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Stability Indicating HPLC Method Development - A Review

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ABSTRACT

High performance liquid chromatography is most powerful tools in analytical chemistry which assessing drug product stability. It is most accurate method for determining the qualitative and quantitative analysis of drug product. Forced degradation plays an important role in development of stability indicating analytical methodology. Stability indicating HPLC methods are used to separate various drug related impurities that are formed during the synthesis or manufacture of drug product. This article discusses the strategies and issues regarding the development of stability indicating HPLC system for drug substance. Forced degradation studies establish degradation pathways of drug substances and drug products. Forced degradation elucidate the possible degradation pathway of the drug substance or the active pharmaceutical ingredient in the drug product. At every stage of drug development practical recommendations are provided which will help to avoid failure.

KEYWORDS: *HPLC, forced degradation, Stability indicating study*

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INTRODUCTION

Stability indicating method is validated quantitative analytical procedure that can detect properties of drug product and substances. Stability testing of drug gives accurate analytical method that quantitatives the Active pharamaceutical ingredient without interference from process impurities, degradation products and potential impurities[(3)]. High performance liquid chromatography is analytical method used for determining drug product stability[(1)].The forced degradation study under a variety of condition, like pH, dry heat, oxidation, light etc.and separation from decomposed product[(4)]. Classical methods deal with the analyses which were carried out by separating the components of interest (the analytes) in a sample by precipitation, extraction or distillation. The separated components were then treated with different types of reagents for qualitative analysis based on the nature of analytes that yielded product that could be identified by their colors, odors, boiling and melting points, solubility study, optical activities or refractive indexes[(2)]. Gravimetric or titrimetric measurements were used to quantify the analytes present in the supplied sample. In titrimetric analysis, the volume of standard reagent required to react completely with the analyte was measured. HPLC has many applications in the environmental, pharmaceutical, forensic, clinical and in food and flavor analysis[(1,5)].

Forced decomposition study may help to facilitate pharmaceutical development as well in manufacturing development, formulation and packaging in which knowledge of chemical behavior can be used to better product.

CHROMATOGRAPHY [(2, 28)]

The term 'Chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.

MODES OF CHROMATOGRAPHY [(1, 29)]

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces are based on the size of the particles (e.g. Size exclusion chromatography)

Different modes of chromatography are as follows -

- Normal Phase Chromatography
- Reverse Phase Chromatography
- Reverse Phase – ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography
- Size Exclusion Chromatography

Reverse Phase Chromatography [29]

Methods can be chosen based on solubility and molecular mass. In most of the cases for non-ionic small molecules (μ < 2000), reversed phase methods are suitable.

In 1960's chromatographers started modifying the polar nature of silanol group by chemically reacting silica with organic silanes. The objective was to make less polar or non-polar so that polar solvents can be used to separate water-soluble polar compounds.

A large number of chemically bonded stationary phases based on silica are available commercially. Silica based stationary phases are still most popular in reversed phase chromatography however other absorbents based on polymer (styrene-divinyl benzene copolymer) are slowly gaining ground.

Simple compounds are better retained by the reversed phase surface, the less water- soluble (i.e. the more non-polar) they are. The retention decreases in the following order: aliphatic > induced dipoles (i.e. CCl_4) > permanent dipoles (e.g.$CHCl_3$) > weak lewis bases (ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong Lewis acids (carboxylic acids). Also the retention increases as the number of carbon atoms increases.

In reverse phase systems the strong attractive forces between water molecules arising from the 3-dimentional inter molecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Non- polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phase.

Chemically bonded octadecyl silane (ODS) an alkaline with 18 carbon atoms, it is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations use ODS-HPLC columns. The solvent strength in reverse phase chromatography is reversed from that of adsorption chromatography (silica gel) as stated earlier. Water interacts strongly highly with silanol groups, so that, adsorption of sample molecules become highly restricted and they are rapidly eluted as a result. Exactly opposite applies in reverse phase system; water cannot wet the non-polar (hydrophobic) alkyl groups such as C_{18} of ODS phase and therefore does not interact with the bonded moiety. Hence water is the weakest solvent of all and gives slowest elution rate. The elution time (retention time) in reverse phase chromatography increases with increasing amount of water in the mobile phase.

