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Review Article

A REVIEW ON INSTRUMENTATION AND VALIDATION METHOD OF UV-VISIBLE SPECTROSCOPY AND HPLC FOR THE ANALYSIS OF DRUGS

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ABSTRACT

The purpose of the present analysis was to identify the application of HPLC and UV/Vis spectroscopy. UV-VIS Spectroscopy is the term used to test the various types of solvents and substances in an analysis. In particular, small-scale enterprises typically prefer spectroscopy, as the cost of equipment is lower and maintenance issues are limited. The analysis approach is based on the calculation of the absorption of monochromatic light in the near ultraviolet direction of a spectrum by colourless compounds (200 400nm). The pharmaceutical analysis requires the necessary procedure for the determination of the "identity, intensity, consistency and purity of such compounds. Analysis of raw materials and intermediates during the drug manufacturing process is also included. The dissociation constant is well known to be the most critical parameter in the production and optimization of a new compound for successful development of formulations. Information on the system of simultaneous equation, difference spectrophotometry, high-performance liquid chromatography (HPLC) or fluid visible ultraviolet spectrophotometry was measured. High performance liquid chromatography (HPLC) is an effective analytical method for drug product evaluation. Methods of HPLC should be able to isolate, detect and quantify the different drugs and drug related degradants that can shape, detect and quantify any drugs and drug related impurities that can be added during synthesis. Validation is the process of determining a method's performance features and limitations and defining the factors that which alter these characteristics and to what extent.

KEYWORDS: Spectroscopy, HPLC, Chromatography, Spectrophotometer, Spectrum

INTRODUCTION

In pharmaceutical research, validation of analytical methods is a major regulatory requirement. High-Performance Liquid Chromatography (HPLC) is widely used in the production and validation of assay methods for drug ingredients and drug substances as an analytical technique. Validation of the procedure offers recorded evidence and a high degree of confidence that an empirical method used for a particular test is sufficient for its intended use. In recent years, regulatory authorities have become increasingly aware of the need to ensure that validated analytical methodology is used to collect the data submitted to them in marketing authorization applications. Accuracy, precision (repeatability and intermediate accuracy), specificity, detection limit, quantitation limit, linearity, range, and stability of analytical solutions are the most commonly used validation characteristics.

The parameters that need validation and the methodology that is followed for each specific case depend on the method's type and applications. It is important that the analytical system itself is properly developed, maintained, calibrated, and validated before undertaking the task of method validation. The first step in process validation is to prepare a protocol, ideally written in a simple step-by-step format with the instructions. Creation and validation of an analytical method by integrating the therapeutic effects of two or more drugs into one product. The analytical chemist responsible for the production and validation of analytical methods can present daunting challenges with these combination products. In pharmaceutical process creation for quality and safety, the detection and quantification of impurities is a critical task. [1, 2]

Analytical chemistry

In order to achieve an understanding of their chemical composition and structure, analytical chemistry is the study of material samples. The science of making quantitative measurements is analytical chemistry. In reality, the quantification of analytes in a complex sample becomes a problem-solving exercise. Sample analysis includes chemical knowledge that can occur in a sample analysis, sample handling methods for a broad range of problems (the tools-of-the-trade), proper data analysis and record keeping to be accurate and reliable.

Qualitative

Qualitative analysis deals with the identification of elements, ions or compounds presents in sample.

Quantitative

Quantitative analysis deals with the determination of how much of one or more constituents are present.

ANALYTICAL METHODS FOR PHARMACEUTICAL ANALYSIS

UV/VIS spectrophotometric methods

Reflectance or spectroscopy in the ultraviolet-visible spectral area, spectroscopy means that light in the visible range is used. In the visual spectrum, absorption or reflectance specifically influences the perceived colour involved. The molecules undergo electronic transformations in that area of the electromagnetic spectrum. This technique is complementary to fluorescence spectroscopy, where fluorescence deals with transitions from the excited state to the ground state, while transitions from the ground state to the excited state are determined by absorption steps. [3]

Electroanalytical methods

Electrochemical methods, combined with the use of simple and inexpensive instrumentation, provide high sensitivity and low detection limits. The electrochemical methods used in dosage formulations for drug analysis are electrogravimetry, coulometry, conductometry, potentiometry, polarography, voltammetry and amperometry. Voltammetric methods, however, are by far the most utilized electrochemical techniques and the literature offers extensive reviews of their use in dosage forms of drug analysis.[4]

Capillary electrophoresis

A powerful isolation technique that can be applied to large and small molecules is capillary electrophoresis. Quantitation of pharmaceuticals deserves to be cited among its many applications. This method has a high separation efficiency, allowing for difficult separations, low analysis time, rapid method growth, low consumption of samples and solvents, and automated and easy instrumentation. [5]

Turbulent-Flow chromatography (TFC)

Turbulent-Flow Chromatography is a very effective choice for reducing measurement times (TFC). In order to distinguish small and large molecules, turbulent flow chromatography is a technique that relies on the differential diffusion coefficients of proteins and small molecules near the surface of the stationary phase in a bulk turbulent flow regime. [6] The online nature of this technology makes it very appealing, with minimal manual manipulation for quick throughput. In the field of bio-analysis, TFC is primarily applied as a sample preparation tool, as it enables the target analytes to be isolated from the large molecules that make up the bulk of the biological matrix.

