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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SOME DRUGS - A REVIEW ARTICLE

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ABSTRACT. High Performance Liquid Chromatography (HPLC) is a very important analytical tool for the assessments of drug product. HPLC techniques should be able to separate, identify and quantify various medicinal products and related detoriating materials that can form on storage or manufacturing or may be incorporated during the synthesis. Validation is the process of establishing the performance and limitations of any technique and identification of various products which may change their characteristics. This article discusses the strategies and the issues pertinent to modelling of HPLC method development and validation.
Key words *- HPLC, Impurities, Method development, Validation*

INTRODUCTION

Analytical chemistry is used to check the qualitative and quantitative composition of material during the research. Both of these criteria are very important to understand the sample materials. The division of analytical chemistry is mainly divided into two branches, quantitative and qualitative analytical chemistry. [1]

A qualitative analysis gives us the knowledge about the nature of sample by identifying the presence or absence of certain components. A quantitative analysis gives a numerical information as the relative amount of one or more of these components. For analyzing the drug samples in bulk, pharmaceutical formulations different analytical methods are used. [1]

In non – instrumental methods, the conventional and physiochemical properties are used to analyze the drug constituents. The instrumental methods are easy, accurate and reproducible as compared to classic methods. Thus, these analytical techniques can be develop using very basic instruments such as spectrophotometer, HPLC and HPTLC which have huge range of applications in maintaining the quality and quantity of raw products and finished products [2].

CHROMATOGRAPHY

Chromatography is the method used for bifurcation of various components of mixture by frequent distribution of the basic materials between two phases in which one phase moving phase (mobile phase) and the other phase (stationary phase) is a continuous one.

The stationary phase is adsorbed on a silica bed and the mixture of various volatile components are allowed to run through it. These volatile content is then uses to move the main Active material to move from its site.

As per USP, Chromatography can be defined as ***"any procedure through which solutes are differentiated by the gradual migration procedure in a system which is consisting of two or more phases, one of which move continuously in a given direction called as flow"***. [3]

Principles of Chromatography [4]

1. *Adsorption Chromatography* - If the stationary phase is particulate or solid and mobile phase is liquid or in gas phase, then it is called Adsorption Chromatography.
   Examples: TLC, Gas and Solid Chromatography.
2. *Partition Chromatography* - When both the phases are liquid, it is called partition chromatography.
   Example: Paper partition chromatography, GLC.

Theory of Chromatography [5]

Two main theoretical approaches are involved in the passage of the solutes through the chromatographic system.

1. *The Plate Theory* - Martin and Synge stated that, a chromatographic system consists of separated layers of virtual theoretical plates. At each of the layer, equilibrium of the solute between the mobile and stationary phases occurs and the movement of this solute is considered as a series of gradual transfers from this plate to plate.

2. *The Rate Theory* - This theory based on the mechanism of movement of solute particles present in the working system as it passes through the voided space between the stationary phase particles in the system as well its kinetic stationary phase.

Phases of Chromatography

1. ***Normal Phase Chromatography*** - In normal phase chromatography, stationary phase is polar and the moving phase is non polar in nature. In this method, the non-polar compounds move faster and are the ones to eluted first. This is due to lower affinity between the non-polar components and the stationary phase. Polar components are retained for longer duration because of their higher affinity with the stationary phase and these compounds. Therefore, it takes more time to elute first. Normal phase of separation is not generally used for pharmaceutical aid because most of the drugmolecules are polar in nature and hence take longer time to elute. [6]

2. ***Reverse Phase Chromatography*** - It is the most popular method for analytical separations of compounds in chemical, pharmaceutical, biologicals, food and biochemical. In this method, the stationary phase is of non-polar functional group bonded with silica gel and the mobile phase is with polar solvent. The polar compounds get eluted first in this mode and non-polar compounds are remained for longer duration.
   As many of the drugs and pharmaceuticals are polar in nature, they are not retained for longer duration. The different columns are used as Octa Decyl Silane or $C_{18,}$ C_8. An aqueous mobile phase gives use of secondary chemical methods like ionization control, ion suppressing, ion pairing to control retention and selectivity. [7]

3. ***Ion Exchange Chromatography*** - The stationary phase containing various ionic groups like NR^+, SO^- which can interact with ionic groups of the given samples. This are suitable for separation and the segregation of charged molecules only. Changing the pH, ions and salts can modify the retention. [6] [8]