Adsorption Chromatography /Normal Phase Chromatography [28]

In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents.

The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase. This forms a weak type of bond with any molecule in the vicinity when any of the following interactions are present.

- Dipole-induced dipole,
- Dipole-dipole,
- Hydrogen bonding,
- π-Complex bonding

These situations arise when the molecule has one or several atoms with lone pair electron or a double bond. The absorption strengths and hence k' values (elution series) increase in the following order. Saturated hydrocarbon < olefins < aromatics < organic halogen compounds < sulphides < ethers< esters < aldehydes and ketones < amines < sulphones < amides < carboxylic acids. The strength of interactions depends not only on the functional groups in the sample molecule but also on steric factors. If a molecule has several functional groups, then the most polar one determines the reaction properties. Chemically modified silica, such as the amino propyl, cyan propyl and diol phases is useful alternatives to silica gel as stationary phase in normal phase chromatography.

The amino propyl and cyan propyl phases provide opportunities for specific interactions between analyse and the stationary phases and thus offer additional options for the optimisations of separations. Other advantages of bonded phases lie in their increased homogeneity of the phase surface. Resolution with water in weak mobile phase may be most conveniently achieved by drying the solvents

and then adding a constant concentration of water or some very polar modifier such as acetic acid or triethylamine (TEA) to the mobile phase. The addition of such polar modifiers serves to deactivate the more polar shape as well as the reproducibility of the retention times.

HPLC SYSTEM [18-23,28,29]

The components of a basic High Performance Liquid chromatograph (HPLC) system are shown in the simple diagram no 2.

The importance of Chromatography is increasing rapidly in pharmaceutical analysis. The exact differentiation selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and intermediates (detection of decomposition products and by-products). As a consequence of the above points, chromatographic methods are occupying an ever-expanding position in the latest editions of the pharmacopoeias and other testing standards.

The modern form of column chromatography has been called high performance, high pressure, and high-resolution and high-speed liquid chromatography.

High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

The essential equipment consists of an eluent, reservoir, a high-pressure pump, and an injector for introducing the sample, a column containing the stationary phase, a detector and recorder. The development of highly efficient micro particulate bonded phases has increased the versatility of the technique and has greatly improved the analysis of multi component mixtures.

The systems used are often described as belonging to one of four mechanistic types, adsorption, partition, ion exchange and size-exclusion. Adsorption chromatography arises from interaction between solutes on the surface of the solid stationary phase. Partition chromatography involves a liquid stationary phase, which is immiscible with the eluent and coated on an inert support. Adsorption and partition systems can be normal phase (stationary phase more polar than eluent) or reversed phase (stationary phase less polar than eluent). Ion-exchange chromatography involves a solid stationary phase with anionic or cationic groups on the surface to which solute molecules of opposite charge are attracted. Size-exclusion chromatography involves a solid stationary phase with controlled pore size. Solutes are separated according to their molecular size, the large molecules enable to enter the pores eluting first.

System Components

Solvent delivery system

The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing, there is a high-pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For normal phase separations eluting power increases with increasing polarity of the solvent but for reversed phase separations, eluting power decreases with increasing solvent polarity. Optimum separating conditions can be achieved by making use of mixture of two 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 important component of HPLC in solvent delivery system is 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 phase should be degassed and filtered before use. Several methods are employed to remove the dissolved gases in the mobile phase. They include heating and stirring, vacuum degassing with an aspirator, filtration through 0.45 filter, vacuum degassing with an air-soluble membrane, helium purging ultra-sonication 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 may be run isocratic ally, i.e., with constant eluent or they may be run in the gradient elution mode in which the mobile phase composition varies during 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 100 samples can be loaded in to the auto injector tray. 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.