Counter current chromatography in analytical chemistry

Counter current chromatography (CCC) is a type of liquid chromatography (LC) that without any solid support uses two immiscible liquid phases. CCC utilises several terms already established for chromatography as an LC technique. As the two liquid stages do not flow counter current to each other, counter current chromatography is inappropriate. CCC is an LC technique with a stationary step of support-free liquid. [7]

Advantages of a support-free liquid stationary phase

Versatility
Unique possibilities
Solutes can make use of the very high volume of stationary phase

Applications

In the science of separation, CCC plays an increasingly significant role. It is possible to retrieve all the components in the sample solution injected into the column and virtually remove the permanent adsorption and contamination of samples. You may directly inject a blunt sample into the column, which simplifies sample preparation. For the separation of organic and inorganic compounds from a complex mixture, CCC is used effectively. [7]

Traditional analytical techniques

Although sophisticated instrumentation governs modern analytical chemistry, traditional techniques, many of which are still used today, are also the roots of analytical chemistry and some of the principles used in modern instruments. [8]

Titration

Titration involves the addition of a reactant to a solution being analysed until some equivalent point is reached. Often the amount of material in solution being analysed may be determined.

Gravimetric analysis

Gravimetric analysis involves determining the amount of material present in the sample. It is done by weighing the sample before and/or after some transformation.

Inorganic qualitative analysis

Inorganic qualitative analysis generally refers to a systematic scheme to confirm the presence of certain, usually aqueous, ions or elements by performing a series of reactions that eliminate ranges of possibilities and then confirms suspected ions with a confirming test.

Miscellaneous techniques

Following types of miscellaneous techniques are used in the drug quantification. [9, 10]

- Dielectric thermal analysis (DEA): dielectric permittivity and loss factor
- Differential thermal analysis (DTA): temperature difference
- Differential scanning calorimetry (DSC): heat difference
- Dilatometry (DIL): volume
- Dynamic mechanical analysis (DMA): mechanical stiffness and damping
- Evolved gas analysis (EGA): gaseous decomposition products
- Laser flash analysis (LFA): thermal diffusivity and thermal conductivity
- Thermogravimetric analysis (TGA): mass
- Thermomechanical analysis (TMA): dimension

Hyphenated techniques

Combinations of the above techniques are called as "hybrid" or "hyphenated" techniques. Several examples are in popular use today and new hybrid techniques are under development. [11, 12]

By using hyphenated techniques separation and quantification of organic sample can be achieved.

- GC-MS (Gas Chromatography – Mass Spectrometry)
- ICP-MS (Inductively Coupled Plasma-Mass Spectrometry)
- GC-IR (Gas Chromatography-Infrared Spectroscopy)
- MS-MS (Mass Spectrometry-Mass Spectrometry)
- LC-MS (Liquid Chromatography- Mass Spectrometry)

Instrumental methods are sensitive, and it needs a small amount of sample. Complex mixtures can be analyzed with or without their prior separation with sufficient reliability and accuracy of results.

Table 1: Comparison of various analytical methods

Technique	Specificity	Properties
GC-MS	High	High resolution, Good for unknowns
HPLC-UV (And DAD)	Good Moderate better with DAD	Good for soy food and conjugates
Fluorescence	Good	Sensitive
ED (and array)	Better with detection array	Suitable for biological samples
MS	High	Ease of use and sensitive
UV (DAD)	Moderate better with DAD	High separation resolution Excellence mass sensitivity
Fluorescence	Moderate	Sensitive
ED (and array)	Moderate	Sensitive
MS	High	Sensitivity
UV and IR spectroscopy	High	High throughput
MALDI-MS	High	High throughput

Spectrophotometry

The branch of science dealing with the study of the interaction between electro-magnetic radiation and chemical samples is spectrophotometry. It requires the calculation and analysis of the absorption or emission of electromagnetic radiation (EMR) when a sample's molecule or atoms or ions shift from one energy state to another energy state. This change may be from ground to excited state or from excited to ground state. The energy of a molecule is the sum of rotational, vibrational and electronic energy at ground level. In other terms, the variations in rotational, vibrational or electronic energies are determined by Spectroscopy. For the study of atomic and molecular structures, it is the most efficient instrument available and is used in the analysis of a wide range of samples. The area of the electromagnetic spectrum between 200 µm and 400 µm is used in optical spectroscopy. The electromagnetic spectrum regions are listed in Table 2.