4. ***Ion Pair Chromatography*** - This technique is also known as reverse phase soap chromatography. It can be used for the segregation of various ionic compounds and this method can also be used as a substitute for ion exchange chromatography. Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs with possible counter ions. [9]

5. ***Affinity Chromatography*** - This method uses highly specific biochemical reactions for separation of system. The stationary phase contains the particular groups of molecules which can absorb the samples if certain charges related conditions can get satisfied. Various macromolecules can get separated by using this method. This technique can be used to separate proteins, enzymes as well as various antibodies from complex components. [10]

6. ***Size Exclusion Chromatography*** - It separates components according to their molecular weights. Largest molecules are eluted first and the smallest ones at last. This method is generally used when the mixture contains components with a molecular mass difference of at least 11%. This method can be further divided into gel permeation chromatography and gel filtration chromatography. [11]

UV- Spectroscopy [12]

The UV–visible spectrophotometer can be used to measure the reflectance. The spectrophotometer measures the intensity of light reflected from a sample to be analyze And compare to the intensity of light reflected a reference sample and the ratio is called as *Reflectance* which is usually expressed in terms of percentage. The basic composition of the spectrophotometer are a light source, a sample holder, a grating source in a monochromator to separate the different wavelengths of light and lastly a detector. The radiation source is mostly a Tungsten filament or a deuterium arc lamp or light emitting diodes (LED) for the visible wavelengths.

The detector is typically photo multiplying tube, a photodiode array of charged device. Single photodiode detectors and photomultiplier tubes are used in combination with the scanning monochromator which filters the light so that light of a single wavelength only reaches to the detector at one time. The scanning monochromator moves the diffraction grating to each wavelength.

Method Development [13]

1. **Physicochemical properties of drug.**

Physicochemical properties of any drug molecule plays an essential role in method development. For one has to study the physical properties like solubility, pKa and pH of the drug molecule to decide the solvent and composition of moving phase. In a non-polar covalent bond, the electrons are divides same between two atoms. A polar covalent bond is identified on the basis of one atom which has a greater affinity towards the electrons than the other atoms. The solubility of any substance can be explained on the basis of the polarity of that drug molecules. [14]

Polar substances like water and non-polar like benzene. In general, like dissolves like, materials with similar polarity are soluble in each other. Selection of diluting substance is based on the solubility of the analyte. The analyte should be dissolve completely in the diluting agents. Thus, the pH of a solution indicates the concentration of hydrogen ions in solution. The concentration of hydrogen ions can be indicated as H^+ or its solvated form in as hydronium ions whose value normally lies between 0 and 14. [15]

The lower the pH, there is more acidity in the solution. The pH of a solution can be changed simply by adding acid or base to the solution for further adjustment. When the pKa and analytes concentration are already known, then the dissociation constant and the pH of the solution can be calculated easily. [16]

2. Set up HPLC conditions

A buffer is a partially neutralized acid which resists the changes in pH. Salts such as sodium citrate or sodium lactate are normally used as buffers.

Buffering Capacity is the ability of the buffers to resist changes in pH. Buffering capacity increases as the molar concentration of the buffer solution increases. The nearer the buffered pH is to the pKa, the greater the Buffering capacity of that compound. [17] Depending on the effect of pH, the type of buffer to use, and its attention, solubility in the organic modifier and its effect on discovery are important in reversed phase chromatography development of ionic analytes. An indecorous choice of buffer, in terms of softening species, ionic strength and pH, can affect in poor or irreproducible retention and trailing rear- phase separation of polar and ionizable composites. [18]

Buffer selection [19]

Selection of buffer is basically governed by the selective pH range. The predetermined pH range for reversed-phase on silica packing is 2 to 8.

It is important to choose a buffer with a pKa value less than 2 unit's pH of the desired mobile phase.

Table No. 01 - HPLC Buffers, pKa Values and Useful pH Range

Buffer	pKa	pH range
Ammonium acetate	4.8, 9.2	3.8 – 5.8, 8.2 – 10.2
Ammonium formate	3.8, 9.2	2.8 – 4.8, 8.2 – 10.2
Phosphoric acid	2.1	1.1 – 3.1
K_2PO_4	7.2	6.2 – 8.2
Potassium acetate	4.8	3.8 – 5.8
Borate	9.2	8.2 – 10.2
Ammonium hydroxide	9.2	8.2 – 10.2
Tri fluoroacetic acid	LT 2	1.5 – 2.5
Potassium formate	3.8	2.8 – 4.8

