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A BRIEF STUDY ON ANALYTICAL METHOD DEVELOPMENT: A REVIEW

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ABSTRACT

Analytical method is an important quality control tool for estimation of percentage of drug in formulation. The developments of analytical methods for the determination of drugs in bulk, in dosage forms or in body fluids have received a considerable attention in recent years because of their importance in quality control, bioavailability and pharmacokinetic study etc. The aim and objective of the present review is to develop new simple, sensitive and validated UV spectrophotometric method for the estimation of drug in marketed formulation and Validation of developed Analytical methods according to standard guidelines.

KEYWORDS

Analytical method, Bioavailability and pharmacokinetic study and Marketed formulation.

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INTRODUCTON

INTRODUCTION TO SPECTROSCOPY[1-3]

Spectroscopy may be a general term for the science that deals with the interaction of assorted kinds of radiation with matter. Spectroscopic analysis and spectroscopical strategies check with the activity of the intensity of radiation with a measurement electrical device or different variety of device. The spectrophotometric assay of medicine seldom involves the activity of absorbance of samples containing just one fascinating part. The pharmaceutical analyst oft encounters the case

wherever the concentration of 1 or additional substances is needed in samples illustrious to contain different fascinating substances, that doubtless interfere within the assay. If the formula of the samples is understood, the identity and concentration of the interferences area unit illustrious and therefore the extent of interference within the assay could also be determined. The idea of all the spectrophotometric techniques for single and multicomponent samples is that the property that in the slightest degree wavelengths:

- The absorbance of an answer is that the add of absorbance of the individual parts or
- The measured absorbance is that the distinction between the entire absorbance of the answer within the sample cell which of the answer within the reference cell.

Following strategies area unit most typically used[4-6].

Synchronic Equation technique (Vierodt's method)

If a sample contains 2 fascinating drug (x and y) every of that absorbs λmax of different, it's going to be potential to see each medication by the technique of synchronic equation providing bound criteria apply. The information required is:-

1. The absorptivities of x at λ_1 and λ_2, ax_1 and ax_2 respectively.
2. The absorptivities of y at λ_1 and λ_2, ay_1 and ay_2 respectively.
3. The absorbances of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.
4. Cx and Cy be the concentrations of x and y respectively in the diluted sample,

So, concentration can be calculated by:

$Cx = (A_2ay_1-A_1ay_2)/ (ax_2ay_1-ax_1ay_2)$

$Cy = (A_1ax_2-A_2ax_1)/ (ax_2ay_1-ax_1ay_2)$

E.g. the B.P. assay of quinine- related alkaloids and cinchonine – related alkaloid in cinchona bark.

Absorption Ratio Method

The absorbance ratio method is a modification of the simultaneous equation method. It depends on the property that for a substance which obeys Beer's law at all wavelengths, the ratio of absorbance's at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilutions of the same substance give the same absorbance ratio A1/A2 = 2.0. In the USP this ratio is reffered to as a Q value. The B.P. also uses a ratio of absorbance at specified wavelength in certain confirmatory test of identity.

E.g. Cynocobalamin exhibits three λmax at 278nm, 361nm and 550nm. The A361/ A550 are required to be 3.30 ± 0.15 and the A361/A278 to be 1.79 ± 0.09.

Geometric correction Method

For elimination of the background irrelevant absorption that may be present in the sample of biological origin, a number of mathematical correction procedures have been developed. The simplest of this procedure is that the 3 - purpose geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected.

Orthogonal Polynomial method

Technique of orthogonal polynomials is another mathematical correction procedure that involves a lot of advanced calculations than the 3 –point correction procedure. The basis of the method is that an absorption spectrum mat be represented in terms of orthogonal functions.

Difference spectrophotometry

The essential options of a distinction spectrophotometric assay square measure that the measured worth is that the distinction absorbance (ΔA) between 2 equimolar solutions of the analyte in several chemical forms that exhibit different spectral characteristics. When absorption spectra of two equimolar solutions of phenylephenerine, in both 0.1 M HCl and 0.1 M NaOH at 257 and 278nm.

Both solutions have identical absorbance and consequently exhibit zero difference absorbance. Such wavelengths of equal absorptivity of the two species are called isobestic or isoabsorptive point. The measured value in quantitative difference spectrophotometric assay is the ΔA.

$\Delta A = A_{alk} - A_{acid}$

ΔA also obeys the Beer-Lambert law and a modified equation may be written as $\Delta A = \Delta abc$

Where ΔA is the difference absorptivity (e.g. ΔA 1% 1cm or ΔЄ) of the substance at the wavelength of measurement.