Table 2: Regions of electromagnetic spectrum

Region	Wavelength
Far (or vacuum) ultraviolet	10-200 nm
Near ultraviolet	200-400 nm
Visible	400-800 nm
Near infrared	0.75- 2.5 µm
Mid infrared	2.5-25 µm
Far infrared	25-300 µm

Ultraviolet spectrophotometers

One of the most widely used methods of pharmaceutical research is UV-Visible Spectrophotometry. It requires the calculation of the amount of ultraviolet or visible radiation that a solution material absorbs. Ultraviolet-Visible Spectrophotometers are an instrument that measures the proportion or function of the ratio of the strength of two light beams in the UV-visible field. [13]

Organic compounds may be classified through the use of a spectrophotometer in qualitative research, if any reported data is available, and quantitative spectrophotometric analysis is used to determine the quantity of radiation absorbing molecular species. The technique of spectrophotometry is quick, rapid, reasonably specific and applicable to small amounts of compounds.

The principle involved in Ultraviolet Spectrophotometry

Radiation in the wavelength range of 200-400 nm is distributed via a compound solution. Inside the molecule, the electrons in the bond become excited so that they occupy a greater quantum state and consume some of the energy passing through the solution in the process. The more loosely kept the electrons are, the longer the wavelength (lower energy) of the absorbed radiation is inside the molecule's bond. Due to absorption, the power of photons passing through a sample containing the analyte is attenuated. This attenuation measurement, which we call absorbance, serves as our signal. [14] Absorption only happens when the energy of the photon equals the energy difference between the two energy levels. An absorbance spectrum is called a plot of absorbance as a function of the energy of the photon. A photon emission occurs when an analyte returns to a lower-energy state from a higher-energy state.

The principle involved in Visible Spectrophotometry (Colorimetry)

The study of the absorption of visible radiation, whose wavelength varies from 400 nm to 800 nm, is concerned with colorimetry. In this wavelength area, any coloured material can absorb radiation. Coloured materials absorb light in a different way from different wavelengths, so we get an absorption curve (absorbance versus wavelength). The wavelength at which maximal radiation absorption occurs in this absorption curve is called λmax. For any coloured material, this λmax is characteristic or unique and this is a qualitative feature that is useful in identifying the substance.

Instrumentation of UV Spectrophotometry

Following are the instrumentation of single beam and double beam UV Spectrophotometry. [15]

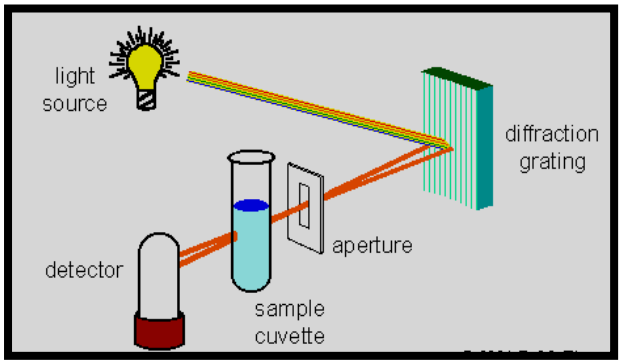

Figure 1: UV Visible single beam Spectrophotometer.

The components of UV-Visible double beam spectrophotometer are

- Source of light
- Monochromators
- Sample cells
- Detector

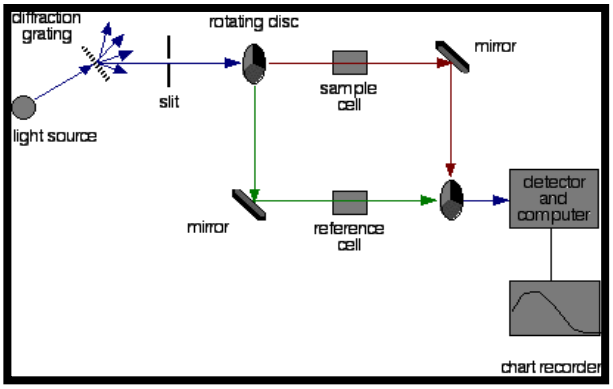

Figure 2: UV Visible double beam Spectrophotometer.

The different sources of light used in UV are

- Hydrogen discharge lamp
- Deuterium lamp
- Xenon discharge lamp
- Mercury arc lamp

Monochromators

Monochromators are better and more efficient than filters in converting a polychromatic light into monochromatic light.

A monochromator has the following units

- Entrance slit
- Collimator
- Grating/ prism
- Exit slit

Most frequently used monochromator in UV is grating monochromators which are made up of quartz. Grating provides a band pass of 0.4- 2 nm.