Liquid chromatographic detectors

The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. Generally, there are two types of HPLC detectors, bulk property detectors and solute 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 refractive index, conductivity and dielectric constant detectors.

Solute property detectors

Solute property detectors respond to a physical property of the solute, which is not exhibited by the pure mobile phase. These detectors measure 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 which do not require the removal of the mobile phase before detection include spectrophotometric (UV or UV-Vis) detector, fluorescence detectors, polar graphic, electro-chemical and radio activity detectors, whilst the moving wire flame ionization detector and electron capture detector both require removal of the mobile phase before detection.

UV-Vis 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 achieve high efficiency of separation, the column material (micro-particles, 5-10 µm size) packed in such a way that highest numbers of theoretical plates are possibleSilica (SiO_2 x H_2O) 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 800 m^2/g. and particle sizes from 3 to 50 µm. The silanol groups on the surface of silica give it a polar character, which is exploited in adsorption chromatography using non-polar organic eluants. Silica can be drastically altered by reaction with Organochloro-silanes or Organoalkoxy silanes giving Si-O-Si-R linkages with the surface. The attachment of hydrocarbon change to silica produces a non-polar surface suitable for reversed phase chromatography where mixtures of water and organic solvents are used as eluants.

The most popular material is octadecyl-silica (ODS-Silica), which contains C_{18} chains, but materials with C_2, C_6, C_8 and C_{22} chains are also available. During manufacture, such materials may be reacted with a small mono functional silane (e.g. trimethylchloro silane) to reduce further the number of silanol groups 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 ion exchangers are also available in which sulphonic acid groups or quaternary ammonium groups are bonded to silica. The useful pH range for columns is 2 to 8, since siloxane linkages are cleaved below pH-2 while at pH-8 or values above silica may dissolve. In HPLC, generally two types of columns are used, normal phase columns and reverse phase columns. Using normal phase chromatography, 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 adsorption on a stationary silica phase. However, it now seems that partition plays an important role, with the compounds interacting with the polar silanol groups on the silica or with bound water molecules.

While normal phase seems the passage of a relatively non-polar mobile phase over a polar stationary phase, reversed phase chromatography is carried out using a polar mobile phase such as methanol, acetonitrile, water, buffers etc., over a non-polar stationary phase. Ranges of stationary phases (C_{18}, C_8, -NH_2, -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.

Derivatization

In HPLC derivatization is used to enhance the sensitivity and selectivity of detection when available detectors are not satisfactory for the underivatized compounds. Both ultra violet absorbing and fluorescence derivatives have been widely used. Ultra

violet derivatization reagents include N-succinimidyl p-nitro phenyl acetate, phenyl hydrazine and 3, 5-dinitro benzyl chlorides, while fluorescent derivatives can be formed with reagents such as dansyl chloride, 4-bromo methyl-7-methoxy-coumarin and fluorescamine. Derivative formation can be carried out before the sample is injected on to the column or by online chemical reactions between the column outlet and the detector.

Stability Indicating Method Development Strategies:

There is no "one set fits all" formula for developing stability indicating analytical method. Before beginning with actual experimentation it would be advantageous to view method development from a broader perspective. The critical component of HPLC methods are : HPLC analysis, sample preparation and standardization (calculations).[2] The method development are high level process map perspective better to define the general steps to achieved end products, stability-indicating method.[1] In method development physiochemical properties of drug are most important. Fluoroscent properties of chromatographic behavior spectrophotometric properties

Of Reduction, oxidation are useful in preliminary experiment and helpful in degradation mechanism and stress study condition. Structure of analytes or functional group indicates active site of degradation.[4] Preliminary experimental condition prepared from unofficial and official methods and literature review. The preliminary condition of HPLC are Experimental should be based on API and properties of drug substance. Proper column and mobile phase selection in HPLC condition.

Steps of stability indicating method development strategies:(1,4)

- physiochemical properties of drug
- Set up preliminary HPLC conditions
- Preparation of sample required for method development
- Developing separation stability indicating chromatographic separation.

