METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULATANEOUS ESTIMATION OF AMLODIPINE AND TELMISARTAN IN PHARAMACEUTICAL DOSAGE FORM BY HPLC

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Submitted by

YAHIA ADAM HASSAN ADAM
Reg.No.:261730702

Under the guidance of

Mr.R.VIJAY AMIRTHARAJ M.Pharm.,
Head of The Department

DEPARTMENT OF PHARMACEUTICAL ANALYSIS
JKKMMRF's - ANNAI JKK SAMPOORANI AMMAL COLLEGE OF PHARMACY, KOMARAPALAYAM-638183

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ABBREVIATIONS

%	-	Percentage
Nm	-	Nanometer
v/v	-	Volume by volume
Min	-	Minute
Mg	-	Milligram
mg	-	Microgram
ng	-	Nanogram
TEA	-	Triethyl amine
LC	-	Liquid chromatography
HPLC	-	High-Performance liquid chromatography
GC	-	Gas chromatography
UV	-	Ultraviolet
HPTLC	-	High-performance thin layer chromatography
TLC	-	Thin Layer chromatography
IPC	-	Ion Pair Chromatography
RPIPC	-	Reverse phase ion pair chromatography
LC-MS	-	Liquid chromatography – Mass spectroscopy
FLU	-	Fluoresence
EC	-	Electrochemical
Mm	-	Millimeter
RSD	-	Relative standard deviation
□mol	-	Micromole
RP-HPLC	-	Reversed-phase high-performance liquid chromatography
RI	-	Refractive index
RRT	-	Relative Retention Time
USP	-	United States Pharmacopoeia
HETP	-	High equivalent theoretical plate
LOD	-	Limit of detection
LOQ	-	Limit of quantification
CV	-	Coefficient of variance

RSD	-	Relative Standard Deviation
NMT	-	Not More Than
NLT	-	Not Less Than
R^2	-	Correlation coefficient
ACN	-	Acetonitrile
ICH	-	International conference of harmonization
Rt	-	Retention time
Min	-	Minutes
S/N	-	Signal to Noise
PDA	-	Photo diode array dector
USFDA	-	United state Food and Drug Administration
PPM	-	Parts per million
WHO	-	World Health Organisation
LQC	-	Low Quality Control
MQC	-	Medium Quality Control
HQC	-	High Quality Control

INTRODUCTION

PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis' plays a vital role in the pharmaceutical product development. Pharmaceutical analysis is a specialized branch of analytical chemistry. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix**. (Skoog, 2004)**

Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken.

A separation step is usually a necessary part of both a qualitative and quantitative analysis. The results of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

Instruments play a key role in the quantitative analysis of pharmaceutical chemistry.

INSTRUMENTAL METHODS OF ANALYSIS

Instrumental methods are exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences. Analytical instrumentation plays an important role in the

production and evaluation of new products and in the protection of consumers and environment.

This instrumentation provides lower detection limits required to assure safe foods, drugs, water and air. Instrumental methods are widely used by Analytical chemists to save time, to avoid chemical separation and to obtain increased accuracy. Most instrumental techniques fit into one of the four-principle areas mentioned below.

a) Spectrophotometric techniques

- UV and Visible Spectrophotometry
- Fluorescence and Phosphorescence Spectrophotometry
- Atomic Spectrophotometry (Emission &Absorption)
- Infrared Spectrophotometry
- Raman Spectrophotometry
- X-Ray Spectrophotometry
- Nuclear Magnetic Resonance Spectroscopy
- Mass Spectroscopy
- Electron spin Resonance Spectroscopy

b) Electrochemical Techniques

- Potentiometry
- Voltametry
- Electrogravimetry
- Conductometry
- Amperometry

c) Chromatographic Techniques

- High Performance Liquid Chromatography
- Gas chromatography
- High Performance Thin Layer Chromatography
- Thin Layer Chromatography
- GC- MS (Gas chromatography - Mass Spectroscopy
- LC-MS (Liquid Chromatography - Mass Spectroscopy

d) Miscellaneous techniques

- Thermal analysis
- Kinetic techniques

Chromatograpllic techniques are predominantly used in the pharmaceutical industry for a large variety of samples. HPLC is one of the chromatographic techniques is widely used for checking thepurity of new drug candidates, monitoring changes or scale ups of synthetic procedures, evaluating formulations, and scrutinizing quality control/assurance of final drug products.

1.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography[2] is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres, that makes it much faster. It also allows using a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other

major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

HPLC employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate, liquid must be pressurized to a few thousands of pounds per square inch. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process. If diffusion is minimized, a faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages. **(Connors KA , 1994)**

- Speed (many analysis can be accomplished in 20 min or less)
- Greater sensitivity (various detectors can be employed)
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Ideal for the substances of low viscosity
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labor)
- Precise and reproducible
- Integrator itself does calculations.

SEPARATION PRINCIPLES OF HPLC

Adsorption chromatography employs high-surface area particles that adsorb the solute molecules. Usually a polar solid such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are used in adsorption chromatography.

In adsorption chromatography, adsorption process is described by competition model and solvent interaction model. Competition model assumes that entire surface of the stationary phase is covered by mobile phase molecules as result of competition for adsorption site, In solvent interaction model the retention results from the interaction of solute molecule with the second layer of adsorbed mobile phase molecules. The differences in affinity of solutes for the surface of the stationary phase account for the separation achieved.

In partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can either be polar or non polar. If the stationary phase is polar and the mobile phase is non polar, it is called normal phase partition chromatography. If the opposite case holds, it is called reverse-phase partition chromatography. In normal phase mode, the polar molecule partition preferentially in to the stationary phase and are retained longer than non-polar compounds. In reverse phase partition chromatography, the opposite behavior is observed.

TYPES OF HPLC TECHNIQUES

Based on modes of chromatographv:

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation:

- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromalography
- Chiral phase chromatography

Based on elution technique:

- Isocratic separation
- Gradient separation

Based on the scale of operation:

- Analytical HPLC
- Preparative HPLC

REVERSED PHASE CHROMATOGRAPHY

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

soluble polar compounds. Since the ionic nature of the chemically modified silica in now reversed i.e., it is non-polar or the nature of the phase is reverted, the chromatographic separation carried out with such silica is referred to as reverse-phase chromatography.

Reverse phase liquid chromatography (RPLC) is considered as the method of choice for the analysis of pharmaceutical compounds for several reasons, such as its compatibility with aqueous and organic solutions as well as with different detection systems and its high consistency and repeatability. Sensitive and accurate RPLC analysis, whether in the pharmaceutical or bioanalytical field, necessitates the use of stationary phases which give symmetrical and efficient peaks.

Therefore, manufacturers of stationary phases are continuously improving and introducing new RPLC products, and the selection of various types of reverse phase stationary phases is high, The needs for consistency as well as the globalization of the pharmaceutical companies require that the methods will be transferred from site to site, using either the same column brands or their equivalents. Therefore, an extensive categorization or characterization of the rich selection of stationary phases has been done in recent years.

The stationary phase in the Reverse Phase chromatographic columns is a hydrophobia support that is consisted mainly of porous particles of silica gel in various shapes (spherical or irregular) at various diameters (1,8, 3, 5, 7,10 urn etc.) at various pore sizes (such as 60, 100, 120, 300).

The surface of these particles is covered with various chemical entities, such as various hydrocarbons (Cl, C6, C4, C8, CIS, etc). In most methods C18 columns

are used currently to separate medicinal materials, which sometimes are called ODS (octedecylsilane) or RP-18. A polar solvent is used as mobile phase.

The parameters that govern the retention in Reversed Phase systems are the following: A. The chemical nature of the stationary phase surface. B. The type of solvents that compose the mobile phase C. pH and ionic strength of the mobile phase**. (Denny R.C, Bassett J, 2003)**

A. The chemical nature of the stationary phase

The chemical nature is determined by the size and chemistry of hydrocarbon bonded on the silica gel surface, its bonding density (units of umole/m^2), and the purity and quality of the silica gel support. As a rule, the more carbons in a bonded hydrocarbon the more it retains organic solutes (as long as similar % coverage is compared). The higher the bonding density the longer the organic solutes are retained. A column is considered relatively hydrophobic if its bonding density exceeds 3 umole/m^2.

Very important modifiers of the stationary phase's surface are surface-active substances used as mobile phase's additives, acting as ion-pair reagents. These are substances such as tri-ethyl am ine or tetrabutylamine or hexyl, heptyl, octylsulfonate. They are distributed between the mobile phase and the hydrophobic surface and cover it with either positive (alkylamines) or negative (alkyl sulfonates) charges. This change of the surface into charged surface affects the retention significantly, especially on charged species in the sample.

B. Composition of the mobile phase

As a rule, the weakest solvent in Reverse Phase is the most polar one, water. The oiher polar organic solvents are considered stronger solvents, where the order of solvent strength follows moreor less their dielectric properties, or polarity. The less polar the **solvent** added to the mobile phase the stronger it gets, shortening the retention times.