General considerations for buffer selection

Phosphate is more answerable in methanol/ water than in acetonitrile/ water or THF/ water. Some swab buffers are hygroscopic. This may lead to changes in the chromatography (increased trailing of introductory composites, and conceivably selectivity differences). Mariners are generally more answerable in organic/ water mobile phases. TFA can degrade with time, is unpredictable, absorbs at low UV wavelengths. Microbial growth can snappily do in softened mobile phases that contain little or no organic modifier. At pH lesser than 7, phosphate buffer accelerates the dissolution of silica and oppressively shortens the continuance of silica grounded HPLC columns. [20]

However, organic buffers should be used at pH lesser than 7 Ammonium bicarbonate buffers generally are prone to pH changes and are generally stable for only 24 to 48 hours, If possible. The pH of this mobile phase tends to come more introductory due to the release of carbon dioxide. After buffers are set, they should be filtered through a0.2- µm sludge. Mobile phases should be degassed. Buffer attention generally, a buffer attention of 10- 50 mm is acceptable for small motes. Generally, no further than 50 organic should be used with a buffer. This will depend on the specific buffer as well as its attention. Phosphoric acid and its sodium or potassium mariners are the most common buffer systems for reversed- phase HPLC. Phosphate buffers can be replaced with sulfate buffers when assaying organophosphate composites. [20]

Selection of sensor - Sensor is a veritably important part of HPLC. Selection of sensor depends on the chemical nature of analytes, implicit hindrance, limit of discovery needed, vacuity and/ or cost of sensor. UV-Visible sensor is protean, binary- wavelength absorbance sensor for HPLC. This sensor offers the high perceptivity needed for routine UV- grounded operations to low- position contamination identification and quantitative analysis. Photodiode Array (PDA) Sensor offers advanced optic discovery for waters logical HPLC results. It's intertwined software and optics inventions deliver high chromatographic and spectral perceptivity. Refractive indicator (RI) Sensor offers high perceptivity, stability and reproducibility, which make this sensor the ideal result for analysis of factors with limited or no UV immersion. [21]

Column selection - The main component of a HPLC system is the column. Changing a column will have the topmost effect on the resolution of analytes during system development. Generally, ultramodern rear phase HPLC columns are made by packing the column casing with globular silica gel globules which are carpeted with the hydrophobic stationary phase. The stationary phase is introduced to the matrix by replied a chlorosilane with the hydroxyl groups present on the silica gel face. The nature of stationary phase has the topmost effect on capacity factor, selectivity, effectiveness and elution. There are several types of matrices for support of the stationary phase will dissolve above pH 7. [22]

In recent times, silica supported columns have been developed for use at high pH. The nature, shape and flyspeck size of the silica support goods separation. Lower flyspeck results in a lesser number of theoretical plates, or increased separation effectiveness.

Still, the use of lower patches also results in increased backpressure during chromatography and the column more fluently becomes plugged. In rear phase chromatography the stationary phase is non-polar and the mobile phase is polar, causing polar peaks to generally elute earlier thannon-polar peaks. To produce a stationary phase for rear phase chromatography on silica support, the free silanols are replied with a chlorosilane with hydrophobic functionality to introduce thenon-polar face. Due to steric constraints, only about1/3 of the face silanols are derivatized. The remaining free silanols can interact with analytes, causing peak trailing. [22]

Generally, after the derivatization of a column with the asked stationary phase, the column is farther replied with chloro trimethyl silane to end cap the remaining free silanols and ameliorate the column effectiveness.

Common stationary phases are C4 (butyl), C8 (octyl), C18 (octadecyl), nitrile (cyanopropyl), phenyl columns. In general, longer alkyl chains, advanced phase lading, and advanced carbon loads give lesser retention of polar analytes.

These columns are generally less stable to hydrolysis than columns with longer alkyl chain. Octyl (C8) columns have wide connection. This phase is less forgetful than the C18 phases, but is still relatively useful for medicinal. Illustration include (Zorbax SB- C8, Luna C8 and YMC- Pack- MOS). Octadecyl (C18, ODS) columns are the most extensively used and tend to be the most forgetful fornon-polar analytes. Exemplifications include Zorbax SB- C18, YMC- Pack ODS and Luna C18. Xterra RP- C18 and Zorbax Extend- C18 columns have been formulated to tolerate high pH systems (pH> 7, typically over to pH 11). [23]