Derivative Spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or the higher derivative spectrum. In this spectrophotometry the normal absorption spectrum is referred to as the fundamental zero order or D0 spectrum.

The first spinoff (D1) spectrum may be a plot of the speed of amendment of absorbance with wavelength against wavelength i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of a da/dλ Vs λ.

The second derivative (D2) spectrum is a plot of the curvature of the D0 spectrum against wavelength or a plot of dA2/dλ2 Vs λ.

Chemical Derivatization

It is an indirect spectrophotometric assay, which is based on conversion of the analyte by a chemical reagent to a derivative that has different spectral properties. When an excess of the reagent is used, to ensure complete conversion, the absorbance of the derivative is usually, but not always, proportional to the concentration of the analyte. In this the derivative of the longer λmax and/ or a higher absorptivity are made.

INSTRUMENTATION[7,8]

The various components of a UV – visible spectrophotometer are as follows:-

Radiation Source

The most commonly used radiation sources are

- Hydrogen or deuterium lamps
- Xenon discharge lamps
- Mercury arcs

Monochromators

The monochromator is used to disperse the radiation according to wavelength. The essential elements of monochromator are an entrance slit, a dispersing element and an exit slit. The dispersing element may be

- Prism
- Grating

Detectors

There are three common types of detectors which are widely used in UV spectrophotometers. These are as follows.

- Barrier Layer cell
- Photocell
- Photomultiplier tube

Recording system

The signal from the photomultiplier tube is finally received by the recording system. The recording is done by recorder pen. The type of arrangement is only done in recording UV - Visible spectrophotometers.

Sample cells

The most commonly used cells are made up of quartz or fused silica. These are readily available even in matched pairs where sample cell is almost identical to the reference cell.

Matched cells

When double beam instrumentation is used, two cells are needed, one for the reference and one of the sample. For most accurate work, matched cells are used.

Power supply

The power supply serves a double function

- It decreases the line voltage to the instruments.
- It converts A.C to D.C with a rectifier.

CHROMATOGRAPHY[1]

Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While natural action could also be applied each qualitatively and quantitatively, it is primarily a separation tool. Quantitative analysis can be carried out by measuring the area of the chromatographic peak.

Chromatography is comparatively a replacement technique, which was first invented by M. Tswett, a botanist in 1906 in Warsaw. In that year, he was successful in doing the separation of chlorophyll, xanthophylls and several other colored substances by percolating vegetable extracts through a column of calcium carbonate. The calcium carbonate column acted as an adsorbent and the different substances got adsorbed to different extent and this gives rise to colored bands at different positions, on the column. Tswett termed methodology} of coloured bands because the recording and also the method as natural action once the Greek words vividness and graphs that means "color" and

"writing" severally. However, in the majority of chromatographic procedures no colored products are formed and the term is a misnomer. Considerable advances have since been made and the method is used to separate colored as well as colorless substances. The column of carbonate, used in Tswett's method, remains stationary and is therefore termed as the stationary phase. The solution of vegetable extracts moves or flows down the column and is thus termed because the mobile section. Chromatography could also be considered a technique of separation within which separation of solutes occur between a stationary section and a mobile section. In 1930's chromatography in the form of thin layer chromatography and ion exchange chromatography was introduced as a separation technique. In 1941, Martin and John Millington Synge introduced partition and chromatography. They introduced gas chromatography in 1952. During the next decade the routine use of chromatography as a separation technique became universal and has been extended to several areas of study, especially chemistry, biology and medicine. Apart from its use in analysis it is becoming a potential technique as a method for the preparation of very pure compounds such as in pharmaceutical industry or in the manufacture of pure chemicals. The recent spectacular developments in the field of biosciences are entirely because of the chromatographic methods of separation of biomolecules.

DEFINITION OF CHROMATOGRAPHY

Chromatography could also be outlined as a way of separating a mix of elements into individual elements through equilibrium distribution between 2 phases.

- The action technique of separation, in general, involves the subsequent steps:
- Adsorption or retention of a substance or substances on the stationary part.
- Separation of the adsorbate substances by the mobile part.
- Recovery of the separated substances.

HIGH PERFORMANCE (PRESSURE) LIQUID CHROMATOGRAPHY[6,7]

One of the early problems with liquid chromatography was the slow rate at which the analysis took place. Early methods used gravity feed, and it was not uncommon for an analysis to take several days to complete. This led to great delay. Also the excessive time on the column inevitably led to loss of resolution by diffusion, and so on. Consequently, for a number of years liquid chromatography was not widely used as means of separating organic compounds. This drawback was mostly overcome by the appearance of high performance liquid natural action (HPLC). In this system pressure is applied to the column, forcing the mobile part through at a lot of higher rate. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC.