C. PH and ionic strength of the mobile phase

When the samples contain solutes of ionizable functional groups, such as amines, carboxyls, Phosphates, phosphonates, sulfates and sulfonates, it is possible to control their ionization degree with the help of buffers in the mobile phase. As a rule, the change of an ionizable molecule to an ion makes it more polar and less available to the stationary phase.

NORMAL PHASE CHROMATOGRAPHY

In normal phase chromatography, the stationary phase is polar adsorbent. The mobile phase is generally a mixture of non-aqueous solvents. The silica structure is saturated with silanol group at the end in normal phase separations. These OH groups are statistically distributed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase.

This forms a weak bond with many molecules in the vicinity when any of the following interactions are present. Dipole-induced dipole, dipole-dipole, hydrogen bonding, n-complex bonding. These situations arise when the molecule has one or several atoms with lone pair electrons or a double bond. The adsorption strengths and hence 'K' value (elution series) increase in the following order. Saturated

hydrocarbon < olefins < aromatic <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 stearic factors, If a molecule has several functional groups, then the most polar one determines the reaction properties.

Chemically modified silica, such as aminopropyl, cyanopropyl and diol phases are the stationary phases alternative to silica gel in normal phase chromatography.

The aminopropyl and cyanopropyl phases provide opportunities for specific interactions between the analyte and the stationary phase and thus offer additional options for the optimization of separations. Other advantages of bonded phases lie in the increased homogeneity of the stationary phase surface.

Polar modifiers such as acetic acid or triethylamine (TEA) are added to the mobile phase, to deactivate the more polar adsorption sites on the surface of stationary phase, which in turn will improve peak shape as well as the reproducibility of the retention times.

ADSORPTION CHROMATOGRAPHY

The stationary phase is an adsorbent (like silica gel or any other silica based packing) and the reparation is based on repeated adsorption-desorption steps.

ION EXCHANGE CHROMATOGRAPHY

Separation is based on the charge-bearing functional groups, anion exchange for sample negative ion, or cation exchange - for sample positive ion. Gradient elution by pH is common.

AFFINITY CHROMATOGRAPHY

Separation is based on a chemical interaction specific to the target species. The more popular reverse phase mode uses a buffer and an added counter-ion of opposite charge to the sample with separation being influenced by pH, ionic strength, temperature, concentration of and type of organic co-solvent(s). Affinity chromatography, common for macromolecules, employs a ligand (biologically active molecule bonded covalently to the solid matrix) which interacts with its homologous antigen (analyte) as a reversible complex that can be eluted by changing buffer conditions.

CHIRAL CHROMATOGRAPHY:

Separation of the enantiomers can be achieved on chiral stationary phases by formation of diastereomers via derivatizing agents or mobile phase additives on a chiral stationary phase. When used as an impurity test method, the sensitivity is enhanced if the enantiomeric impurity clutes before the enantiomeric drug.

ISOCRATIC SEPARATION

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

GRADIENT SEPARATION

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

ANALYTICAL HPLC

In this only analysis of the samples are done. Recovery of the samples for reusing is normally not done, since the samples used are very low.

PREPARATIVE HPLC

Where analysis of the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused.

ION-PAIR CHROMATOGRAPHY

Ion Pair Chromatography (IPC) is used to separate ionic analytes on a Column. An Ion Pair reagent is added to modulate retention of the ionic analytes. Ion-pair chromatography is commonly used in combination with UV detection, in which case it is referred as reverse phase ion-pair chromatography (RPIPC).

Principle of ion-pairing:

With the aid of ion pair chromatography it is possible to separate the same analytes as in ion exclusion chromstography, but the separation mechanism is completely different. The stationary phases used are completely polar reverse phase materials such as are used in distribution chromatography. A so-called ion pair regent is added to the clueivs; this consists of anioniccationic surfactants such as tetra alkyl ammonium salts or n-alkylsulfonic acids. Together with the rppositely charged analyte ions the ion pair reagents form an uncharged ion pair, which can be retarded at the stationary phase by hydrophobic interactions. Separation is possible because of the formation constants of the ion pairs and their different degrees of adsorption. Figure 1 shows a simplified static ion exchange model in which it is

assumed that interactions with the analytes only i-ccur after adsorption of the ion pair reagent at the stationary phase.

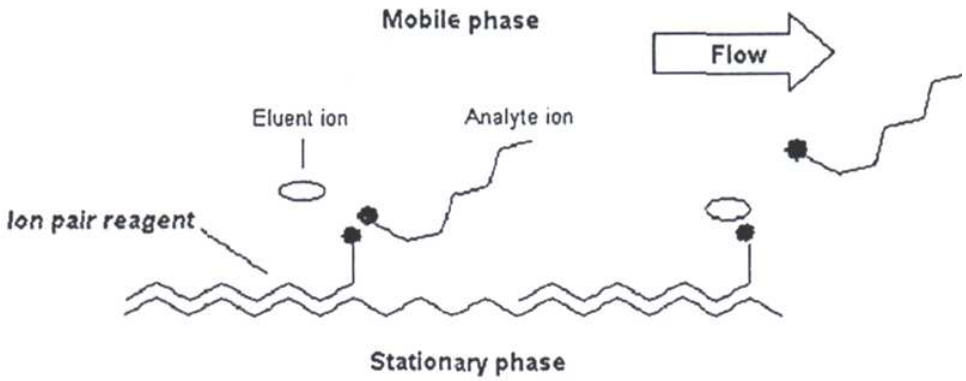


Fig No.1: Ion Pairing

Separation mechanism:

Ion-exchange selectivity is mediated by both the mobile phase and stationary phase. In contrast the selectivity of an ion-pair separation is determined primarily by the mobile phase. The two major components of aqueous mobile phase are the ion-pair reagent and the organic solvent; the type and concentration of each component can be varied to achieve desired separation. The ion-pair reagent is a large ionic molecule that carries a charge opposite to analyte of interest. It usually has both hydrophobic region to interact with the analyte. Stationary phases used for ion-pair are neutral, hydrophobic resins such as polystyrene, divinyl benzene or bonded silica. A single stationary phase can be used for either anion or cation analysis.

Although the retention mechanism of ion-pair chromatography is not fully understood, three major theories have been proposed:

- Ion pair formation
- Dynamic ion exchange
- Ion interaction

In first model, the analyte and ion-pair reagent form a neutral pair, which is then partitions between the mobile phase and stationary phase. Retention can be controlled by varying the concentration of crganic solvent in the mobile phase as in reverse phase chromatography.

The dynamic ion-exchange model maintains that the hydrophobic portion of ion-pair reagent adsorbs to the hydrophobic stationary phase to form a dynamic ion-exchange surface. The analyte is retained on this surface, as it would in classic Ion Chromatography. Using this scenario, solvents used in the mobile phase can be used to impede interaction of ion-pair reagent with the stationary phase, there by altering the capacity of the column.

A third model describes an electrical double layer that is formed when ion-pair reagent permeates the stationary phase, carrying with it an associated counter-ion. Retention of analyte ion in this model is dependent upon a combination of factors including those described in first two models.

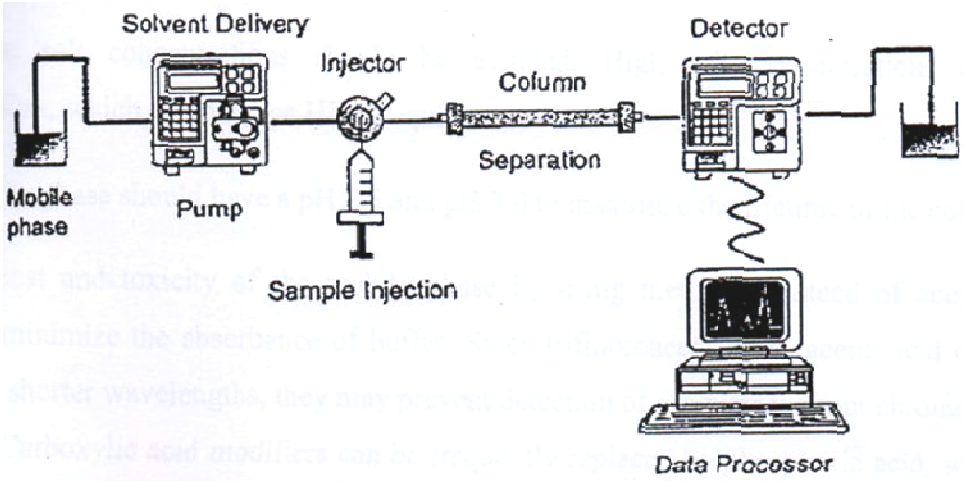
Typical IP reagents are divided into two categories

- Cationic: These are used for anion analysis. Cationic ion-pair reagents include ammonium and tetra methyl-, tetraethyl-, tetra propyl-, and tetra butyl ammonium.
- **Anionic:** These reagents used for cation analysis. Anionic ion-pair reagents include hydrochloric, perchloric, and perfluorocarboxylic acids and pentane-, hexane-, heptane, and octane sulfonic acids.

INSTRUMENTATION OF HPLC

The individual components HPLC and their working functions are described below.