Important Parameters of Method Development

- Solvent type
- Mobile phase
- Isocratic and gradient mode
- Column temperature
- Peak purity

Solvent type

Solvent type (methanol, acetonitrile, tetrahydrofuran) will affect selectivity. The choice between acetonitrile and methanol dependent on buffer and solubility of analyte. Tetrahydrofuran less polar than methanol, acetonitrile, often large changes in selectivity and also low wavelength detection[(1,11,12)].

Mobile phase

There is no separation, when sample is eluted with mobile phase and sample is eluted with void volume. This is because sample is not retained; but retention is observed when mobile phase strength is decreases. Optimization of mobile phase can be increased in combination with increased in optimization. The bond spacing of solute of goal should be range of 4 to 9 and a run time of about 15 minute to 20 minutes at stability runs and most routine product release[(1,11,2)].

Isocratic and Gradient mode

Isocratic and gradient mode selection depend upon number of active component to be separated and resolved. In deciding whether isocratic mode would be required or whether gradient mode would be adequate, an initial gradient run is performed. The ratio between total gradient time and the difference in gradient time between first and last component are calculated. The calculated ratio is <0.25 isocratic is adequate, when the ratio is >0.25 gradient would be beneficial. Generally gradient mode for stability assessment and isocratic mode is used for product release because the isocratic mode less than 15 minute and decomposition product would be monitered[(1,2,3)].

Column temperature and Role of column

Column is the heart of HPLC system. During method development the resolution of analytes will have greatest effect on changing column. The stationary phase, the matrix and the hardware are three main components of HPLC column. The column of modern reverse phase HPLC are made by packaging the column using the spherical silica gel bed which are coated with hydrophobic stationary phase. The hydroxyl group present on silica gel surface by reacting a chlorosilicane which introduced in stationary phase. The nature of stationary phase has greatest effect on capacity factor, efficiency, elution and selectivity. Silica, polymers, alumina and zirconium are several types of matrices for support of stationary phase. In HPLC column silica is most common matrix. Silica matrices are robust, easily derivative, manufactured to consistent sphere size. Smaller particles are also results in increased in backpressured during chromatography and column are easily become plugged. For this reason, 3 A^0 column less frequently used than 5 A^0 in development work. The silica particle also results in better resolution with narrower particle size distribution[(2)]. The nature of stationary phase will be determining whether a column can be used reversed phase or

normal phase. Normal phase chromatography determines non-polar mobile phase and polar stationary phase. Generally, more polar compound elutes later than non-polar compound. Types of column include for normal phase chromatography include derivative silica, nitrile, amino, nitro and glycerol column. Normal phase conditions chiral separation is usually performed. The column life should be extending to use of highly polar and ionic compound are retained normal phase column, guard column and silica gel sample. In reverse phase chromatography stationary phase is non-polar and mobile phase is polar. Temperature control column is long term method reproducibility as temperature can affect selectivity. Good reproducibility temperature in the range of 30^{0}-40^{0}. Advantages of elevated temperature for several reason. First, a temperature is higher than ambient reduces the viscosity of mobile phase thus the overall backpressure on column. Lower system process allows for faster flow rates and faster analyses. Temperature also effect the selectivity pattern[1,2,15,16,13]

Peak purity

An essential requisite of a separation analysis is the ability to verify the purity of the separated species, that is, to ensure that no co eluting or co migrating impurity contributes to the peak response. Peak purity (or peak homogeneity) analysis of the main peak, to assess for the presence of impurities under the main peak, is an essential part of the validation of a SIM. Direct evaluation can be performed in-line by employing PDA detection, LC-MS or LC-NMR. Indirect evaluation of peak purity can be accomplished by changing one or more chromatographic parameters (column, mobile phase, gradient composition, etc.) that will significantly impact the separation selectivity. The resulting impurity profile is then compared against that of the original method. If the number of degradant peaks is the same in both separations, and if the percent of the main component is the same in both separations, then there can be reasonable confidence that all the degradants have been resolved from the main component. Automated versions of this approach have been successfully utilized in a multi-dimensional screening with instrumentation capable of systematically evaluating several different columns and eluents for impurity analysis[3,20,18,22]