Varying the pH can affect selectivity and resolution of polar analytes, especially for ionizable composites. Phenyl columns offer unique selectivity from the alkyl phases and are generally less forgetful than C8 or C18 phases. Phenyl columns are generally used to resolve sweet composites. Exemplifications include Zorbax SB-Phenyl, YMC- Pack Phenyl and Luna Phenyl- Hexyl. Nitrile (CN or cyano) columns are polar and can be used for both rear and normal phase operations. This phase is frequently used to increase retention of polar analytes. The type of column chosen for a particular separation depends on the emulsion and the end of analysis. [23]

Column temperature [22]

Column temperature control is important for long- term system reproducibility as temperature can affect selectivity. A target temperature in the range of 30- 40 °C is typically sufficient for good reproducibility. Use of elevated temperature can be profitable for several reasons. First, operating at a temperature advanced than ambient reduces the density of the mobile phase and therefore the overall backpressure on the column. Lower system pressures allow for faster inflow rates and therefore briskly analyses.

The temperature may also affect selectivity patterns because analytes will respond diversely to different temperatures. Eventually, use of a column roaster eliminates variability due to normal oscillations in the air temperature girding the column. While temperature is a variable that can affect selectivity 'α' its effect is fairly small. Also, the k' generally decreases with an increase in temperature for neutral composites but lower dramatically for incompletely ionized analytes. Some effect when there's a significant difference in shape and size.

Overall, it's better to use solvent strength to control selectivity than to use temperature; its effect is much more dramatic. An increase of 1 °C will drop the k' by 1 to 2, a both ionic and neutral samples are reported to show significant changes in a with temperature changes. Possible temperature oscillations during system development and confirmation, it's recommended that the column be thermostat to control the temperature.

Mobile phase - (Detergent type) Solvent type (methanol, acetonitrile, and tetrahydrofuran) will affect selectivity. The choice between methanol and acetonitrile may be dependent on the solubility of the analyte as well as the buffer used. Tetrahydrofuran is least polar among these three detergents, frequently responsible for large changes in selectivity and is also inharmonious with the low- wavelength discovery needed for utmost pharmaceutical composites.

The mobile phase goods resolution, selectivity and effectiveness. In rear phase chromatography, the mobile phase consists of a waterless buffer and anon-UV active water miscible organic detergent. The effect of the organic and waterless phase and the proportions in which they're mixed will affect the analysis of the medicine patch. Selection of the mobile- phase and grade conditions is dependent on the ionogenic nature of the analyte and the hydrophobicity of the analytes in the admixture independently. The waterless buffer serves several purposes.

At low pH, the mobile phase protonates free silanols on the column and reduces peak trailing. At sufficiently low pH introductory analytes are protonated when ionized the analyte will elute more snappily but with bettered peak shape. Acidic analytes in buffers of sufficiently low pH will remain unchanged, adding retention. Again, at advanced pH neutral introductory composites will be more retained, and ionized acidic composites will elute before.

Peak splitting may be observed if the pKa of an emulsion is analogous to the pKa of the buffer, and the analyte elutes as both a charged and uncharged species. The pH of a buffer won't greatly affect the retention of non-ionizable sample factors. Generally, a 10- 50 mm result of a waterless buffer is used. The most generally used waterless phase is H3PO4 in water i.e., phosphate buffer. [23]

pH - The pH of a phosphate buffer is fluently acclimated by using mono, di or tribasic phosphate mariner's pH of Mobile phase when the sample is eluted with a mobile phase with 100 organic there's no separation, as the sample is eluted in the void volume. This is because the sample isn't retained; but retention is observed when the mobile phase detergent strength is dropped to allow equilibrium competition of the solute motes between the clicked phase and the mobile phase.

When the separation is complex, that is, numerous factors are to be separated, and when the solvent strength is dropped and there's still no resolution between two close peaks, another organic detergent of a different opposition or indeed an admixture of two organics may need to be tried to prompt separation. Mobile phase optimization can be enhanced in combination with clicked phase optimization (i.e., substituting C18/ C8 with cyano or phenyl). A thing for the band distance of a solute (K ') should be in the range of 4 to 9 and a run time of about 15 twinkles or 20 twinkles at most for utmost routine product release or stability runs. [23]

Separation Techniques [24]

1. Isocratic separations

Isocratic, constant eluent composition means equilibrium conditions in the column and the actual composites moving through the column are constant; analyte- eluent and analyte-stationary- phase relations are also constant throughout the whole run. This makes isocratic separations more predictable, although the separation power (the number of composites which could be resolved) isn't veritably high. The peak capacity is low and longer the element is retained on the column, the wider is attendant peak.