- Mobile phase and reservoir
- Solvent degassing system
- Pump
- Injector
- Column
- Detector
- Data System



MOBILE PHASE AND RESERVOIR

The most common type of solvent reservoir is a glass bottle. Most of the manufacturers supply these bottles with special caps, Teflon tubing and filters to connect to the pump inlet and to the purge gas (helium) used to remove dissolved air.

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 ;:1 vents, which need to be considered for a successful separation, are boiling point, viscosity, ir.ector compatibility, flammability and toxicity.

Mobile phases used for HPLC typically are mixtures of organic solvents and water or aqueous ruffers. Isocratic methods are preferable to gradient methods. Gradient methods will sometimes be -t.quired when the molecules being separated have vastly different partitioning properties. When a elution method is used, care must be taken to ensure that all solvents are miscible.

The following points should also be considered when choosing a mobile phase

It is essential to establish that the drug is stable in the mobile phase for at least the duration of the analysis.

Excessive salt concentrations should be avoided. High salt concentrations can result in precipitation, which can damage HPLC equipment.

The mobile phase should have a pH 2.5 and pH 7.0 to maximize the lifetime of the column.

Reduce cost and toxicity of the mobile phase by using methanol instead of acetonitrile when possible minimize the absorbance of buffer. Since trifluoroacetic acid, acetic acid or formic acid absorb at shorter wavelengths, they may prevent

detection of products without chromophores above 220 nm. Carboxylic acid modifiers can be frequently replaced by phosphoric acid, which does not absorb above 200 nm.

Use volatile mobile phases when possible, to facilitate collection of products and LC-MS analysis. Volatile mobile phases include ammonium acetate, ammonium phosphate, formic acid, acetic acid, and trifluoroacetic acid. Some caution is needed as these buffers absorb below 220 nm.

lonizable compounds in some cases can present some problems when analyzed by reverse phase chromatography. Two modifications of the mobile phase can be useful in reverse phase HPLC for ionizable compounds. One is called ion suppression and other ion pairing chromatography. In both techniques, a buffer is used to ensure that the pH of the solution is constant and usually at least 1.5 pH units from a pKa of the drug to ensure that one form predominates.

If pH is approximately equal to pKa, peak broadening can occur. In ion suppression chromatography, the pH of the aqueous portion of the mobile phase is adjusted to allow the neutral form of the drug to predominate. This ensures that the drug is persistent in only one form and results in improvement of the peak shape and consistency of retention times.

In ion pairing chromatography, the pH of the mobile phase is adjusted so that the drug is completely ionized. If necessary to improve peak shape or lengthen retention time, an alkyl sulfonic acid salt or bulky anion such as trifluoroacetic acid is added to the ion pair to cationic drugs or a quaternary alkyl ammonium salt is added to ion-pair to anionic drugs. Ion pairing chromatography also allows the

simultaneous analysis of both neutral and charged compounds. . **(Gurdeep.R.Chatwal, Sham.K.Anand, 2008)**

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, degassing with an air-soluble membrane, helium purging ultra signification or purging combination of these methods.

Helium purging and storage of the solvent under helium is not sufficient for degassing aqueous solvents. It is useful to apply a vacuum for 5-10 min and then keep the solvent under a helium atmosphere.HPLC systems are also provided an online degassing system, which continuously removes the solved gases from the mobile phase.**(Skoog, 2012)**

PUMP:

High pressure pumps are needed to force solvents through packed stationary phase beds. Smaller bed particles require higher pressures. There are many advantages to using smaller particles, but they may not be essential for all separations.

The most important advantages are: higher resolution, faster analyses, and increased sample load capacity. However, only the most demanding separations require these advances in significant amounts.

Many separation problems can be resolved with larger particle pickings that require less pressure. Flow rate stability is another important pump feature that distinguishes pumps. Very stable flow rates are usually not essential for analytical chromatography.

However, if the user plans to use a system in size exclusion mode, then there must be a pump which provides an extremely stable flow rate. An additional feature found on the more elaborate pumps is external electronic control. Although it adds to the expense of the pump, external electronic control is a very desirable feature when automation or electronically controlled gradients are to be run.

Alternatively, this becomes an undesirable feature (since it is an unnecessary expense) when using isocratic methods. The degree of flow control also varies with pump expense. More expensivePumps include such state of-the-art technology as electronic feedback and multi headed igurations.

It is desirable to have an integrated degassing system, either helium purging, or membrane filtering.

INJECTOR

Sample introduction can be accomplished in various ways. The simplest method is to use an injection valve.

In more sophisticated LC systems, automatic sampling devices are incorporated where the sample is introduced with the help of auto samplers and microprocessors. In liquid chromatography, liquid samples may be injected directly and solid samples need only be dissolved in an appropriate solvent. The solvent need

not be the mobile phase, but frequently it is judiciously chosen to avoid detector interference, column/component interference, and loss in efficiency or all of these.

It is always best to remove particles from the sample by filtering over a 5 urn filter, or centrifuging, since continuous injections of paniculate material will eventually cause blockages in injection devices or columns. Sample sizes may vary widely.

The availability of highly sensitive detectors frequently allows use of the small samples which yield the highest column performance. Sample introduction techniques can be used with a syringe or an injection valve.

COLUMN

The heart of the system is the column. The choice of common packing material and mobile phases depends on the physical properties of the drug.

Many different reverse phase columns will provide excellent specificity for any particular separation. It is therefore best to routinely attempt separations with a standard Cg or Cig column and determine if it provides good separations. If this column does not provide good separation or the mobile phase is unsatisfactory, alternate methods or columns should be explored.

Reverse phase columns differ by the carbon chain length, degree of end capping and percent carbon loading. Diol, cyano and amino groups can also be used for reverse phase chromatography.

Typical HPLC columns are 5,10,15 and 25 cm in length and are filled with small diameter (3, 5 or 10 um) particles. The internal diameter of the columns is

usually 4.6 mm; this is considered the best compromise for sample capacity, mobile phase consumption, speed and resolution.

However, if pure substances are to be collected (preparative scale), then larger diameter columns may be needed. Packing the column tubing with small diameter particles requires high skill and specialized equipment.

For this reason, it is generally recommended that all but the most experienced chromatographers purchase prepacked columns, since it is difficult to match the high performance of professionally packed LC columns without a large investment in time and equipment.

In general, LC columns are fairly durable and one can expect a long service life unless they are used in some manner which is intrinsically destructive, as for example, with highly acidic or basic duents, or with continual injections of'dirty' biological or crude samples.

It is wise to inject some test mixture (under fixed conditions) into a column when new, and to retain the chromatogram. If questionable results are obtained later, the test mixture can be injected again under specified conditions.

The two chromatograms may be compared to establish whether or not the column is still useful.

DETECTOR

Today, optical detectors are used most frequently in liquid chromatographic systems. These detectors pass a beam of light through the flowing column effluent as it passes through a low Volume (~10ul) flow cell.

The variations in light intensity caused by UV absorption, fluorescence emission or change in refractive index, from the sample components passing through the cell, are monitored as changes in the output voltage.

These voltage changes arc recorded on a strip chart recorder and frequently are fed into a computer to provide retention time and peak area data. The most commonly used detector in LC is the ultraviolet absorption detector.

A variable wavelength detector of this type, capable of monitoring from 190 to 400 run, will be found suitable for the detection of the majority samples.

Other detectors in common use include: Photo Diode Array UV detector (PDA), refractive index), fluorescence (FLU), electrochemical (EC).

The RI detector is universal but also the less sensitive one. FLU and EC detectors are quite sensitive *(up* to 10-15 pmole) but also quite selective.

DATA SYSTEM

Since the detector signal is electronic, using modem data collection techniques can aid the signal analysis. In addition, some systems can store data in a retrievable form for highly sophisticated computer analysis at a later time.

The main goal in using electronic data systems is to increase analysis accuracy and precision, while reducing operator attention. There are several types of data systems, each differing in terms of available features. In routine analysis, where no automation (in terms of data management or process control) is needed, a pre-programmed computing integrator may be sufficient.

If higher control levels are desired, a more intelligent device is necessary, such as a data station or minicomputer. The advantages of intelligent processors in chromatographs are found in several areas.

First, additional automation options become easier to implement. Secondly, complex data analysis becomes more feasible. These analysis options include such features as run parameter optimization and deconvolution (i.e. resolution) of overlapping peaks.

Finally, software safeguards can be designed to reduce accidental misuse of the system.

HPLC THEORY:

System Suitability Parameters

High performance liquid chromatography is defined as a separation of mixtures of compounds due to differences in their distribution equilibrium between two phases, the stationary phase packed inside columns and the mobile phase, delivered through the columns by high pressure pumps.

Components whose distribution into the stationary phase is higher, are retained longer, and get icparated from those with lower distribution into the stationary phase. The theoretical and practical Bundations of this method were laid down at the end of 1960s and at the beginning of 1970s.

The theory of chromatography has been used as the basis for System-Suitability tests, which are set of quantitative criteria that test the suitability of the chromatographic system to identify and quantify drug related samples by HPLC at any step of the pharmaceutical analysis.