Grating monochromators are of two types

- Diffraction gratings
- Transmission gratings

Sample cells

Sample cells are made up of quartz only since glass cells will absorb UV radiation. The path length of these cells is 10 mm or 1cm.

Choice of solvent

Suitable solvent for UV Spectroscopy should meet the following requirements

a. It should not absorb radiations in the region under investigation.
b. It should be less polar, so that it has minimum interaction with the solute molecules.
c. The solvent used should be of high purity.

Detectors

A detector is a transducer that converts EMR into an electron flow and subsequently, into a current flow or voltage in the readout circuit. Photoelectric or Photo multiplier tubes are generally used as detectors. The detector must have the following important requirements. It must respond to radiant energy over a broad wavelength range.

- It should be sensitive to low levels of radiation power.
- It should rapidly respond to the radiation and produce an electrical signal that can be readily amplified.
- It should have a relatively low noise level (for stability).
- The signal produced is directly proportional to the power of beam striking it.

Photo Multiplier Tubes

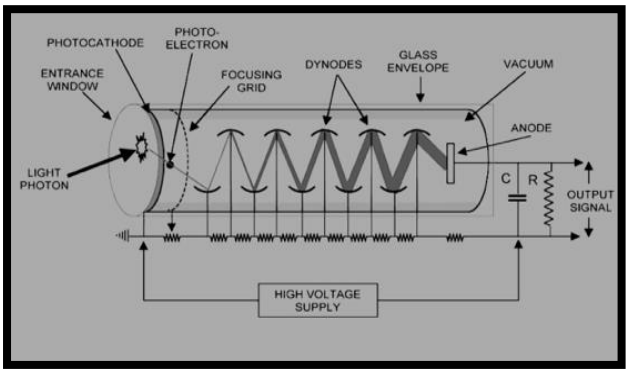

Figure 3: Photo Multiplier Tube.

This detector is most sensitive, expensive and used in sophisticated instruments. The principle employed in the detector is that the multiplication of photoelectrons by secondary emission of electrons as indicated in Figure3. This is achieved by using a photocathode and a series of anodes up to 10 dynodes. Each dynode is maintained at 75-100 V higher than preceding one. At each stage the electron emission is multiplied by a factor of 4 or 5 due to secondary emission of electrons.

QUANTITATIVE ANALYSIS IN UV SPECTROPHOTOMETRY

Assay of substances in a single component sample

The assay of single component samples, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal procedures.

The various methods used for the calculation of sample concentration are

- Using absorptivity value (E 1%, 1cm)
- Calibration curve method or Multiple standard method
- Single standard or Direct comparison method

Using absorptivity (E 1%, 1cm) values

In the standard absorptive value method, the use of standard A (1%, 1cm) or E values are used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. [16]

Calibration curve method or multiple standard methods

In the calibration curve method, of the absorbances of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte within the sample solution is read from the graph because the concentration like the absorbance of the answer.

Single standard or direct comparison method

The single point standardization procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The concentration of the substances within the sample is calculated from the proportional relationship that exists between absorbance and concentration.

Assay of substances in multicomponent samples

The basis of all the spectrophotometric techniques for multi-component samples is the property that all wavelengths

The absorbance of a solution is the sum of absorbances of the individual components; or
The measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell. In multi-component formulations the concentration of the absorbing substance is calculated from the measured absorbance using one of the following procedures. [18]

Principle of Chromatography and HPLC

In general, the strategy involves a liquid sample being passed over a solid adsorbent packed into a column employing a flow of liquid solvent. Each analyte within the sample interacts slightly differently with the adsorbent, thus retarding the flow of the analytes. If the interaction is weak, the analytes flow away the column during a short amount of time, and if the interaction is powerful, then the elution time is long. HPLC has been used in medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of comparable synthetic chemicals from each other), and manufacturing [19] (e.g. during the production process of pharmaceutical and biological products). Chromatography is often described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, resulting in the separation of the sample components. The active component of the column, the sorbent, is usually a granular material made from solid particles (e.g. silica, polymers, etc.), 2-50µm in size. The components of the sample mixture are separated from one another because of their different degrees of interaction with the sorbent particles. The pressurized liquids are typically a mix of solvents (e.g. water, acetonitrile and/or methanol) and are referred to as a "mobile phase" [20]

Advantages of HPLC

- Speed (analysis can be accomplished in 20 minutes or less).
- Greater sensitivity (various detectors can be employed).
- Improved resolution (wide variety of stationary phases).
- Reusable columns (expensive columns but can be used for many analysis).
- Ideal for the substances of low volatility.
- Easy sample recovery, handling and maintenance.
- Instrumentation tends itself to automation and quantitation
- Precise and reproducible.
- Calculations are done by integrator itself.
- Suitable for preparative liquid chromatography on a much larger scale.