2. Gradient separation

Grade separation significantly increases the separation power of a system substantially because of the dramatic increase of the apparent effectiveness (drop of the peak range). The condition where the end of the chromatographic zone is always under the influence of a stronger eluents that leads to the drop of the peak range. Peak range varies depending on the rate of the eluent composition variation (grade pitch).

Changing grade- grade elution is employed for complex multicomponent samples since it may not be possible to get all factors eluted between k 1 and 10 using a single detergent strength under isocratic conditions. This leads to the general elution problem where no bone set of conditions is effective in eluting all factors from a column in a reasonable time period while still attaining resolution of each element. This perpetration of a grade allows for carrying differences in the chromatographic selectivity. This is attributed to the different pitches of the retention versus organic composition for each analyte in the admixture.

When a grade system is used, the column must be allowed to disequilibrate at the starting mobile phase conditions previous to the coming sample injection and the launch of the coming grade run. Selection of isocratic or grade mode depends on the number of active factors to be resolved or separated. In deciding whether a grade would be needed or whether isocratic mode would be acceptable, an original grade run is performed, and the rate between the total grade time and the difference in grade time between the first and last factors are calculated. The advised rate is 0.25, grade would be salutary.

3. Sample Preparation

The medicine substance being anatomized should be stable in result (diluent). During original system development, medications of the results in amber steins should be performed until it's determined that the active element is stable at room temperature and doesn't degrade under normal laboratory conditions. The sample result should be filtered; the use of a0.22 or0.45 µm severance- size sludge is generally recommended for junking of particulates.

Filtration is a preventative conservation tool for HPLC analysis. Sample medication is a critical step of system development that the critic must probe. The effectiveness of the hyper pollutants is largely determined by their capability to remove pollutants undoable factors without filtering undesirable vestiges into the filtrate. However, also the diluents must be filtered to determine if a leachable element is coming from the hyper sludge, if any fresh peaks are observed in the filtered samples.

4. Method Optimization

The experimental conditions should be optimized to get asked separations and perceptivity after getting applicable separations. Stability indicating assay experimental conditions will be achieved through planned systemic examination on parameters including pH(if ionic), mobile phase factors and rate, grade, inflow rate, temperature, sample quantities, Injection volume and diluents solvent type.

5. Method Validation

Logical procedure is the process by which it's established, by laboratory studies, that the performance characteristics of the procedure meet the conditions for its intended use. The styles evidence process for logical procedures begins with the planned and regular collection by the applicant of the evidence data to support logical procedures. All logical styles that are intended to be used for assaying any clinical samples will need to be validated. The evidence of logical styles is done as per ICH guidelines.

Validation parameters [25]

The following are typical analytical performance characteristics which may be tested during methods validation,

1. Accuracy is the nearness of a measured value to the true or accepted value. Delicacy indicates the divagation between the mean value set up and the true value. It's determined by applying the system to samples to which known quantities of analyte have been added. These should be anatomized against standard and blank results to insure that no hindrance exists. The delicacy is also calculated from the test results as a chance of the analyte recovered by the assay. It may be expressed as the recovery by the assay of known, added quantities of analyte.

2. Precision is an analytical method in which the degree of identification among individual test results obtained when the method is applied to various aliquots sampling of a same sample. Precision is the measure of the repeated results of the whole analytical method. It consists of two further components: repeatability and intermediate precision.

3. Repeatability is the variation experienced by a single analyston a single instrument. It does not distinguish between variations from the instrument or system alone and from the sample preparation process. During validation, it is performed by analyzing multiple replicates of an assay composite sample by using the analytical method. The recovery value is calculated. Intermediate precision is the variation within a laboratory such as different days, with different instruments, and by different analysts.

4. Linearity is the ability of analytical procedure to get a response that is directly proportional to the concentration of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the limit around the slope of the regression line.

5. Detection Limit or limit of discovery (LOD) of an individual procedure is the smallest quantum of analyte in a sample that can be detected but not inescapably quantitated as an n exact value. In logical procedures that parade birth noise, the LOD can be grounded on a signal- to- noise(S/ N) rate (31), which is generally expressed as the attention of analyte in the sample. The signal- to- noise rate is determined by s = H/ h Where H = height of the peak corresponding to the element. h = absolute value of the largest noise change from the graph of the chromatogram of a blank result.

6. Particularity is the capability to assess unequivocally the analyte in the presence of factors that may be anticipated to be present similar as contaminations, declination products, and excipients. Particularity measures only the asked element without hindrance from other species that might be present; separation isn't inescapably needed.