Retention Time (tn), Capacity Factor k' & Relative Retention Time (RRT)

The time elapsed between the injection of the sample components into the column and their detection is known as the Retention Time (t_R).The retention time is longer when the solute has higher affinity to the stationary phase due to its chemical nature. For example, in reverse phase chromatography, the more lypophilic compounds are retained longer.

Therefore, the retention time is a property of the analyte that can be used for its identification. Ar.on retained substance passes through the column at a time to, called the Void Time.

Retention factor is calculated as follows:

$$k_1 = \frac{t_R - t_0}{t_o}$$

The Capacity Factor describes the thermodynamic basis of the separation and its definition is the ratio of the amounts of the solute at the stationary and mobile phases within the analyte band inside die chromatographic column:

$$k_1 = \frac{C_s}{C_m}$$

Where C_s is the concentration of the solute at the stationary phase and C_{in} is its concentration at the mobile phase and phi is the ratio of the stationary and mobile phase volumes all within the chromatographic band.

Retention Factor is used to compare the retention of a solute between two chromatographic is, normalizing it to the column's geometry and system flow rate.

The retention factor value should be in between 1-20. The need to determine the void time can be ticky sometimes, due to the instability of the elution time of the void time marker, t_0, therefore, when the chromatogram is complex in nature, and one. known component is always present at a artain retention time, it can be used as a retention marker for other peaks. In such cases the ratio between the retention time of any peak in the chromatogram and the retention time of the marker is used ($t_{R(peak)}/t_{R(Marker)}$) and referred to as the Relative Retention Time (RRT).

RRT is also used instead of the capacity ratio for the identification of the analyte as well as to compare its extent of retention in two different chromatographic systems.

The sharpness of a peak relative to its retention time is a measure of the system's efficiency, calculated as N, plate count. Band-broadening phenomena in the column such as eddy diffusion, molecular diffusion, and mass-transfer kinetics and extra-column effects reduce the efficiency of the separation.

The sharpness of a peak is relevant to the limit of detection and limit of quantification of the chromatographic system. The sharper the peak for a specific area, the better is its signal-to-noise; hence the system is capable of detecting lower concentrations.

Therefore, the efficiency of the chromatographic system must be established by the system suitability test before the analysis of low concentrations that requires high sensitivity of the system, such as the analysis of drug impurities and degradation products.**(Kasture AV et al., 2010), (Lindsay S , 1991)**

Efficiency: Plate Count N and Peak Capacity P_c

The efficiency of the separation is determined by the Plate Count N when working at isocratic conditions, whereas it is usually measured by Peak Capacity P_e when working at gradient conditions.

The following equation for the plate count is used by the United States Pharmacopoeia (USP) to calculate N:

$$N = 16 \times \left(\frac{t_R}{W(\text{Baseline})}\right)2$$

Where w is measured from the baseline peak width calculated using lines tangent to the peak width x 50 % height. European and Japanese Pharmacopoeias use the peak width at 50% of the peak height, hence the equation becomes:

$$N = 5.54 \times \frac{(t_R)}{W_{(50\%)}}$$

Peak Capacity P_c is defined as number of peaks that can be separated within a retention window for i specific pre-determined resolution. In other words, it is the runtime measured in peak width units 54). It is assumed that peaks occur over the gradient chromatogram.

Therefore, Peak Capacity can be calculated from the peak widths w in the chromatogram as follows:

$$1 + \frac{t_g}{(1/n)\sum_1^n w}$$

Where n is the number of peaks at the segment of the gradient selected for the calculation, t_e. Thus peak capacity can be simply the gradient run time divided by the average peak width. The sharper the peaks the higher is the peak capacity, hence the system should be able to resolve more peaks at the selected run time as well as detect lower concentrations.

Another measure of the column's chromatographic efficiency is the Height Equivalent to Theoretical Plate (HETP) which is calculated from the following equation:

HETP = (L/N)

Where L is column length and N is the plate count. HETP is measured in micrometer.The behavior of HETP as function of linear velocity has been described by various equations. It is frequently called "The Van-Deemter curve", and it is frequently used to describe and characterize various chromatographic stationary phases' performance and compare them to each other.

Lower are the values of HETP, the more efficient is the chromatographic system, enabling the toection of lower concentrations due to the enhanced signal-to-noise ratio of all the peaks in the chromatogram.

Ptak Asymmetry Factor A_f and Tailing Factor T

The chromatographic peak is assumed to have a Gaussian shape under ideal conditions, describing normal distribution of the velocity of the molecules populating the peak zone migrating through the stationary phase inside the column.

Any deviation from the normal distribution indicates non-ideality of the distribution and the migration process therefore might jeopardize the integrity of the

peak's integration, reducing the accuracy of the quantification. This is the reason why USP Tailing is a peak's parameter almost always measured in the system suitability step of the analysis.

The deviation from symmetry is measured by the Asymmetry Factor, Af or Tailing Factor T. The calculation of Asymmetry Factor, Afis described by the following equation:

$$A_f = \frac{A_{(10\%k)}}{B_{(10\%k)}}$$

Where A and B are sections in the horizontal line parallel to the baseline, drawn at 10% of the peak height

The calculation of Tailing Factor, T, which is more widely used in the pharmaceutical industry, as suggested by the pharmacopeias, is described by the following equation:

Where A and B are sections in the horizontal line parallel to the baseline, drawn at 5% of the peak height. The USP suggests that Tailing Factor should be in the range of 0.5 up to 2 to assure a precise and accurate quantitative measurement.

Selectivity Factor: Alfa and Resolution Factor Rs

The separation is a function of the thermodynamics of the system. Substances are separated in a chromatographic column when their rate of migration differs, due to their different distribution between the stationary and mobile phases.

The Selectivity Factor, a, and Resolution Factor, Rs, measure the extent of separation between two adjacent peaks. The Selectivity Factor accounts only for the

ratio of the Retention Factors, k', of the two peaks (k'2/k'1), whereas the Resolution Factor, Rs, accounts for the difference between the retention times of the two peaks relative to their width.

The equation that describes the experimental measurement of the Resolution Factor, Rs, is as follows:

Where t_R is the retention time of peaks 1 and 2 respectively and w is their respective peak width at the tangents' baseline. According to the pharmacopeias Rs should be above 1.5 for an accurate quantitative measurement.

The resolution is a critical value when working with complex samples such as drug impurities and degradation products, or when the formulation is complex and excipients might interfere with the quantitative measurements. Therefore, it is an essential part of the system suitability measurement stage before the quantitative work of these types of samples.

The sample used for the measurements of Rs during the system suitability runs is sometimes called Resolution Solution, It usually contains the components that are the most difficult to resolve.

The theoretical description of the Resolution Factor Rs equation is shown in Equation. It includes some of the above parameters, the plate count N, the selectivity a and the average of the two peaks' capacity factors k[1]

$$RS= \frac{\sqrt{N}}{4}\left(\frac{\propto-1}{\propto}\right)\left(\frac{k_{ave}1}{k_{ave}1+1}\right)$$

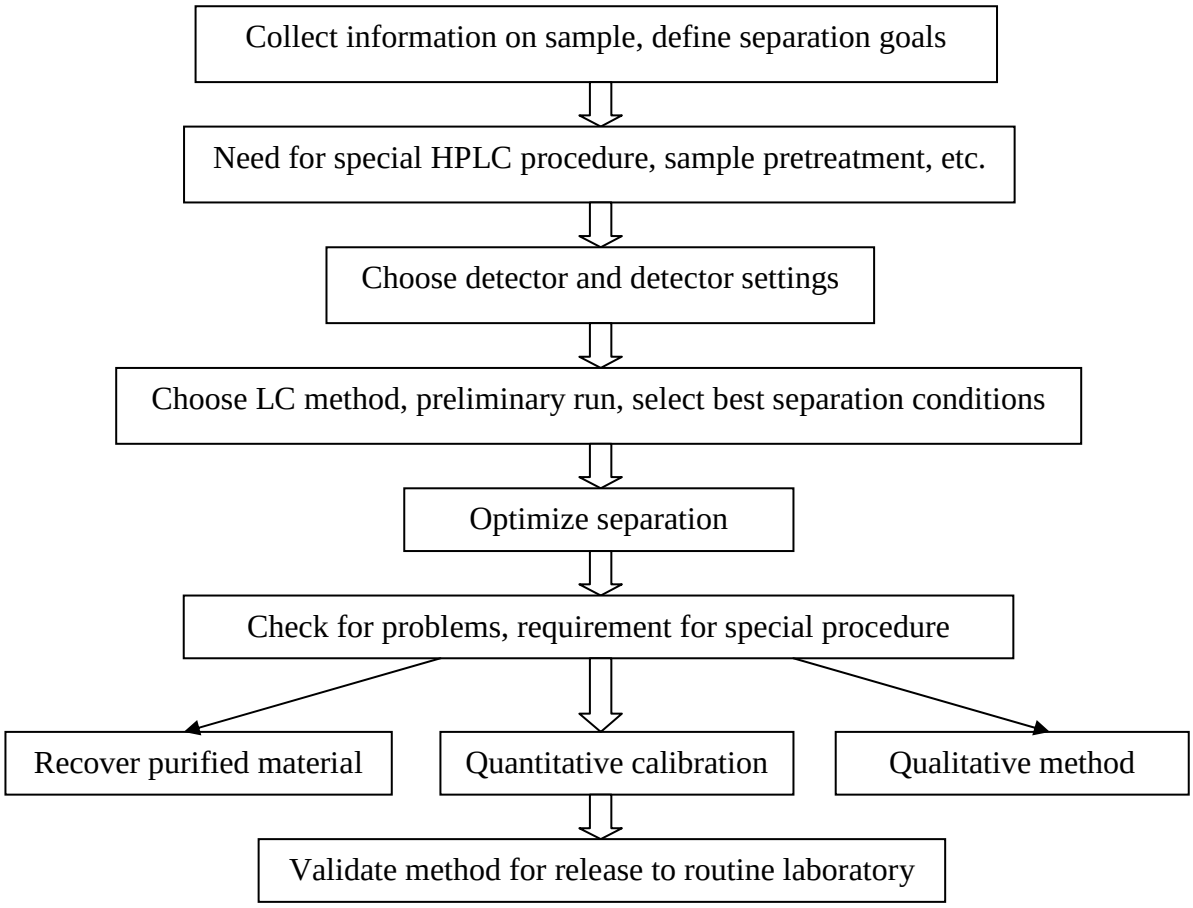
It can be clearly seen from this equation that the plate count is the most effecting parameter in the increase of the chromatographic resolution. Since the plate count increases with the reduction in particle diameter, it explains the reduction