Modern high performance liquid chromatography (HPLC)

The highly sophisticated, reliable and fast liquid chromatographic (LC) separation techniques are become a requirement in many industries like pharmaceuticals, agrochemicals, dyes, petrochemicals, natural products and others. Early LC used gravity fed open tubular columns with particles 100 s of microns in size; the human eye was used for a detector and separations often to develop.

Instrumentation of HPLC

Isocratic and Gradient LC System operation two basic elution modes are used in HPLC. The first is called isocratic elution. In this mode, the mobile phase, either a pure solvent or a mixture, remains the same throughout the run.

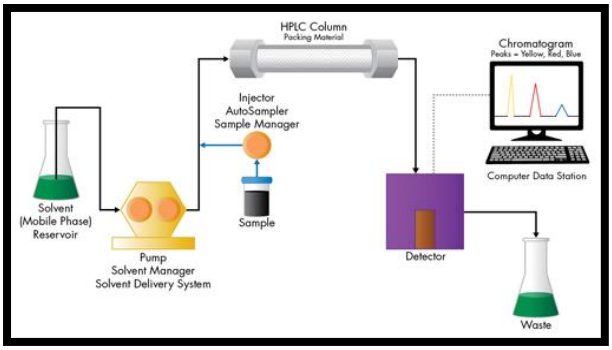

Figure 4: Isocratic HPLC system

The second type is called gradient elution, wherein, as its name implies, the mobile phase composition changes during the separation. This mode is useful for samples that contain compounds that span a wide range of chromatographic polarity. As the separation proceeds, the elution strength of the mobile phase is increased to elute the more strongly retained sample components.

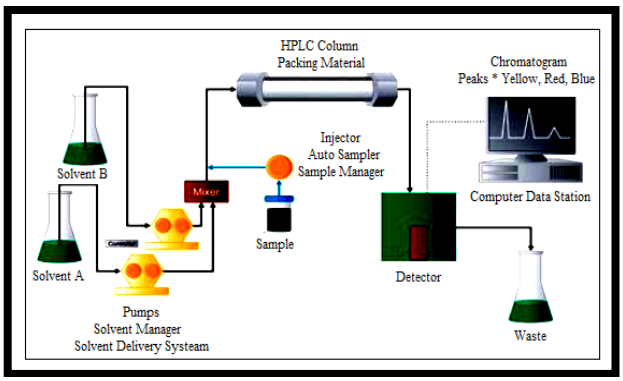

Figure 5: High Pressure Gradient HPLC System

In the simplest case, shown in Figure 5, there are two bottles of solvents and two pumps. The speed of each pump is managed by the gradient controller to deliver more or less of each solvent over the course of the separation. The two streams are combined in the mixer to create the actual mobile phase composition that is delivered to the column over time. At the beginning, the mobile phase contains a higher proportion of the weaker solvent [Solvent A]. [21]

Over time, the proportion of the stronger solvent [Solvent B] is increased, according to a predetermined timetable. Note that in the mixer is downstream of the pumps; thus, the gradient is created under high pressure. Other HPLC systems are designed to mix multiple streams of solvents under low pressure, ahead of a single pump. A gradient proportioning valve selects from the four solvent bottles, changing the strength of the mobile phase over time.

HPLC Gradient mixtures

To maintain a reproducible gradient profile, HPLC gradient mixers must provide very precise control of the solvent composition. The tiny volumes of elution needed by many systems can complicate this in HPLC. When mixing small volumes, it is much more difficult to create a constant gradient than when mixing large volumes. This requires great precision in the process and low dispersion flows in the mixer for low pressure systems. It requires very precise control of the flow rate for multi-

pump high pressure systems, thus allowing very minor adjustments in the flow rate. [22]

HPLC Pumps

Modern LC pumps need to work safely and efficiently at pressures of 10,000 psi or at least 6,000 psi, due to the small particles used in modern HPLC. HPLC pumps typically have sapphire pistons, stainless steel cylinders and return valves equipped with sapphire balls and stainless-steel sheets to work at these pressures and to remain sensitively inert to the wide range of solvents used. HPLC pumps should have flow rates ranging from 0 to 10 mL/min for analytical purposes, but flow rates in excess of 100 mL/min may be needed for preparatory HPLC. Providing a very steady flow rate at very low flow rates is extremely difficult. If 1% is considered acceptable then for 1 mL/min a flow variation of less than 10μL/min is required. This level of constancy is required because most HPLC detectors are flow sensitive and errors in quantization will result from changes in flow rate.