All of the factors affecting separation in liquid chromatography apply to HPLC. The factors affecting plate height, the sample distribution between the stationary and mobile phases, and the selection of stationary and mobile phases still pertain even under the conditions of HPLC. The principal advantage of the system is the speed at which separation takes place. Because of the decrease in time, diffusion in the column is reduced and resolution improved.

Emphasis has been placed on the size of the particles making up the substrate. It has been found that the smaller size, the better the resolution. Pressures used normally range from 30 to 200atm, depending on the type of column used. The pressure is varied to provide the optimum linear flow rate of the mobile phase. It is that pressure which gives the smallest theoretical plate.

THEORIES OF HPLC[8]

Two theories have been put forward regarding the rate of migration of solute and development of peaks in the chromatogram.

PLATE THEORY

According to plate theory developed by Martin and Synge, a action column consists of a series of distinct nevertheless continuous horizontal layers, that area unit termed because the theoretical plates. Equilibrium of the solute between the stationary and the mobile phases takes place at each of these plates. Migration of the solute is then assumed to occur by a series of stepwise transfers between one plates to the other immediately below. The efficiency of separation in chromatographic column gets increased as the number of theoretical plate increases. This is because the number of equilibriums will also correspondingly increase. The number of theoretical plates of N refers to a measure of column efficiency. If the length the column is L and the height equivalent of a theoretical plate is H, then N is given by

$$N = L / H$$

The height equivalent of a theoretical plate (HETP) refers to the height of a layer of the column, such that the solution leaving the layer is in equilibrium with the average concentration of the matter within the stationary section throughout the layer.

RATE THEORY

The rate theory is able to explain the effect of variables, such as mobile phase velocity and adsorb abilities, which determine the width of an elution band. It also relates the effects of these variables on the time taken by a solute to make its appearance at the end of the column. Migration of solute particles in a column occurs in a state of confusion, each solute molecule progressing in a stop and go sequence independent of any other molecule. If a molecule is attached to the stationary phase, its migration down the column is temporarily stopped, but the zone passes on. That is to say, one molecule may get immobilised temporarily on the column while other molecules migrate. In this manner, a molecule alternates rapidly between adsorbed and desorbed states. The time a molecule spends in either phase is highly irregular and it depends on an accidental energy gain by a molecule from its environment so as to affect a reverse transfer. A particle can migrate only if it is present in the mobile phase and as a result the migration down the column is highly irregular. Consequently, some solute molecules may migrate rapidly whereas others may lag behind. The net result of all these random individual processes is a symmetric distribution of velocities around the mean value, which represents the behaviour of the most common or average particle. The width of zone increases as it migrates down the column, because more time is needed for migration to take place. Hence, the zone width is directly related to the retention time on the column and inversely proportional to the mobile phase velocity. If the best use of a chromatographic column is to be made, a study of the factors that determine the time of retention of a molecule by either phase or the factors that decide zone spreading must be made.

PRINCIPLE OF SEPARATION IN HPLC

The principle of separation in traditional part mode and reverse part mode is surface assimilation. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationery phase. The part that has additional affinity towards the adsorbent travels slower. The part that has less affinity towards the stationary part travels quicker. Since no 2 parts have an equivalent affinity towards the stationary part, the parts are separated.

TYPES OF HPLC TECHNIQUES[9-11]

BASED ON MODES OF SEPARATION

Normal phase chromatography

Mechanism

Retention by interaction of the stationary phase's polar surface with polar parts of the sample molecules.

Stationary phase

It is bonded siloxane with polar functional group like SiO_2, Al_2O_3.

Mobile phase

Nonpolar solvents like heptane, *n*-hexane, cyclohexane, chloroform, ethyl, ether and dioxane.

Application

Separation of non-ionic, nonpolar to medium polar substances.

Sample elution Order

Least polar components are eluted first.

Reverse phase chromatography

Mechanism

Retention by interaction of the stationary phase's nonpolar hydrocarbon chain with nonpolar parts of sample molecules.

Stationary phase

It is bonded siloxane with nonpolar functional groups like n-octadecyl (C_{18}) or n- octyl (C_8), ethyl, phenyl, -(CH_2)n-diol, - (CH_2)n-CN.