in particle diameter of the stationary phase material Airing the last 3 decades of HPLC.

This is also the rationale behind the recent trend in HPLC, the use of sub 2 micron particle columns and the development of a specially design of ultra performance HPLC systems to accommodate such columns. :

1.2 ANALYTICAL METHOD DEVELOPMENT

Method development has following steps:



A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling.

The important factors, which are to be taken into account to obtain reliable quantitative analysis, are:

1. Careful sampling and sample preparation.

2. Appropriate choice of the column.

3. Choice of the operating conditions to obtain the adequate resolution of the mixture.

4. Reliable performance of the recording and data handling systems.

5. Suitable integration/peak height measurement technique.

6. The mode of calculation best suited for the purpose

7. Validation of the developed method.

Sample preparation

Samples come in various forms:

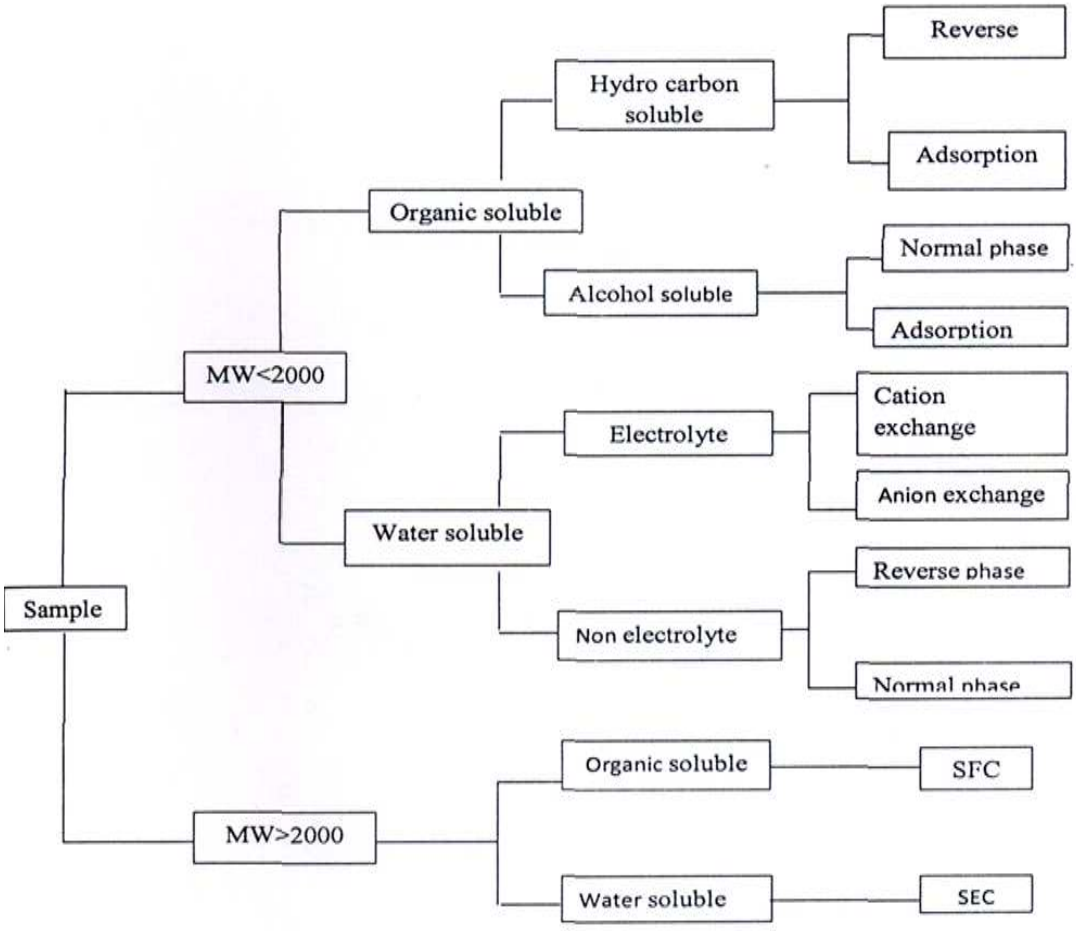
- Solutions ready for injection
- Solutions that require dilution, buffering, addition of an internal standard or othervolumetric manipulation
- Solids must be dissolved or extracted

- Samples that require pretreatment to remove interferences and/or protect the column orequipment from damage.

Most samples for HPLC analysis require weighing and /or volumetric dilution before injection. Best results are often obtained when the composition of the sample solvent is close to that of the mobile phase since this minimizes baseline upset and other problems. Some samples require a partial separation (pretreatment) prior to HPLC, because of need to remove interferences, concentrate sample analytes or eliminate "column killers".**(SatinderAhuja and Michael W. Dong , 2006)**

SELECTING HPLC METHOD AND INITIAL CONDITIONS:

The mode of HPLC can be selected based on the following chart:



In many cases the development of an adequate sample pretreatment can be challenging for achieving a good HPLC separation. The samples may be of two types, regular or special. The regular samples are typical mixtures of small molecules (<2000Da) that can be separated by normal starting conditions.

THAT ARE TO BE ACHIEVED IN METHOD DEVELOPMENT

Table No.l: Goals in method development

Goal	Comment
Separation time	<5-10 min is desirable for routine procedures.
Qualification	52% for assays; S5% for less-demanding analyses <15% for trace analyses.
Pressure	<150 bars is desirable, <2QO bars is usually essential (new column assumed).
Peak height	Narrow peaks are desirable for large signal/noise ratios.
Solvent consumption	Minimum mobile-phase use per run is desirable.

OPTIMIZATION OF HPLC METHOD

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts, symmetry, capacity factor, elution time, detection limits, limit of quantification and overall ability to quantify the specific analyte of interest.

Optimization of a method can follow either of two general approaches:

- Manual
- Computer driven

The manual approach involves varying one experimental variable at a time, while holding all other constant and recording changes in response. The variables might include flow rate, mobile or stationary phase composition, temperature, detection wavelength and pH.

The univariate to system is slow, time consuming and potentially expensive. However, it may provide a better understanding of the principles and theory involved and of interactions of the variables.

In the second approach, computer driven automated method development, efficiency is optimized experimental input is minimized. Computer driven automated approaches can be applied to applications. In addition, they are capable of significantly reducing the time, energy and cost, instrumental method development.

The various parameters that include to be optimized during method development are

- Selection of mode of separation.
- Selection of stationary phase,
- Selection of mobile phase.
- Selection of detector.

Selection mode of separation

In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For Ae separation of polar or moderately polar compounds, the most preferred mode is reverse phase.

The nature of the analyte is the primary factor in the selection of the mode of separation. A second factor is the nature of the matrix.

A useful and practical measurement of peak shape is peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10% of full peak 'height and peak tailing factor at 5%. Reproducibility of retention times and capacity factor is important for developing a rugged and repeatable method.

Buffers if any and its Strength

Buffer and its strength play an important role in deciding the peak symmetries and separations. Some of the most commonly employed buffers are

- Phosphate buffers prepared using salts like $KH_2PO^{\wedge}$, K_2HPO_4, NaH_2PO_4, Na_2HPOi etc.
- Phosphoric acid buffers prepared using HjPO.
- Acetate buffers-Ammonium acetate, Sodium acetate etc.
- Acetic acid buffers prepared using CH_3COOH.