7. Range is defined as the interval between the upper and lower attention of analyte in the sample for which it has been demonstrated that the logical procedure ash a suitable position of perfection, delicacy, and linearity.

8. Robustness is defined as the measure of the capability of a system to remain by small but deliberate variations in system parameters and provides a suggestion of its trustability during normal operation. Determination of robustness is a methodical process of varying a parameter and measuring the accuracy.

9. System Suitability Parameters System Suitability Parameters is the evaluation of the factors of a logical system to show that the performance of a system meets the norms needed by a system. These parameters can be calculated experimentally to give a quantitative system felicity test report number of theoretical plates, capacity factor, separation, resolution, trailing factor, relative standard divagation. These are measured on a peak or peaks of known retention time and peak range. System felicity parameters studied are as follows,

Capacity factor (K') - Capacity factor is defined as the ratio of the number ofmolecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column or TLC plate during an isocratic separation. The ideal value of k' ranges from 2-10.

Column efficiency (N) - It is the count of band spreading of the peak. If band spread is smaller, higher is no. of theoretical plates, indicates good column efficiency and system performance. Columns with N ranging from 5,000 to 100000 Plates/m are ideal for good system.

Advantages of Analytical Method Validation [25]

1. It builds a degree of confidence, not only for the inventor but also to the stoner.
2. Although the confirmation exercise may appear expensive and time consuming, it proves to be affordable by barring reiterations and leads to better time operation in the end.
3. The system confirmation absorbs the shock of variations of logical conditions and pays for further than invested on the process.

REFERENCES

1. Shethi PD. Quantitative Analysis of Pharmaceutical Formulations, 1st Edition 2001
2. United States Pharmacopoeia and National Formulary, (24th) Asian Edition, The United States Pharmacopoeia Convention Inc. U.S.A. 2126.
3. Sethi PD, HPLC: Quantitative Analysis of Pharmaceutical Formulation, New Delhi, CBS Publishers and Distributors, 1996; 113-202.
4. Sankar SR, Text book of Pharmaceutical Analysis. 5th Edition 2006. Rx publications, Tirunelveli. 2006; 13-1,2.
5. Jeffery GH, Bassett J, Mendham J, Denny RC, Vogel's Textbook of Quantitative Chemical Analysis, fifth edition, Longman scientific &technical.
6. Kaushal C, Srivastava B, A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, 2010; 2(2): 519-545.
7. Buffers and pH Buffers. Accessed April 05, 2013.
8. Understanding pH Buffers: which one to use, and at what concentration. April 05, 2013.
9. Technical Tips: Selecting Buffers pH in Reversed-phase HPLC. April 05,2013.
10. Reversed-phase HPLC Buffers: High Quality Buffers HPLC. April 05, 2013.
11. Effective HPLC method development. April 05, 2013.
12. Changhe Wen, Designing HPLC Methods for Stability Indication and Forced Degradation Samples for API, American Pharmaceutical Review. April 05, 2013.
13. Chandra M., Buffers: A guide for preparation and use of buffers in biological system. April 05, 2013.
14. Lindholm J, Development and Validation of HPLC Method for Analytical and Preparative Purpose, Acta Universities Upsaliensis Uppsala, 2004; 13-14.
15. Snyder LR, Practical HPLC method development. 2nd edition. 1997; 233-291.
16. C.T. Rhodes, Columns. April 05, 2013.
17. Wagaw S, Tedrow J, Grieme T, Bavda L, Wang W, Viswanath S et al. HPLC Guide; Departments R450, R452, R45R.
18. Weston A, Brown PR, HPLC and CE Principles and Practice, Academic press, California, 1997.
19. Donald D. Hong, Development and validation of HPLC Stability-indicating Assays, Drug Stability-Principle & Practice. 3rd Edition. New York: Marcel Dekker. 2008;
20. FDA Guidance for Industry: Analytical procedures and methods validation. Aug2000.
21. Ngwa G, Forced Degradation Studies. Forced degradation as an Integral part of HPLC Stability Indicating Method Development Drug Delivery Technology. 2010; 10(5).
22. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, October 1994, Geneva.
23. ICH, Q2B, Validation of Analytical Procedures, Methodology, International Conference on Harmonization, November.1996**;** Geneva
24. ICH, Stability testing of new drug substances and products (QIAR) international conference on harmonization IFPMA, 2000; Geneva.
25. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Mott MG. Available Guidance and Best Practices for Conducting Forced Degradation Studies. Pharmaceutical Technology, 2002; 48-56.