Mobile phase

Polar solvents like methanol, acetonitrile, water or buffer, (Sometimes with additives of THF or dioxane)

Application

Separation of non-ionic and ion forming nonpolar to medium polar substances. (Carboxylic acids hydrocarbons)

Sample elution order

Most polar components are eluted first.

BASED ON PRINCIPLE OF SEPARATION

Adsorption Chromatography

The principle of separation is adsorption. Separation of components takes place because of the differences in affinity of compounds towards stationary phase. This principle is seen in normal phase as well as reverse phase mode, where adsorption takes place.

Ion Exchange Chromatography

The principle of separation is activity, which is reversible exchange of functional groups. In ion exchange chromatography, an ion exchange resin is used to separate a mixture of similar charged ions. For cations, a cation exchange resin is used. For anions, an anion exchange resin is used.

Ion Pair Chromatography

In particle try natural action, a reverse phase column is converted temporarily into ion exchange column by using ion pairing agents like pentane or hexane or octane sulphonic acid sodium salt, tetraethyl or tetraethyl ammonium hydroxide, etc.

Size Exclusion or Gel Permeation Chromatography

In this type of chromatography, a mixture of components with different molecular sizes is separated by using gels. The gel used acts as molecular sieve and therefore a combination of gear with completely different molecular sizes is separated. Soft gels like dextran, agarose or polyacrylamide are used. Semi rigid gels like styrene, alkyl dextran in non-aqueous medium are also used. The mechanism of separation is by steric and diffusion effects.

Affinity Chromatography

Affinity natural action uses the affinity of the sample with specific stationary phases. This technique is used mostly in the field of Biotechnology, Microbiology and Biochemistry etc.

Chiral Phase Chromatography

Separation of optical isomers may be done by victimisation chiral stationary phases. Different principles operate for different types of stationary phases and for different samples. The stationary phases used for this kind of natural action area unit largely with chemicals secured colloid.

BASED ON ELUTION TECHNIQUE

Isocratic Separation

In this technique, the same mobile combination is used throughout the process of separation. The same polarity or extraction strength is maintained throughout the method.

Gradient Separation

In this technique, a mobile phase combination of lower polarity or strength is used followed by gradually increasing the polarity or elution strength.

BASED ON THE SCALE OF OPERATION

Analytical HPLC

Where only analysis of the samples is done. Recovery of the samples for reusing is normally not done, since the samples used in very low.

Preparative HPLC

Where the individual fractions of pure compounds are often collected exploitation fraction collector. The collected samples are reused.

BASED ON THE TYPE OF ANALYSIS

Qualitative Analysis

This is used to identify the compound, detect the presence of impurities, to find out the number of components, etc. This is done by using retention time values.

Quantitative Analysis

It is done to determine the quantity of the individual or several components in a mixture. This is done by

comparison the height space of the quality and sample.

INSTRUMENTATION OF HPLC

The various components of a HPLC system are herewith

SYSTEM COMPONENTS

Solvent delivery system

The mobile part is pumped-up besieged from one or many reservoirs and flows through the column at a continuing rate. With micro particulate packing, there is a high-pressure drop across a chromatography column. Eluting power of the mobile part is set by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For traditional part separations eluting power will increase with increasing polarity of the solvent except for reversed part separations, eluting power decreases with increasing solvent polarity. Optimum separating conditions are often achieved by creating use of mixture of 2 solvents. Some other properties of the solvents, which need to be considered for a successful separation, are boiling point, viscosity, detector compatibility, flammability and toxicity.

The most necessary element of HPLC in solvent delivery system is that the pump, because its performance directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems (direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc.

Solvent degassing system

The constituents of the mobile part ought to be degassed and filtered before use. Several methods are employed to remove the dissolved gases in the mobile phase. They embrace heating and stirring, vacuum degassing with an aspirator, filtration through 0.45 filter, vacuum degassing with an air-soluble membrane, helium purging ultrasonication or purging or combination of these methods. HPLC systems are also provided an online degassing system, which continuously removes the dissolved gases from the mobile phase.

Gradient elution devices

HPLC columns is also run isocratically i.e., with constant eluent or they will be run within the gradient extraction mode within which the mobile part composition varies throughout run. Gradient elution is a means of overcoming the problem of dealing with a complex mixture of solutes.

Sample introduction systems

Two means for analyte introduction on the column are injection in to a flowing stream and a stop flow injection. These techniques can be used with a syringe or an injection valve. Automatic injector is a microprocessor-controlled version of the manual universal injector. Usually, up to one hundred samples is loaded in to the automobile appliance receptacle. The system parameters such as flow rates, gradient, run time, volume to be injected, etc. are chosen, stored in memory and sequentially executed on consecutive injections.