HPLC Sample Valves

Since sample valves come between the pump and the column, it follows that pressure up to 10,000 psi must also be tolerated by HPLC sample valves. The sample volume for analytical HPLC should be selectable from sub micro litres to a few micro litres, where the sample volume can be even greater than 10 mL, as in preparative HPLC. The sample valve must be designed to have very low dispersion characteristics to preserve device performance, this is valid not only for flow dispersion, but also for the less apparent dispersion problems caused by sample adsorption/desorption on valve surfaces and the diffusion of the sample between valve moving parts into and out of the mating surfaces. It goes without saying that the valves must deliver a very constant sample size, but this is usually attained by the use of a constant size sample loop.

HPLC Columns

HPLC columns have very fine particles in their box (usually a few microns in diameter). To achieve the low dispersion that gives the high plate counts expected of modern HPLC, very fine particles are needed. With modern columns, plate counts in excess of 25,000 plates per column are possible, however these quite high efficiencies are quite rarely found with real samples due to the dispersion associated with injection valves, detectors, data acquisition systems and dispersion due to the greater molecular weight of real samples compared to traditional test samples. [23]

It is a difficult technical problem to load these small particles into the column, but even with good packaging, the column end fittings and the inlet and outlet link must be given a great deal of care to keep dispersion to a minimum. The primary consideration for HPLC is the much wider range of solvents and packaging materials that can be used because of the much smaller amounts required for both. In particular, to make Chiral HPLC stationary phases, very costly optically pure compounds can be used and can also be used as (disposable) HPLC solvents.

HPLC Detectors

The most widely employed detectors are UV/Vis spectrophotometers, including diode array detectors. It is also possible to use fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, detectors for light dispersion, radioactivity detectors or other unique detectors. A flow-through cell placed at the end of the column consists of a detector. In the flow cell and into the detector, a beam of UV radiation passes through. They pass into the cell as a compound solution from the column and absorb radiation, resulting in observable changes in the energy level. Fixed (mercury lamp), variable (deuterium or high-pressure xenon lamp), and multi-wavelength detectors are widely available. Modern variable wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi-wavelength detectors measure absorbance at two or more wavelengths simultaneously.

In multi-wavelength detectors in the diode array, continuous radiation is passed through the sample cell and then resolved into its constituent wavelengths, which are detected individually by the array of photodiodes. Throughout the entire UV-visible spectrum, these detectors obtain absorption data, thus providing the analyst with chromatograms at different, selectable wavelengths, eluting peak spectra and also peak purity. The discrepancy between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographic compounds as it emerges from the column is determined by the differential refractometer detectors. For the detection of UV absorbing substances, refractive index detectors are used. Fluorometric detectors are susceptible to compounds which are naturally fluorescent or which can be transformed to fluorescent derivatives either by chemical compound transformation or by coupling with fluorescent reagents in particular functional groups. Electrochemical potentiometric, voltametric, or polarographic detectors are useful for quantifying species that can be oxidised or reduced by working electrodes. The detector requirement is likely to retain high sensitivity, as this typically relies on providing a greater volume of cells. [24, 25]

Table 3: Normal vs. Reversed Phase Chromatography Comparison

Parameters	Normal	Reverse
Packing polarity	High	Low
Solvent polarity	Low	High
Elution order	Non-polar first, then polar	Polar first, then non-polar
Effect of increasing solvent polarity	Decrease retention time	Increases retention time

ANALYTICAL METHOD VALIDATION

An analytical method used to measure the quality of pharmaceutical products has been developed. It is important to ensure that the performance characteristics of the analytical procedure established fulfil the requirements for the analytical application intended. As method validation, the procedure that provides assurance for the same by means of laboratory studies is specified. Validation of the system is the process of demonstrating that the analytical methods are sufficient for their intended use and that they facilitate the identification, intensity and consistency of drug substances and drug products for quantification. The prime objective of any pharmaceutical plant is to manufacture products of requisite attribute and quality consistently, at the lowest possible cost. Although validation studies have been conducted in the pharmaceutical industry for a long time, there is an ever-increasing interest in validation owing to the industry's greater emphasis in recent years on quality assurance program and is fundamental to an efficient production operation. Validation is a concept that has evolved in united states

in 1978The concept of validation has expanded through the years to embrace a wide range of activities from analytical methods used for the quality control of drug substances and drug products to computerized systems for clinical trials, labelling or process control, Validation is founded on, but not prescribed by regulatory requirements and is best viewed as an important and integral part of cGMP. The word validation simply means assessment of validity or action of proving effectiveness. Validation is a team effort where it involves people from various disciplines of the plant. [26]

Importance of validation

- Assurance of quality
- Time bound
- Process optimisation
- Reduction of quality cost.
- Nominal mix-ups, and bottle necks
- Minimal batch failures, improved
- Efficiently and productivity.
- Reduction in rejections.
- Increased output.
- Avoidance of capital expenditures

Planning for validation

All validation activities should be planned. The key elements of a validation programme should be clearly defined and documented ina validation master plan (VMP) or equivalent documents.