Method Optimization

The experimental conditions should be optimized to get desired sensitivity and separation after getting proper seperations. The development of mobile phase parameters is always considered first as this is much convenient and easier than stationary phase development. Primary control factors in the development of liquid chromatography (LC) methods are the different constituents of the mobile phase determining acidity, gradient, flow rate, solvent strength, temperature, injection volume, sample amount, and diluents solvent type. This is used to find the desired balance between analysis time and resolution after satisfactory selectivity has been achieved. The parameters involved include column package particle size, flow rate and column dimension. These parameters may be changed without affecting capacity factors or selectivity[3,19].

Forced Degradation Studies in Stability-Indicating Method Development

Stability indicating method must be able to observed a change in the physical, chemical, and microbiological properties of drug product over time. The ability of the method to observed a change in the chemical properties of the drug over time, regularly calls for a stress testing study to be done on the drug product and drug substance. Forced degradation on the drug product and substance will also provide the following information:

1. Determination of degradation pathways of drug substances and drug products;
2. Discernment of degradation products in preparation that are related to drug substances versus those that are related to excipient;
3. Structure interpretation of degradation products;
4. Determination of the intrinsic stability of a drug substance molecule in solution and solid state; and
5. Reveal the hydrolytic, oxidative, photolytic, and thermolytics degradation mechanism of the drug product and drug substance[1,2])
6. To creat stability indicating nature of the developed method.
7. To know the chemical properties of drug particle.
8. To developed more stable formulations
9. To solve stability related problems[3,24,21,25])

According and FDA and ICH guidance document, three main purpose of forced degradation study: The drug product and drug substance which provide stability assessment; to interprete degradation pathway of the active pharmaceutical drug product and drug substance; and to examine the stability indicating the power of analytical procedure which applied on drug substance and drug product.[1] Forced degradation plays important part not just in the development of stability indicating methods, but also

in providing effective information about the degradation products and degradation pathways that could during storage.[2] *Q1A (R2) – Stability Testing of New Drug Substances and Products*, states: "Stress testing is likely to be carried out on a single batch of the drug substance. The testing should contains effect of temperature (in 10°C increments (i.e., 50°C, 60°C) above that for accelerated testing), humidity (i.e., 75% relative humidity or greater) where appropriate photolysis and oxidation of drug substance[2,26,27].

Appropriate timing

The stability testing of the drug substance should assess stress studies in different pH solutions in the presence of oxygen and light and at humadity level and elevated temperature.

These studies are most effective if development in phase 1 preclinical trial or preclinical development. A forced degradation study on drug substance at this stage will provide timely guidance for development in manufacturing process, proper selection of stability-indicating analytical techniques and ensure there is enough time for degradation pathway interpretation, degradation pathway identification and development of stress condition.

Experimental Design

In designing forced degradation studies, it must be remembered that more tough condition than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used.

The forced degradation condition should be followed-

1. Acid and base hydrolysis,
2. Hydrolysis at various ph,
3. Thermal degradation,
4. Photolysis, and
5. Oxidation.[2,27]

Initially the experiment should be focused on determining the condition that degrade the drug approximately 10%. The condition should be summarized below in table 2.

CONCLUSION

Stability-indicating method is an analytical procedure which is used to check purity of drug sample or drug substance. Stability-indicating method is an analytical procedure that is capable of discriminating between the major active (intact) pharmaceutical ingredients (API) from any degradation (decomposition) product(s) formed under defined storage conditions during stability evaluation period. Forced degradation studies are indespensible in development of stability indicating and degradant monitoring method as part of validation protocol. Chromatographic factor should be evaluated to optimized of the stability-indicating of HPLC method for detection for all potentially relevant degradant. An appropriate sample solvent and mobile phase must be found that afford suitability and compatibility with the component of interest as well as impurities and degradants.