Detectors

The perform of the detector in HPLC is to observe the mobile section because it emerges from the column. Generally, there are 2 sorts of HPLC detectors, bulk property detectors and matter property detectors.

Bulk property detectors

These detectors are based on differential measurement of a property, which is common to both the sample and the mobile phase. Examples of such detectors are index of refraction, conduction and material constant detectors.

Solute property detectors

Solute property detectors reply to a property of the matter, that isn't exhibited by the pure mobile section. These detectors live a property, which is specific to the sample, either with or without the removal of the mobile phase prior to the detection. Solute property detectors that don't need the removal of the mobile section before detection embody spectrophotometric (UV or UV-Vic) detector, fluorescence detectors, polarographic, electro-chemical and radio activity detectors, whilst the moving wire flame ionisation detector and electron capture detector both require removal of the mobile phase before detection. UV-Visible and fluorescent detectors are suitable for gradient elution, because many solvents used in HPLC do not absorb to any significant extent.

Column and Column-packing materials

The heart of the system is the column. In order to attain high potency of separation, the column

material (micro-particles, 5-10μm size) packed in such a way that highest numbers of theoretical plates are possible.

Silica (SiO2 X H2O) is the most widely used substance for the manufacture of packing materials. It consists of a network of siloxane linkages (Si-O-Si) in a rigid three dimensional structure containing inter connecting pores. Thus a wide range of commercial products is available with surface areas ranging from 100 to 800m2/g. and particle sizes from 3 to 50μm.

The silanol teams on the surface of oxide provides it a polar character, that is exploited in sorption natural process mistreatment non-polar organic eluents. Silica can be drastically altered by reaction with organo chloro silanes or organo alkoxy silanes giving Si-O-Si-R linkages with the surface. The attachment of organic compound amendment to oxide produces a non-polar surface appropriate for reversed section natural process wherever mixtures of water and organic solvents are used as eluents. The most popular material is octadecyl-silica (ODS-Silica), which contains C18 chains, but materials with C2, C6, C8 and C22 chains are also available. During manufacture, such materials may be reacted with a small mono functional silane (e.g. trimethyl chloro silane) to scale back more the quantity of silanol teams remaining on the surface (end-capping). There is a vast range of materials which have intermediate surface polarities arising from the bonding to silica of other organic compounds which contain groups such as phenyl, nitro, amino and hydroxyl. Strong particle exchangers {are also|also ar|are} offered during which sulfonic acid teams or quaternary ammonium ion teams are guaranteed to oxide. The helpful pH vary for columns is two to eight, since siloxane linkages are cleaved below pH-2 while at pH values above eight silica may dissolve.

In HPLC, usually 2 sorts of columns are used, normal phase columns and reversed phase columns. Using traditional section natural process, particularly of non-polar and moderately polar drugs can make excellent separation. It was originally believed that separation of compounds in mixture takes place slowly by differential sorption on a stationary oxide section. However, it currently looks that partition plays a very important role, with the compounds interacting with the polar silanol groups on the silica or with bound water molecules.

While traditional section looks the passage of a comparatively non-polar mobile section over a polar stationary section, reversed phase chromatography is carried out using a polar mobile phase such as methanol, acetonitrile, water, buffers etc., over a non-polar stationary section. Ranges of stationary phases (C18, C8, -NH2, -CN, -phenyl etc.) are available and very selective separations can be achieved. The pH of the mobile phase can be adjusted to suppress the ionisation of the drug and thereby increase the retention on the column. For highly ionised drugs ion-pair chromatography is used.

PERFORMANCE CALCULATIONS

The following values (which can be included in a custom report) are used to access overall system performance.

1. Relative retention
2. Theoretical plates
3. Capacity factor
4. Resolution
5. Peak asymmetry
6. Plates per meter

The parameters used to calculate these system performance values for the separation of two chromatographic components. (Note: Where the terms W and t both appear in the same equation they must be expressed in the same units).