The retention also depends on the molar strengths of the buffer-Molar strength is increasingly proportional to retention times. The strength of the buffer can be increased, if necessary to achieve the required separations. The solvent strength is a measure of its ability to pull analyte from the column. It is generally controlled by the concentration of the solvent with the highest strength.

It is important to maintain the pH of the mobile phase in the range of 2.0 to 8.0 as most columns does not withstand to the pH which are outside this range. This is due to the fact that the siloxane linkages are cleaved below pH 2.0, while pH values above 8.0, silica may dissolve.

Mobile Phase Composition

Most chromatographic separations can be achieved by choosing the optimum mobile phase composition. This is due to the fact that fairly large amount of selectivity can be achieved by choosing the qualitative and quantitative composition of aqueous and organic portions.

Most widely used solvents in reverse phase chromatography are Methanol and Acetonitrile. Experiments should be conducted with mobile phases having buffers with different pH and different organic phases to check for the best separations of analyte peak.

A mobile phase which gives separation of analyte peak and which is rugged for variation of both aqueous and organic phase by at least ± 0.2% of the selected mobile phase composition should be used.

Selection of Detector

The detector was chosen depending upon some characteristic property of the analyte like UV absorbance, florescence, conductance, oxidation, reduction etc. The characteristics that are to be fulfilled by a detector to be used in HPLC determination are,

- High sensitivity facilitating trace analysis
- Negligible baseline noise to facilitate lower detection
- Large linear dynamic range.
- Low dead volume
- Inexpensive to purchase and operate

Pharmaceutical ingredients do not absorb all UV light equally, so that selection of detection wavelength is important. An understanding of the UV light absorptive properties of the organic impurities and the active pharmaceutical ingredient is very helpful.

For the greatest sensitivity it should be used. Ultra violet wavelengths below 200nm should be avoided because detector noise increases in this region. Higher wavelengths give greater selectivity.

Performance Calculations

Carrying out system suitability experiment does the performance calculations. System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision.

The requirements for system suitability are usually developed after method development and validations have been completed. 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 can be determined during validation.

System suitability might then require that retention times fall within a ±3 SD range during routine performance of the method.

The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. These parameters include plate number (n), tailing factor (T), resolution (Rs) and relative standard deviation (RSD) of peak height or peak area for respective injections.The RSD of peak height or area of five injections of a standard solution is normally accepted as one of the standard criteria. For assay

method of a major component, the RSD should typically be less than 1% for these five respective injections.

The plate number and' or tailing factor are used if the run contains only one peak. For chromatographic separations with more than one peak, such as an internal standard assay or an impurity method expected to contain many peaks, some measure of separations such as RSis recommended. Reproducibility of tR or k value for a specific compound also defines system performance. The column performance can be defined in terms of column plate number. As the plate count is more the column is more efficient. **(Khopkar S.M, , 2008)**

1.3 ANALYTICAL METHOD VALIDATION

Method validation can be defined as *(ICH)* "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

Method validation is an integral part of the method development; it is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose and demonstrating that analytical procedures are suitable for their intended use that they support the identity, quality, purity, and potency of the drug substances and drug products. Data thus generated become part of the methods validation package submitted to Center for Drug Evaluation and Research (CDER). Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose.

All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, and detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters includes namely.

- System suitability
- Specificity
- Accuracy
- Precision
- Linearity
- Limit of Detection
- Limit of Quantification
- Ruggedness
- Robustness

SYSTEM SUITABILITY

According to the USP, system suitability tests are an integral part of chromatographic methods. I These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment,electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole.

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

Similar to the analytical method development, the system suitability test Strategy should be revised as the analysts develop more experience with the assay. In general, consistency of system performance. (E.g.: Replicate injections of the standard) and chromatographic suitability.(Eg: Tailing factor, column efficiency and resolution of the critical pair) are the main components of system suitability.

During the early stage of the method development process some of the more sophisticated system suitability tests may not be practical due to the lack of experience with the method. In this stage, usually a more "generic" approach is used. For example, evaluation of the tailing factor to check chromatographic suitability, and replicate injections of the system suitability solution to check injection precision may be sufficient for an HPLC impurities assay. As the method matures more experience is acquired for this method, a more sophisticated system suitability test may be necessary.

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns.

Parameters such as plate count, tailing factors; resolution and reproducibility (%RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method. These parameters are measured during the analysis of system suitability "sample" that is a mixture of main components and expected by-products. **(Beckett, J.B. Stenlak, 2001), (Willard H, 1988)**

Table No.2: System suitability parameters and recommendations

Capacity Factor (k')	The peak should be well-resolved from other peaks and the void volume, generally k' 1 to 20
Repeatability	RSD < 1% for N > 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interfering (impurity, excipient, degradation product, internal standard, etc.
T of >0.5and <2	Tof>0.5and<2
Theoretical Plates (N)	N > 3000

SPECIFICITY/SELECTIVITY

The terms selectivity and specificity are often used interchangeably. According to ICH, the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate.

Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. Studies are designed to evaluate the degree of interference, if any, which can be attributed to other analytes, impurities, degradation products, reagent "blanks" and excipients. This provides the analyst with a degree of certainty that theresponse observed is due to the single analyte of interest. The degree of specificity testing varies depending on

the method type and the stage of validation. Specificity should be evaluated continually through the drug development process. Specificity is sometimes used interchangeably with the term "selectivity". The argument over which term is more correct is one of semantics. Although there is some dissention, the term "specificity" has been adopted by the regulatory guidance documents and should be used to prevent further confusion.

Non-interference of Placebo

This portion of specificity evaluation applies to the finished drug product only. Excipients present in the formulation should be evaluated and must not interfere with the detection of the analyte. Individual solutions of each excipient prepared at several times the normal concentration of the component in the drug product ensure that any detector response from the excipient will be readily visible. Injecting individual solutions of each excipient into the HPLC system in comparison with a standard solution of the analyte is one means of performing this experiment. The absence of apeak eluting at the retention time of the active ingredient is sufficient to demonstrate specificity for excipients.

Challenge Study

Injecting solutions of known process impurities, degradation products, intermediates, homologues, dimers, etc. further challenges the specificity of a method. Identification of these compounds may require an extensive search in order to identify all possible species that may be present in the sample. For new chemical entities (NCE), this information may not be readily available. Probable suspects should be identified by careful review of the synthetic route and manufacturing process to identify any likely species that may be present in the sample.

DEGRADATION STUDIES

Degradation studies involve exposing the sample to a variety of stressed conditions to further evaluate the specificity of degradation products. In this study, the drug substance, drug product, and the combined excipients (or placebos) are each exposed to the stressed conditions.

These may include, but are not limited to, heat, light, acidic media, alkaline media, and oxidative environments. Other conditions may be used depending on the nature and chemistry of the test subject.

Forced degradation is usually evaluated with not more than 20% degradation of the drug substance, although more may be acceptable depending on the particular properties of the drug. A reasonable effort should be made to degrade samples in order to identify possible degradation products. If the planned experiments do not show any appreciable degradation, the strength and/or exposure time of the stress condition may be increased, but degradation is not required for every condition studied. There is a point beyond which the stress condition becomes extreme and unrealistic. Sound scientific judgment should be used to determine the extent and degree of degradation studies.

ACCURACY

Accuracy is the measure of how close the experimental value is to the true value. Accuracy should be established across the specified range of the analytical procedure.

Assay:

Drug Substance

Several methods of determining accuracy are available:

a) Application of an analytical procedure to an analyte of known purity (e.g. reference material);
b) Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.
c) Accuracy may be inferred once precision, linearity and specificity have been established.

Drug Product

Several methods for determining accuracy are available:

a. Application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analyzed have been added.
b. In cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyteto the drug product or to compare the results obtained from a second, well Characterized procedure, the accuracy of which is stated and/or defined.
c. Accuracy may be inferred once precision, linearity and specificity have been established.

Impurities (Quantification)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities. In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used. It should be clear how the individual or total impurities are to be determined e.g., weight/weight or area percent, in all cases with respect to the major analyte.

Recommendations

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations / 3 replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

PRECISION

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. Ruggedness as defined in USP XXII <1225>, 1990 incorporates the concepts described under the terms "intermediate precision", "reproducibility" and "robustness" of this guide.

Repeatability

(1)Injection Repeatability

Sensitivity is the ability to detect small changes in the concentration of the analyte in the sample. Sensitivity can be partially controlled by monitoring the specification for injection reproducibility (system suitability testing).

The sensitivity or precision as measured by multiple injections of a homogeneous sample (prepared solution) indicates the performance of the HPLC instrument under the chromatographic conditions and day tested.

The information is provided as part of the validation data and as a system suitability test. The specification, as the coefficient of variation in % or relative standard deviation (RSD), set here will determine the variation limit of the analysis. The tighter the value, the more precise or sensitive to variation one can expect the results. This assumes that the chromatograph does not malfunction after the system suitability testing has been performed. Keep in mind, however, that it does not consider variations due to the drug product manufacturing and laboratory sample preparation procedures. The set of four duplicate samples were injected sequentially. Variations in peak area and drift of retention times are noted.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term 'set' is defined as referring to a number (N) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of

squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation S, is given by

$$S = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(xi - x)2}$$

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 sometimes 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

Recommendations

As part of method validation, a minimum of 10 injections with an RSD of 2% is recommended. With the methods for release and stability studies, an RSD of 2% for precision of the systemsuitability tests for at least five injections (n=5) for the active drug either in drug substance or drug product is desirable. For low-level impurities, higher variations may be acceptable.