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FIGURES:

Figure 1- HPLC system

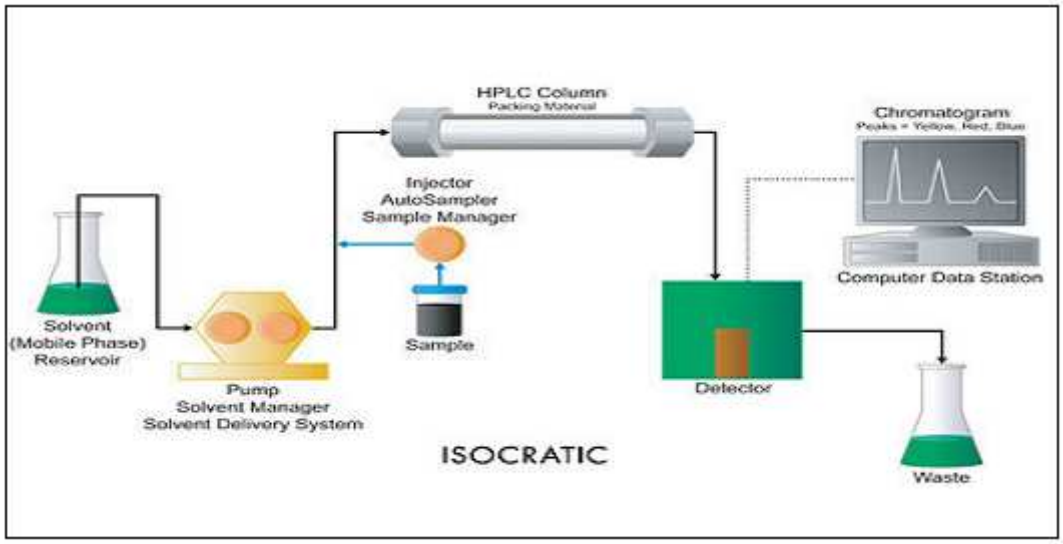

Figure-2 Instrumentation of HPLC system

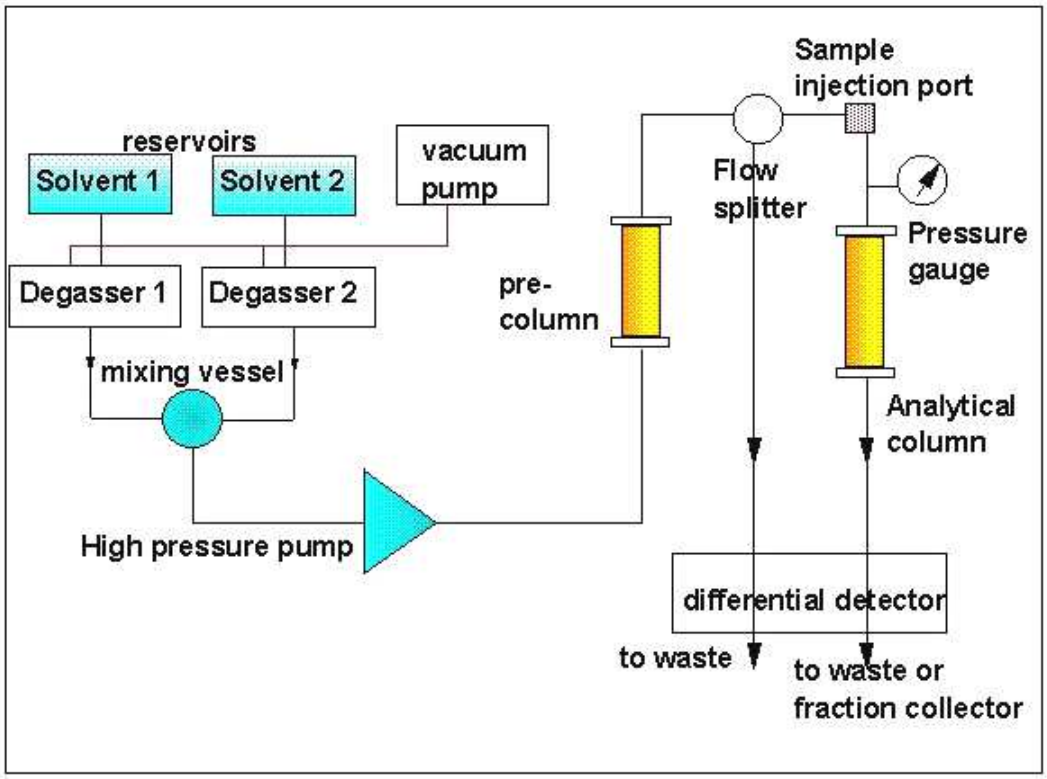

TABLE:

Table 1 Classification of Chromatographic methods

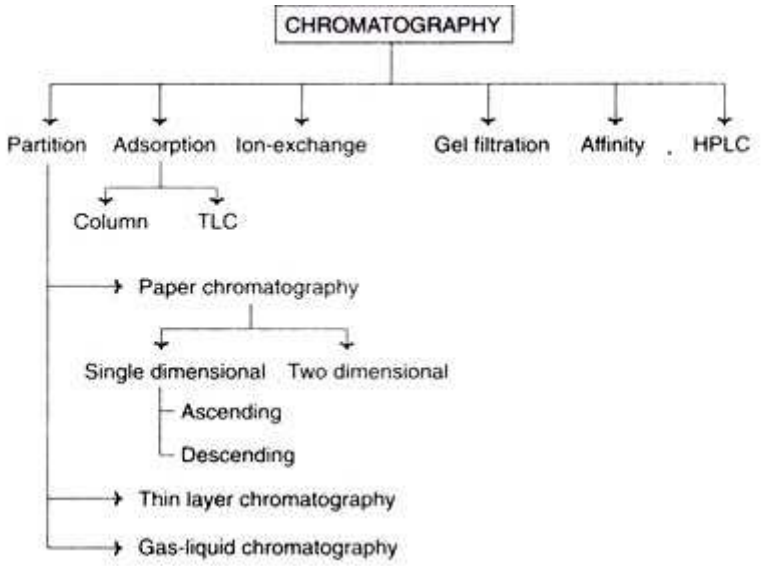

Table 2- Conditios Generally Employed For Forced Degradation[2, 3]

CONDITIOS GENERALLY EMPLOYED FOR FORCED DEGRADATION			
	Experimental Condition	**Storage Condition**	**Sampling Time**
Degradation Type	Control API (no acid or base)	40^0 C, 60^0 C	1, 3, 5 days
	0. 1N HCL	40^0 C, 60^0 C	1, 3, 5 days
	0. 1N NAOH	40^0 C, 60^0 C	1, 3, 5 days
	Acid Control (no API)	40^0 C, 60^0 C	1, 3, 5 days
	Base Control (no API)	40^0 C, 60^0 C	1, 3, 5 days
Oxidation	pH: 2, 4, 6, 8	25^0 C, 60^0 C	1, 3, 5 days
	Peroxide Control	25^0 C, 60^0 C	1, 3, 5 days
	Azobisisobutyronitrile	40^0 C, 60^0 C	1, 3, 5 days
	AIBN Control	40^0 C, 60^0 C	1, 3, 5 days
Photolytic	Light, 1X ICH	NA	1, 3, 5 days
	Light, 3X ICH	NA	1, 3, 5 days
	Light control	NA	1, 3, 5 days
Thermal	Heat chamber	60^0 C	1, 3, 5 days
	Heat chamber	60^0 C/ 75^0 C	1, 3, 5 days
	Heat chamber	80^0 C	1, 3, 5 days
	Heat chamber	80^0 C/ 75% RH	1, 3, 5 days
	Heat control	Room Temperature	1, 3, 5 days

Table 3 –Forced degradation study