RELATIVE RETENTION (SELECTIVITY)

$\alpha = (t_2 - t_a) / (t_1 - t_a)$

THEORITICAL PLATES

$n = 16 (t / W)^2$

CAPACITY FACTOR

$K' = (t_2 - t_a / t_a)$

RESOLUTION

$R = 2 (t_2 - t_1) / (W_2 + W_1)$

PEAK ASYMMETRY

$T = W0.05 / 2f$

PLATES PER METER

$N = n / L$

HETP

$HETP = L/n$

Where, α = Relative retention.

t_2 = Retention time of the second peak measured from point of injection.
t_1 = Retention time of the first peak measured from point of injection.
t_a = Retention time of an inert peak not retained by the column, measured from point of injection.
n = Theoretical plates.
t = Retention time of the component.
W = Width of the base of the component peak using tangent method.
K' = Capacity factor.
R = Resolution between a peak of interest (peak 2) and the peak preceding it (Peak 1).
W_2 = Width of the base of component peak 2.
W_1 = Width of the base of component peak 1.
T = Peak asymmetry, or tailing factor.
W0.05 = Distance from the leading edge to the tailing edge of the peak, measured at a point 5 % of the peak height from the baseline.
f = Distance from the peak maximum to the leading edge of the peak.
N = Plates per meter.
L = Column length, in meters.

METHOD VALIDATION[11-13]

Method validation are often outlined as (ICH) "Establishing documented proof that provides a high degree of assurance that a selected activity can systematically turn out a desired result or product meeting its preset specifications and quality characteristics".

Method Validation, however, is usually a one-time method performed once the strategy has been developed to demonstrate that the strategy is scientifically sound which it serves the supposed analytical purpose. All the variables of the strategy ought to be thought-about, together with sampling procedure, sample preparation, activity separation, and detection and information analysis. For activity ways employed in analytical applications there's additional consistency in validation observe with key analytical parameters together with

Recovery

The absolute recovery of analytical technique is measured because the response of a processed spiked matrix commonplace expressed as a proportion of the response of pure commonplace, that has not been subjected to sample pre-treatment and indicates whether or not the strategy provides a response for the whole quantity of analyte that's gift within the sample. It's best established by examination the responses of extracted samples at low, medium and high concentrations in replicates of a minimum of half-dozen with those non-extracted standards, that represent 100% recovery.

Response of perform

In activity ways of study, peak space or peak height is also used as response perform to outline the linear relationship with concentration referred to as the activity model. It's essential to verify the activity model selected to make sure that it adequately describes the link between response perform (Y) and concentration (X).

Sensitivity

The method is claimed to be sensitive if little changes in concentration cause giant changes in response perform. The sensitivity of associate analytical technique is decided from the slope of the activity line. The bounds of quantification (LOQ) or operating dynamic vary of bio analytical technique are outlined because the highest and lowest concentrations, which may determined with acceptable accuracy. It's advised that, this be set at V-J Day for each the higher and lower limit of quantitation severally. Any sample concentration that falls outside the activity vary cannot be interpolated from the activity line and extrapolation of the activity curve is discouraged. If the concentration is over vary, the sample ought to be diluted in sober matrix and re-assayed.

Exactness

The purpose of closing a determination is to get a sound estimate of a 'true' price. Once one considers the standards in line with that associate analytical procedure is chosen, exactness and accuracy are sometimes the primary things to return to mind. Exactness and accuracy along confirm the error of a private determination. They're among the foremost vital criteria for judgement analytical procedures by their results.

Precision refers to the reliableness of activity inside a group, that is, to the scatter of dispersion of a group concerning its central price. The term 'set' is outlined as bearing on variety (n) of freelance replicate measurements of some property. One in all the foremost common applied mathematics terms

utilized is that the variance of a population of observation. Variance is that the root of the total of squares of deviations of individual results for the mean, divided by one but the quantity of leads to the set. The quality deviation S, is given by

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is some times multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

% Relative standard deviation = S x 100 / x

Accuracy

Accuracy normally refers to the difference between the mean of the set of results and the true or correct value for the quantity measured. According to IUPAC accuracy relates to the distinction between results (or mean) and also the true worth. For analytical ways, there area unit 2 potential ways that of crucial the accuracy, absolute methodology and comparative methodology.

Accuracy is best reported as percentage bias, which is calculated from the expression

$$\% \text{ Bias} = \frac{(\text{Measured value} - \text{True value})}{\text{True value}} \times 100$$

Since for real samples the true value is not known, an approximation is obtained based on spiking drug – free matrix to a nominal concentration. The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug - free matrix sampler.

Limit of detection (LOD)

The limit of detection (LOD) of associate degree analytical technique could also be outlined because the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopical techniques or different ways that depend on a activity curve for quantitative measurements, the IUPAC approach employs the quality deviation of the intercept (Sa), which can be related to LOD and the slope of the calibration curve, b, by

LOD = 3 Sa / b

Limit of quantitation (LOQ)

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

LOQ = 10 Sa / b

Where, Sa is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs. b is slope of the corresponding calibration curve.