(ii)Analysis Repeatability

Determination, expressed as the RSD, consists of multiple measurements of a sample by the same analyst under the same analytical conditions.

For practical purpose, it is often combined with accuracy and carried out as a single study.

(iii)Intermediate Precision

Intermediate precision was previously known as part of ruggedness. The attribute evaluates the reliability of the method in a different environment other than that used during development of the method. The objective is to ensure that the method will provide the same results when similar samples are analyzed once the method development phase is over. Depending on time and resources, the method can be tested on multiple days, analysts, instruments, etc.

Intermediate precision in the test method can be partly assured by good system suitability specifications. Thus, it is important to set tight, but realistic, system suitability specifications.

LINEARITY

The linearity of a method is its ability to obtain test results that are directly proportional to the analyte concentration over a given range. For HPLC methods, the relationship between analyte concentration and detector response (peak area or height) is used to make this determination.

Concentration Ranges

The concentration range used for linearity should be large enough to encompass the desired range of the method. A minimum of five concentration ranges should be investigated and a plot of the detector response vs. the sample concentration should be generated. It is important that the concentration ranges selected for the linearity study are relatively equally spaced throughout the range of the method (e.g., 50%, 75%, 100%, 125% and 150%), and not clustered, as this will provide a skewed estimation of linearity.

Acceptance Criteria

Acceptance criteria should be evaluated to ensure that they are meaningful when compared with the performance of the method. Table 6 gives a list of suggested acceptance criteria for use in evaluating method linearity. The ranges in Table 6 are suggestions only and should be adjusted to ensure that all specification limits are within the validated linear range for any given method, Under most circumstances, regression coefficient (r) is 0.999. Intercept and slope should be indicated.

Statistical Analysis

Linearity data should be evaluated using appropriate statistical methods. A simple regression line of the detector response vs. the analyte concentration is the most common means of evaluation. Regulatory agencies require the submission of the correlation coefficient, y-intercept, slope of the regression line, and the residual sum of squares for linearity evaluation.

A graphical representation of the linearity data should also be generated. Additional analysis of the deviation of the actual values from the regression line is suggested, especially when the method uses a single-point calibration standard. The percent y-intercept is calculated by dividing the y-intercept by the detector response at the nominal concentration expressed as a percentage. For single-point calibration, this value should be less than 1 -2% to ensure accurate results.

Table No.3: Statistical analysis

Test	Level	Range	Acceptance criteria
Assay	5	50% to 150%	R> 0.999
Dissolution	5-8	10% to 150%	R > 0.99
Impurity	5	LOQ to 2%	R > 0.98

LIMIT OF DETECTION

These limits are normally applied lo related substances in the drug substance or drug product. Specifications on these limits arc submitted with the regulatory impurities method relating to release and stability of both drug substance and drug product.

Limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions. With UV detectors, it is difficult to assure the detection precision of low-level compounds due to potential gradual loss of sensitivity of detector lamps with age, or noise level variation by detector manufacturer.

At low levels, assurance is needed that the detection and quantification limits are achievable with the test method each time. With no reference standard for a given impurity or means to assure detectability, extraneous peak(s) could "disappear/appear." A crude method to evaluate the feasibility of the extraneous peak detection is to use the percentage claimed for detection limit from the area counts of the analyte. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental.

Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on Signal-to-Noise

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

Based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as:

$$D.L. = \frac{3.3\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of s may be carried out in a variety of ways, for example

Based on the Standard Deviation of the Blank

Analyzing an appropriate number of blank samples and calculating the standard deviation of these responses perform measurement of the magnitude of analytical background response.

Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Recommendations

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

LIMIT OF QUANTIFICATION

Limit of quantification is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Several approaches for determining the quantification limit

are possible, depending on whether the procedure is a non-instrumental or instrumental.

Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The quantification limit is generally determined by the analysis of samples with known concentrations of anatyte and by establishing the minimum level at which the analyte can be quantified with, acceptable accuracy and precision.

Based on Signal-to-Noisc Approach

This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analytewilh those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

Based on the Standard Deviation of the Response and the Slope

TJhe quantification limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of s may be carried out in a variety of ways.

Based on Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the Calibration Curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

Recommendations

The quantification limit and the method used for determining the quantification limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantification limit. Otherwise the information that is expressed as % area or height of the drug substance peak from the same HPLC chromatogram willbe biased. It should also be noted that the extraneous peak using area count does not consider the detection response that depends on the UV extinction coefficient or absorptivity of the compound.

RUGGEDNESS

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, analysts, instruments, reagents, elapsed assay times, assay temperatures, or days. It is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method.

Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Recommendations

The ruggedness of an analytical method is determined by analysis of aliquots from homogeneous lots in different laboratories, by different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay.

The degree of reproducibility of test results is then determined as a function of the assay variables. This reproducibility may be compared to the precision of the assay under normal conditions to obtain a measure of the ruggedness of the method.

ROBUSTNESS

ICH defines robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Robustness can be partly assured by good system suitability specifications. The evaluation of

robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters(e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- Stability of analytical solutions
- Extraction time

In the case of liquid chromatography, examples of typical variations are

Influence of variations of pH in a mobile phase

- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate.

In the case of gas chromatography, examples of typical variations are

- Different columns (different lots and/or suppliers)
- Temperature.

LITERATURE REVIEW

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORM BY RP-HPLC, WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES,

A simple, sensitive and rapid reverse phase HPLC method was developed for the simultaneous estimation of Amlodipine besylate and Telmisartan. A Phenominex-luna C18 column (250x4.6 mm i.d 5µ) was used with a mobile phase containing a mixture of acetonitrile and phosphate buffer in the ratio of 56:44%v/v.pH was adjusted with orthophosphoric acid to 4.The flow rate was 1ml/min and the eluents were monitored at the detector wavelength of 236nm.The retention times of Amlodipine besylate and Telmisartan were found to be 4.32 and 5.32 minutes respectively.The validation of the proposed method was carried out for its specificity, accuracy, pecision, linearity, limit of detection and limit of quantification for both Amlodipine and Telmisartan. **(Rajeswari A1, et al..; 2013)**

Validation method for simultaneous estimation of amlodipine and telmisartan by RP-HPLC in bulk and pharmaceutical dosage form.

A simple, rapid, precise, accurate and highly sensitive High Performance Liquid Chromatographic method has been developed and validated for the simultaneous quantitative estimation of Amlodipine and Telmisartan in bulk pharmaceutical dosage form. Chromatography was carried on Boston pH lex C18 ODS (150 x 4.6 mm) 5µ Column with mobile phase based comprising mixture of pH 3.0 ammonium acetate buffer: acetonitrile – 70: 30 v/v. The flow rate was

adjusted to 0.75 mL/min with UV detection at 236 nm. The retention times of Amlodipine and Telmisartan were found to be 2.21 and 6.72 min respectively. The different analytical parameters such as linearity, accuracy, precision, ruggedness and robustness were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response is linear in the range of 1-7 µg/mL and 8-56 µg/mL respectively. In the linearity study, the regression equation and coefficient of correlation for Amlodipine and Telmisartan were found to be (y = 39.68x + 0.494, r = 0.999), (y = 649.5x+ 116.9, r = 0.998) respectively. The results of the all validation parameters were within their acceptance values. The proposed method was successfully applied for quantitative estimation of Amlodipine and Telmisartan. **(A. Aroon, D. Suchitra , 2016)**

DEVELOPMENT AND VALIDATION OF REVERSED-PHASE HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE IN TABLET DOSAGE FORM, was achieved on (Waters symmetry C18 250mm x 4.6mmµm) analytical column with mobile phase consisting mixture of Potassium dihydrogen phosphate (0.02M, pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in ratio (60:40 v/v) at flow rate of 1.5ml/min and detector wavelength 237 nm. The retention time of Amlodipine and telmisartan was found to be 3.5 and 8.1 minutes respectively. The validation of the proposed method was carried out for its specificity, linearity, accuracy, precision, limit of detection and quantification for both Telmisartan and Amlodipine. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation.simple, precise and accurate reversed phase liquid chromatographic method was developed and validated for simultaneous

estimation of Telmisartan and Amlodipine in tablet formulations. The chromatographic separation **(SURESH KUMAR GV a*, RAJENDRAPRASAD Yb, 2010)**