- The VMP should be a summary document, which is brief, concise and clear.
- The VMP should contain data on at least the following:
- Validation policy.
- Organisational structure of validation activities.
- Summary of facilities, systems, equipment and processes to be validated.
- Documentation format: The format to be used for protocols and reports.
- Planning and scheduling.
- Change control.
- Reference to existing document.
- In case of large projects, it may be necessary to create separate validation master plans.

Strategy for the validation of methods

Creation of methods and validation are an iterative process. At the validation stage, which was not done during the method's development/optimization phases, the effect of operating parameters on the performance of the method can be evaluated. The most critical argument posed for validation is that only by laboratory experiments can the validity of a method be demonstrated. Simply reviewing historical findings is not enough; instead, experimental studies that are intended to confirm the particular approach must be performed, and those studies should be pre-planned and defined in the required documentation. This report should clearly demonstrate the intended use and operating principles of the method, as well as the parameters of validation to be analysed, and a justification for the choice of this method and these parameters. [26] .It must also include pre-defined conditions for admission and a description of the analytical method.

TYPES OF VALIDATION

Prospective validation

It is defined as the existing written proof that, based on a pre-planned protocol, a system does what it purports to do. This validation is typically conducted prior to the delivery of either a new product or a revised production process product. Performed in at least three output sizes in succession (Consecutive batches). The validation protocol is performed in Prospective Validation before the procedure is placed into commercial use. The manufacturing process can be divided into individual phases during the product development phase. To determine the critical parameters which can affect the quality of the finished product, each move should be evaluated on the basis of experience or theoretical considerations. To assess the criticality of these considerations, a series of experiments should be planned. Experiments should be prepared and thoroughly recorded in the approved protocol.

Concurrent Validation

This is achieved in two cases, i.e. for existing equipment, and proper installation verification is
done along with unique operational checks. Dates are collected from at least three successful trials in the case of a current, frequently made product. It is similar to prospective, except that during the certification runs, the operating company will sell the product to the public at its selling price, and also similar to retrospective validation. This validation includes in-process inspection and product testing of important processing steps.

Retrospective Validation

It is defined as the existing record proof that a system does what it intends to do on historical information review and analysis. This is done by evaluating the historical testing data for manufacturing to show that the process has always been in order. This form of process validation for a product that is already being sold. For well-established systems, retrospective validation is only permissible and would be unacceptable if there have been recent adjustments in the composition of the product, operating procedures or equipment. It is important to base the validation of such processes on historical data. The steps involved include a particular procedure to be prepared and the results of the data analysis to be published. It leads to a conclusion and is a tribute. The data source for this validation should include, but not be limited to, records of batch processing and packaging, process control charts, logbooks of maintenance, records of staff changes, studies of process capacity, finished product data, including trend cards and results of storage stability. Batches selected for retrospective validation should be representative of all batches made during the review period, including any batches that failed to meet the specifications, and should be sufficient in number to demonstrate process consistency.

Re-validation

Provides evidence that process features, and product quality are not negatively affected by improvements in a process and/or process environment that are implemented. The specifications for documentation would be the same as for initial method validation. To confirm that they remain accurate, services, systems, equipment and processes, including cleaning, should be regularly reviewed. If no substantial changes have been made to the validated status, the need for revalidation is satisfied by a check with proof that services, structures, equipment and processes meet the specified criteria. Revalidation becomes necessary in certain situations. Some of the changes that require validation are as follows: properties such as density, viscosity,
particle size distribution and moisture etc.

- Changes in the source of active raw material manufacturer.
- Changes in packaging material.
- Changes in the plant/facility.
- Changes in the process (e.g., mixing time, drying temperatures and batch size)

- Changes in the equipment (e.g., addition of automatic detection system). Changes of equipment which involve the replacement of equipment on a "like for like" basis would not normally require re-validation except that this new equipment must be qualified.
- A decision not to perform revalidation studies must be fully justified and documented.[28]

CONCLUSION

UV spectroscopic and HPLC methods for simultaneous estimation of different drugs have been mentioned in this review paper. And how the methods of validation influence the analytical process of any study of drugs. There are plenty of validation parameters for the proper validation and estimation of analytical processes that are included in this article. All of the instrumentation knowledge is very important if we are running any instrument. Validation must be sufficient at the end of any calculation for a better outcome.