Ruggedness

Method huskiness is outlined because the dependability of results once the tactic is performed below actual use conditions. This includes completely different analysts, laboratories, columns, instruments, supply of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Robustness

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 robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, percentage organic solvent strength and buffer concentration etc. To determine the lustiness of the tactic experimental conditions area unit intentionally altered and natural process characters area unit evaluated.

Stability

To generate reproducible and reliable results, the samples, standards and reagents used for the HPLC method must be stable for a reasonable time (e.g. one day, one week, one month depending upon need). For example, the analysis of even a single sample may require ten or more chromatographic runs to determine the system suitability, including standard concentrations to create a working analytical curve and duplicate or triplicate injections of the sample to be assayed.

System suitability

System suitability experiments can be defined as tests to ensure that the method can generate results with acceptable accuracy and precision. The requirements for system quality area unit sometimes

developed when method development and validation have been completed (or) the USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The criteria selected will be based on the actual performance of the method as determined during its validation. For example, if sample retention times form part of the system suitability criteria, their variation (SD) during validation can be determined system suitability might then require that retention times fall at intervals a three SD vary throughout routine performance of the tactic.

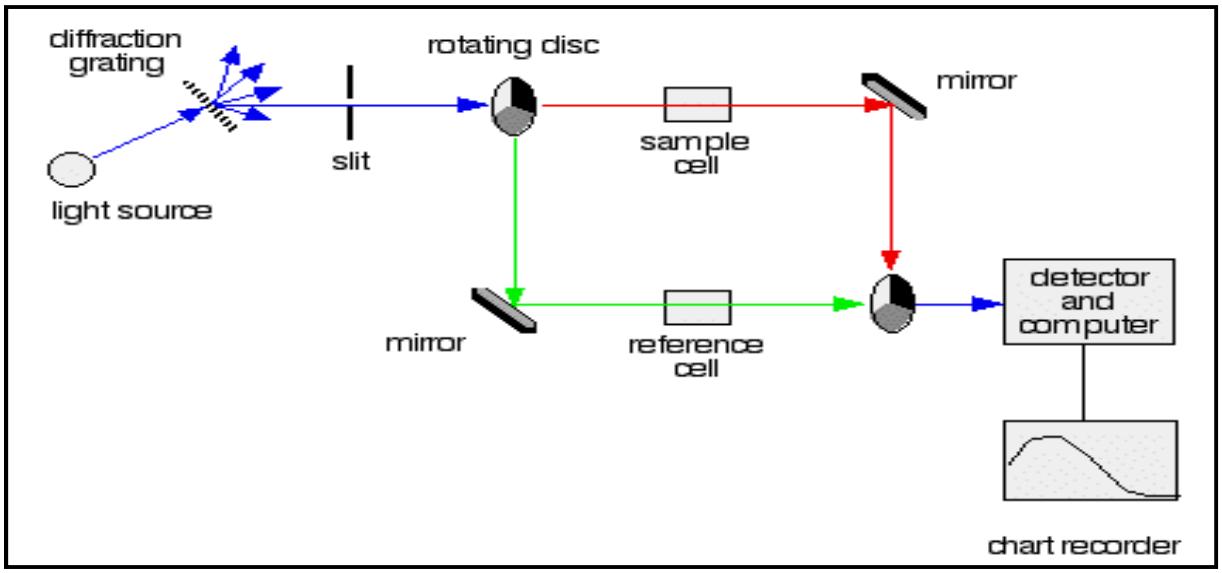

Figure No.1: Block Diagram of Double Beam UV - Visible Spectrophotometer

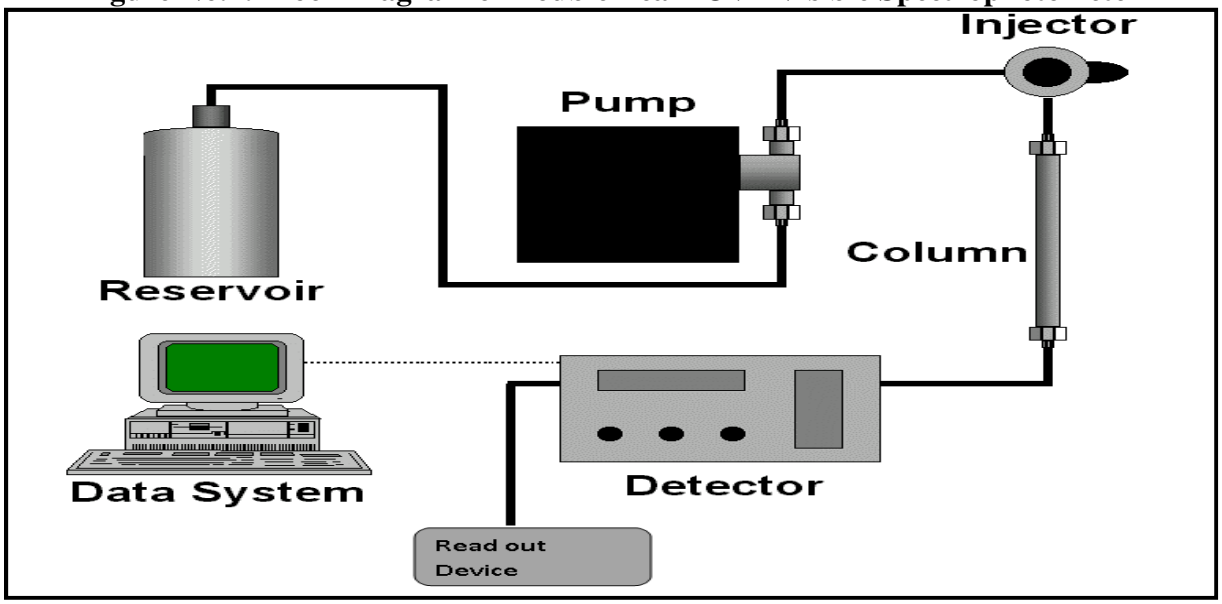

Figure No.2: Instrumentation of HPLC

CONCLUSION

