, purity and identity. In view of the foregoing discussion the

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assaying and stability testing in pharmaceutical analysis[1] occupies an important role to meet the requirement of statutory certification of drugs and their formulations by the industry. The analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. The best way to characterize the quality of a bulk drug is to determine its purity. There are two possible approaches to reach this goal.

In the early years of drug analysis, when chromatographic techniques were not yet available the characterization of the purity of drugs was based on the determination of the active ingredient content by non-specific titrimetric and photometric methods supported by the determination of physical constants and some limit tests for known impurities based mainly on color reactions. The deficiencies of this approach are well known. As a consequence of the enormous development of the analytical technology in the last two decades entirely new possibilities have been created for the determination of the purity of drug materials. Among the several instrumental techniques [HPLC/UPLC GC, CE (Capillary electrophoresis), Fluorimetry, NMR, mass spectroscopy, spectrophotometry covering IR, NIR, Raman, UV and visible regions] available for the assay of drugs, usually visible spectrophotometric technique is simple and less expensive. The selectivity and sensitivity of the visible spectrophotometric method depends only on the nature of chemical reactions involved in color development and not on the sophistication of the equipment. Spectrophotometric analytical procedures are not generally stability indicating. Most widely used methods are based on HPLC/UPLC, GC. Capillary electrophoresis and super critical fluid chromatography are slowly gaining ground in recent years. Rao et al.[2-19] have published their results on different oxide materials, luminescent materials, polymers, glasses and on different drugs in their earlier studies.

UV-Visible absorption spectrophotometry

The principle of UV-Vis spectrophotometry[27] is based on the ability of molecule to absorb ultraviolet and visible light. The absorption of light corresponds to the excitation of outer electrons in the molecule. When a molecule absorbs energy and the outer electrons in the molecule excited from the Highest Occupied Molecular Orbital (HOMO) to Lowest Unoccupied Molecule Orbital (LUMO). The occupied molecular orbitals with lowest energy are known the σ orbitals, at slightly higher energy are called π orbitals and at still higher energy are known non-bonding orbitals (unshared pair electrons). The π^* and σ^* are called the highest energy state. The absorption can be measured at a single wavelength or on spectral extended range. Ultraviolet and visible spectroscopy are enough energetic to excite

outer electrons to high energy level and it is very useful for quantity measurement. The Beer-Lambert law is used to determine the concentration of analyte by measuring the absorbance at various wavelengths. Beer-Lambert Law is the relationship between absorbance and concentration.

Two radiation sources are generally used in UV-Visible spectrometers which together cover the range from 200-800 nm. For measurements below 320 nm a deuterium or a hydrogen lamp at low pressure is used for emitting a continuous spectrum. For measurements above 320 nm compact tungsten halogen sources in quartz envelope are often used. This type of source is used in the wavelength range of 350-2500 nm. Tungsten/halogen lamps are very efficient and their output extends well into the ultraviolet region. If a tungsten halogen lamp is used to emit radiations below 400 nm, special filters are often included in the optical path, to reduce the stray radiation. Wavelength selectors are needed to guarantee monochromatic radiation, since a narrow bandwidth is required in order to enhance the sensitivity of the absorbance measurements. A variety of detectors are available for UV-Visible measurements.

High performance UV-Visible spectrophotometers utilize photomultiplier tube technology from the ultraviolet into the visible region. Very few reactions are specific for a particular substance, but many give colors for a small group of related substances only, that is they are selective. By altering and controlling of pH, close approximation to specificity is obtained. For visual colorimeters it is important that the color intensity should increase linearly with the concentration of the substance to be determined, since a calibration curve may be constructed relating the instrumental reading of the color with the concentration of the solution. It is desirable that the system follows Beer's law even when photo electric colorimeters are used. The color procedure should be sufficiently stable to permit an accurate reading to be taken. This applies also to those reactions in which colors tend to reach a maximum after a time; the period of maximum color must be long enough for precise measurements to be made. In this connection the influence of other substances and of experimental conditions (temperature, pH, stability in air etc.) must be known.

The colorimetric procedure must give reproducible results under specific experimental conditions. The reaction need not necessarily represent stoichiometrically quantitative chemical change. The solution must be free from precipitate if comparison is made with a clear standard. Turbidity scatters as well as absorbs the light. High sensitivity: It is desirable, particularly when minute amount of substances are to be determined, that the color reaction be highly sensitive and that the reaction product absorbs strongly in the visible

rather than the UV region. The interfering effect of other substances in the UV region is usually more pronounced. The solvent to be used in colorimetric or spectrophotometric determination must be a good solvent for the substance under determination. It should not interact with the solute and must show significant absorption at the wavelength to be employed in the determination. Calibration is one of the most important steps in spectrophotomertic analysis. Good precision and accuracy can only be obtained when a good calibration procedure is used.