REFERENCES

1. Knox J. H., Done J. N. High-Performance Liquid Chromatography. *International Journal of Pharmaceutical Compounding*. 2004; 8(3):223-225.
2. Simpson C.F. Practical High-Performance Liquid Chromatography. *International Journal of Pharmaceutical Compounding*. 2004;8(3): 116-118.
3. Rudy B., Magali A., Herida S., Recent applications of analytical techniques for quantitative pharmaceutical analysis, A review. *WSEAS transaction on biology and biomedicine*.2010; 7(4): 316-338.
4. Alain B. Lain B., Tatyana M., Boris S., Oleg S., Counter current chromatography in analytical chemistry. *International Union on Pure and Applied Chemistry.* 2009; 81(2): 355-387.
5. Istvan B. Modern analytical techniques in the pharmaceutical and bioanalysis. Budapest, 2011; 1-60.
6. Malviya R., Bansal V., Omprakash P., Sharma P., High performance liquid chromatography: A short review. *Journal of Global Pharma Tehnology*, 2010; 2(5): 22-26.
7. Guido F. P., Samuel M. Pro., J. Brent F. Countercurrent Separation of Natural Products. *Journal of Natural Product*. 2008; 71(8): 1489-1508.
8. Sathish K. K., Sujana. K; Development and validation Of UV Spectroscopic Method for Determination of Atazanavir Sulphate in Bulk and Formulation; *International Journal of Pharmacy and Pharmaceutical Sciences*; 2012, 4(3): 109-112
9. Khanage S.G., Deshmukh V.K., Mohite P.B., Dhamak V.M., Appala Raju; Development of Derivative Spectrophotometric Estimation of Atazanavir sulfate in Bulk Drug and Pharmaceutical Dosage Forms; *International Journal of Pharm & Health Science*, Vol. 1 (3), 2010, 149-154
10. Kitson F. G., Larsen B. S., McEwen C. N. Gas Chromatography and Mass Spectroscopy-A Practical Guide. *Academic Press*, London.6th Edn , 1996; 522-536.
11. Scott P. W. Gas Chromatography. *Library Science*, UK. 2003; 245-250.
12. Kennedy J. H. Analytical Chemistry Principles. *Harcourt Brace Jovanovic*, London.6th Edn, 1984; 540-549.
13. Lena O., Antony J. S. Handbook of Pharmaceutical Analysis. *Marel Dekker Inc.* New York, Basel. 2002; 462.
14. Christian G. D. Analytical Chemistry. *John Wiley and Sons*, New York, 6th Edn. 2003; 471.
15. Chatwal G. R., Sham K. A. Instrumental methods of chemical Analysis, *Himalaya Publishing house*, 5th Edn . 2002; 107-116.
16. Becket A. H., Stenlake J. B. Practical pharmaceutical chemistry, *CBS Publication*, New Delhi. 4th Edn ., 2001; 284-288.
17. Skoog D. A., Holler F. J., Crouch S. R. Principle of Instrumental Analysis, *Thomson Publications*, USA, 6th Edn., 2007; 1-3.
18. David H. Modern analytical chemistry, *McGraw Hill Higher Education*, 3rd Edn ., 2000; 38-84.
19. Dey S., Subhasis P., Sureshbabu N., Murthy P.N., Panda S.K., Development And Validation Of A Stability Indicating Rp-Hplc Method For Estimation Of Atazanavir Sulphate In Bulk. *Journal of Pharmaceutical Analysis*. 2012; 7 (3): 149-154.
20. Ahuja S., Rasmussen H. Overview of HPLC Method Development for Pharmaceuticals, *Elsevier Academic Press*. 2007; 45-83.
21. Lloyd R.S., Joseph L., Kirklan J., Rihard W.A., Practical HPLC method development, E*lsevier Analytica Chemia Acta*, Wiley, New York.1991; 277-288.
22. Maccrehan W. A., Differential pulse detection in liquid chromatography and its application to the measurement of organometal cations *Analytical Chemistry*,1981, 5(1); 74-77.
23. Kissinger P. T., Szulc M. E., Fischer D. H., Krull I. S., Marcel D. In Electrochemical Detection in Liquid Chromatography and Capillary Electrophoresis. *Chromatography Science Series*. New York. 1997; 137.
24. Kissinger P. T., Heineman W. R., Laboratory Techniques in Electroanalytical Chemistry, *Marcel Dekke*r, New York, 2nd . Edn ., 1984; 51- 125.
25. Snyder L. R., Joseph J., Kirikland J. Practical HPLC method development. J*ohn Wiley and Sons*, 2nd Edition. 1988; 740-746.
26. Jeevana Jyothi B., Uv Spectrophotometric Method For Estimation Of New Drug, Daclatasvir Dihydrochloride. *International Research Journal of Pharmacy*, 2016, 7(9): 1-3.
27. Shabir A.M., Moloy M., Bhasin P.S., Hplc Method Development and Validation: A Review. *International Research Journal Of Pharmacy*, 2015, 4(4): 39-46
28. Indian Pharmcopoeia 2014, Volume I., Government of India Ministry Of Health & Family Welfare., 7th Edn, January 2014: 770

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