Analytical method is an important quality control tool for estimation of percentage of drug in formulation. Fexofenadine Hydrochloride plays an important role in the maintenance of human health in case of verity of disorder like Hypersensitivity reaction, treatment of Gastric ulcer etc. This is where analytical method plays important role to estimation the content of drug in marketed formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Skoog D A, West D M and Holler J F. Principles of Instrumental Analysis, *Saunders college Publishing,* 6th Edition, 1994, 336-362, 817-818.
2. Ewing G W. Instrumental Methods of Chemical Analysis, McGraw Hill Book company, Inc, New York, 2nd Edition, 1960, 2-3.
3. Corners K A. A Text Book of Pharmaceutical Analysis, *John Wiley and Sons, NJ,* 3rd Edition, 2002, 581-622.
4. Sharma B K. Instrumental Methods of Chemical Analysis, *Goel Publishing Housing, Meerut,* 18th Edition, 1999, 1-77.
5. Willard H H, Merritt L L, Dean J A and Settle F A. Instrumental Methods of Analysis, *CBS Publishers and Distributors, New Delhi,* 7th Edition, 2001, 592-604.
6. Chatwal G R, Anand S K. Instrumental Methods of Chemical Analysis, *Himalaya Publishing House, Mumbai,* 5th Edition, 2005, 2.150-2.181, 2.624-2.639.
7. Rabisankar S. Text Book of Pharmaceutical Analysis, *R_x Publications,* 2nd Edition, 2.1-2.13, 18.1-18.15.
8. Kasture A V, Mahadik K R, Wadodkar S G, More H N. Pharmaceutical Analysis-Instrumental Method, Part-II, *Nirali Prakashan, Pune,* 9th Edition, 2003, 6-7, 10, 49-50, 156-159.
9. Snyder L R, Kirklad J J, Glajch L J, Limbird L E. Practical HPLC Method Development, *Wiley Interscience Publishing Inc, Co., USA,* 2nd Edition, 1996, 1, 3, 15, 631.
10. Meyer V R. Practical High Performance Liquid Chromatography, *John Wiley and Sons,* 4th Edition, 2004, 6-9, 87-92, 114, 228-234, 261-263.
11. Sethi P D. High Performance Liquid Chromatography- Quantitative Analysis of Pharmaceutical Formulations, *CBS Publication and Distributors, New Delhi,* 5th Edition, 2001, 15, 101-102.
12. *International Conference on the Harmonization,* Draft guideline on Validation of analytical Procedure for Pharmaceutical Availability, Federal Register, 59(220), 1994, 9750.
13. *International Conference on the Harmonization*, Draft guideline on Validation of analytical Procedure for Pharmaceutical Availability, Federal Register, 60(40), 1995, 11260.
14. Kemp W. Organic Spectroscopy, *Mac Milan Press Ltd, Hampshire,* 3rd Edition, 1996, 1-7.

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