High performance liquid chromatography

High performance liquid chromatography [HPLC] is one mode of chromatographic techniques widely used to separate a wide variety of chemical mixtures and pharmaceutical compounds in mixtures. HPLC utilizes a stationary phase can be a liquid or a solid phase and a liquid mobile phase to separate the components of a mixture. There are three basic types of molecular forces: ionic forces, polar forces and dispersive forces on which each specific technique capitalizes one of these specific forces. Polar forces are the dominant type of molecular interactions employed in Normal Phase-HPLC. Dispersive forces are employed in Reversed Phase-HPLC. NP-HPLC explores the differences in the strength of the polar interactions of the analytes in the mixture with the stationary phase. The stronger the analyte-stationary phase interaction, the longer the analyte retention. Analyte molecules compete with the mobile phase molecules for the adsorption sites on the surface of the stationary phase. The stronger the mobile phase interactions with the stationary phase, the lower the difference between the stationary phase interactions and the analyte interactions, and thus the lower the analyte retention. Mobile phases in NP-HPLC are based on nonpolar solvents (such as Hexane, Heptane, etc.) with the small addition of polar modifier (i.e., Methanol, Ethanol).

Packing materials traditionally used in NP-HPLC are usually porous oxides such as Silica (SiO_2) or Alumina (Al_2O_3). Surface of these stationary phases is covered with dense population of OH groups, which makes these surfaces highly polar. Chemically modified stationary phases can also be used in NP-HPLC. RP-HPLC employs mainly dispersive forces (hydrophobic or vander wall's interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobic and mobile phase is polar, where mainly water-based solutions are employed. RP-HPLC is by far the most popular mode of Chromatography. Almost 90% of all the analysis of low-molecular-weight samples are carried out using RP-HPLC. Adsorbents employed in this mode of Chromatography are porous rigid materials with hydrophobic

surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous Silica.

HPLC systems are also provided an online degassing system which continuously removes the dissolved gases from the mobile phase. In HPLC system it is achieved by heating, stirring, vacuum degassing with an aspirator, filtration through 0.45 μm filters, vacuum degassing with an air-soluble membrane, Helium purging ultra sonification. Two means for analyte introduction systems are used in the HPLC systems. (1) Flowing stream and (2) a stop flow injection. These two techniques are used with a syringe or an injection valve. The most useful and widely used sampling device for modern LC is the micro sampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow even at elevated temperatures. The heart of the any HPLC system is the column which decides the efficiency of separation. Silica (SiO_2. X H_2O) is the most widely used packing materials inside the column. It consist of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing inter connected pores. Thus, a wide range of commercial products are available in the market with surface areas ranging from 100 to 800 m^2/g and particle sizes from 3 to 50 μm.

Silica is altered by reaction with organo Chloro Silanes or organo Alkoxy Silanes giving Si-O-Si-R linkages with the surface. This attachment of hydrocarbon chain to Silica produces a non-polar surface suitable for reversed phase chromatography where mixtures of water and organic solvents are used as eluents. The most popular material is Octa Decyl Silica (ODS), which contains C_{18} chains, but material with C_2, C_6, C_8 and C_{22} chains are also available. During manufacture various organic compounds, which contain groups such as Phenyl, Nitro, Amino and Hydroxyl are reacted with a small mono functional silane (eg: Trimethyl Chlorosilane) of the column to reduce further number of silanol groups remaining on the surface (End -Capping). In HPLC, generally two types of columns are used, normal phase column and reversed phase column. In normal phase chromatography, analysis is carried out on the passage of a relatively non polar mobile phase over a polar stationary phase, while in reversed phase the analysis is carried out using a polar mobile phase such as Methanol, Acetonitrile, Water, Buffer etc. Method validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristic. HPLC method validation studies include system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification, stability of samples.

Prior to the analysis of samples by HPLC, the operator must establish that the HPLC system and the procedure developed should be capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and Precision. The requirements for system suitability are usually developed after method development. The linearity of a method is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data so obtained is then processed using a linear least-squares regression and the resulting plot slope, intercept and correlation coefficient provides the desired information on linearity. The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose true value is known) is analyzed and the measured value is identical to the true value. Typically, accuracy is represented and determined by recovery studies.

The terms selectivity and specificity are often used interchangeably. According to ICH, the term specific generally refers to a method that produces a response for a single analyte only while the term selective refers to a method which provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate. The analyte should have no interference from other extraneous components and be well resolved from them. To achieve this either a representative Chromatogram or a profile should be generated that should show the extraneous peaks either by addition of known compounds or samples from stress testing are baseline resolved from the parent analyte.

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method. The concept of robustness of an analytical procedure has been defined by the ICH as a measure of its capacity to remain unaffected by small, but

deliberate variations in method parameters. The variable method parameters adapted to carryout robustness in HPLC technique may involves the studies on the flow rate, column temperature, sample temperature, pH and mobile phase composition.

CONCLUSION

The analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. HPLC is one mode of chromatographic techniques widely used to separate a wide variety of chemical mixtures and pharmaceutical compounds in mixtures. The variable method parameters adapted to carryout robustness in HPLC technique may involves the studies on the flow rate, column temperature, sample temperature, pH and mobile phase composition. The concept of robustness of an analytical procedure has been defined by the ICH as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters.

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