Handbook of Pharmaceutical Analysis for HPLC

Develop and validate a simple and rapid isocratic reversed-phase high performance liquid chromatographic method (RP-HPLC) for the simultaneous estimation of amlodipine and telmisartan in combined dosage form. Methods: The chromatographic separation was achieved by using mobile phase acetonitrile and 0.05M sodium dihydrogen phosphate buffer (60:40) adjusted to pH 6.0, a C-18 column, perfectsil target ODS3 (150 mm 4.6 mm i.d., 5 m). The mobile phase was pumped at a flow rate of 0.8 mL/min and the eluents were monitered at 254 nm. Results: Retention times were 4.0 min and 8.2 min for amlodipine and telmisartan respectively. The method was validated in terms of accuracy, precision, linearity, range, specificity, limit of detection and limit of quantitation. Linearity for amlodipine besylate and telmisartan was established in the range of 5-30 and 10-60 g/mL, respectively. The recoveries for the two compounds were above 96%. Conclusions: This method was found to be efficient, accurate, precise, specific and economic and is suitable for routine quality control analysis. **(Saurabh K et al., 2012).**

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE BESYLATE IN TABLET DOSAGE FORM BY RP-HPLC, WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Telmisartan and Amlodipine besylate in combination. The separation was carried out using a mobile phase consisting of acetonitrile: water: triethyelamine (68:31.8:0.2 v/v) with pH 4 adjust by using ortho-phosphoric acid. The column used was C-18 column (Inertsil ODS-3 250*4.6 mm) with flow rate of 1 ml / min using PDA detection at 240 nm. The described method was linear over a concentration range of 4-60 μg/ml and 10-150 μg/ml for the assay of Amlodipine and Telmisartan respectively. The retention times of Amlodipine and Telmisartan were found to be 2.3 and 2.7 mins respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Amlodipine and Telmisartan were found to be 0.0046 μg/ml and 0.0018 μg/ml, 0.014 and 0.0056 μg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Amlodipine and Telmisartan bulk drug and in its pharmaceutical dosage form. (**PathuriJnana Nagarjuna1, SreeVidyaParvataneni,2014)**

Method development and validation for simultaneous estimation of telmisartan and amlodipine by RP-HPLC

A novel, precise, accurate, rapid and effective isocratic RP-HPLC method was developed, optimized and validated for the estimation of Telimsartan (TEL) and

Amlodipine (AML) in pharmaceutical dosage forms (tablet). The drugs were estimated using Symmetry C18 (250 x 4.6 mm, 5 µm) column. A mobile phase composed of phosphate buffer of pH 6 and acetonitrile in the ratio of 40:60, v/v), at a flow rate of 0.8 ml/min was used for the separation. Detection was carried out at 243 nm. The linearity range obtained was 16-48 µg/ml for TEL and 2-6 µg/ml for AML with retention times (Rt) of 3.209 min and 5.351 min for TEL and AML respectively. The correlation coefficient values were found to be 0.999. Precession studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of TEL and AML were in the range of 98.01-101.62% and 99.30-101.40% respectively. The assay results of TEL and AML were 99.60% and 99.75% respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The developed validated method was successfully used for the quantitative analysis of commercially available dosage form. **(Vatchavai B R, 2017)**

AIM AND PLAN OF WORK

AIM

- To develop and validate a new isocratic RP-HPLC method for the determination of Amlodipine and Telmisartan in tablet dosage form.
- To validate the method with respective linearity, precision and accuracy.
- To develop the method suitable for the routine analysis of Amlodipine and Telmisartanin Pharmaceutical formulation.

OBJECTIVE

- The objective of validation of analytical procedure is to demonstrate that it is suitable for intended purpose.

PLAN OF WORK

- Solubility data, Analytical data is to be studied to develop the initial conditions.
- Selection of initial separation conditions and trails for assay of Amlodipine and Telmisartanin pharmaceutical dosage form.
- To develop a method for the assay of Amlodipine and Telmisartanin pure and marketed sample by HPLC.
- To validate the developed assay method according to 1CH method validation parameters.

Method development by HPLC method for Amlodipine and Telmisartan:

- Development of suitable mobile phase.
- Optimization of the chromatographicconditions
- Selection of suitable detection wavelength
- Preparation of standard calibration curve of Amlodipine and Telmisartan
- Assay of pure mixed standards and formulation
- Validation of the developed method.

The parameters that will be validated are

- System suitability
- Linearity
- Precision
- Accuracy

DRUG PROFILE

Amlodipine:

IUPAC Name : (RS)-3-ethyl 5-methyl 2-[(2-aminoethox)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridne-3,5-dicarboxylate.

Molecular formula : $C_{20}H_{25}ClN_2O_5$

Molecular weight : 409-879 g/mol

Category : Anti-hypertension

Structure :

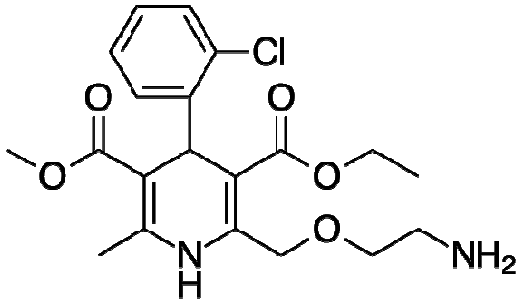


Fig No 3 : chemical structure of amlodipine.

Physical and chemical properties :

Appearance **:** Amlodipine is white and solid .

Solubility : water soluble -75.3mg/L

Melting point **:** 178-179 ^{0} c

Mechanism of action :

- Amlodipine is angioselective calcium channel blocker and inhibits the movement of calcium ions into vascular smooth muscle cells and cardiac muscle cells and which inhibits the contraction of cardiac muscles and vascular smooth muscle. This causes vasodilatation and reduction in peripheral vascular resistance, thus lowering BP and prevent excessive construction of Coronary arteries.

Adverse effect :

- Some common dose-dependent adverse effects of amlodipine include vasodilatory effects, peripherals edema, dizziness, palpitations, and flushing. Amlodipine may increase the risk of worsening angina or acute myocardium infraction, especially in those with severe obstructive coronary artery disease , upon dosage initiation or increase. **(www.merriam – webste.com, 2006)**

Medical use:

- Amlodipine treats hypertension and coronary artery disease in people with either stable angina or vasopastic angina and without heart failure **(Wang, J G ,2009).**

TELMISARTAN:

IUPAC Name **:** 2-[4-[[4-methyl-6-(1-methylbenzimidazole-2-yl)-2-prepylbenzimidazole-1-yl]methyl]Phenyl]benzoic acid **(http//www.drugbank.ca/drugs./DB009966)**

Molecular formula **:** $C_{33}H_{30}N_4O_2$

Molecular weight **:** 514-629 g/ml

Category **:** Anti hypertension

Structure :

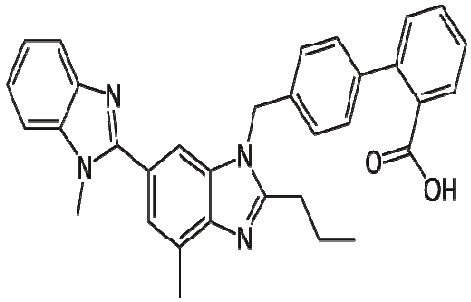


Fig no.4 : chemical structure of Telmisartan.

Physical and Chemical Properies :

Appearance **:** Telmisartar is a white to slightly yellowish solid

Melting point : 261-236°C

Solubility **:** Practically insoluble in water and in the pH range of 3 to 9

Mechanism of action :

- Telmisartan is an angiotensin II receptor blocker that shows high a inffinity for the angiotension II acceptor type 1, with a binding affinity 3000 times greater for AT_1 than AT_2

- In addition to blocking the RAS, telmisartan acts as a selective molecular of peroxisome proliferators–activated receptor gamma (PPAR-r) a central regulator of insulin and glucose metabolism. It is believed that telmisartan'sdual made of action may provide benefits against the vascular and renal damage caused by diabetes and cardiovascular disease(CVD)

Adverse Effect:

- Side effects are similar to other angiatensin 11 receptor antagonists and include tachycardia and bradycardia (fast or slow heart beat),hypertension (low blood pressure), edema and allergic reactions

Medical use:

- Telmisartan is used to treat high blood pressure, heart failure and diabetic kidney disease. **(Drug.com, 2006)**

MATERIALS AND METHODS

Drug (Active Pharmaceutical Ingredients)

Sl. No.	Drug Name	Supplier
1	Telmisartan	Glenmark Pharmaceuticals Ltd.,
2	Amlodipine	Glenmark pharmaceutical Ltd.,

List of Instruments

S.No	Instruments
1	LC –Zolocht, Shimadzu
2	Aegispak C_{18} – F 5 µm, 120 A, 4.6 X 250 mm P/N = 05AF04626 S/N = 05AFdf11204
3	Semi micro balance – Shimadzu
4	Ultra Sonic cleaner Citizon
5	Vaccum filter pump
6	Denver Instrument
7	HI2215 PH/ORP meter

LC Solution software

Chemicals and Solvents used

Sl. No.	Chemical and Solvents
1	Acetonitrile (HPLC grade)
2	Water (HPLC grade)
3	Methanol (HPLC grade)
4	Acetic acid (A.R grade)

ANALYTICAL METHOD DEVELOPMENT :

- Proper selection of HPLC method development depends upon the nature of the sample, its molecular weight and solubility .
- For success method development, various chromatographic parameters such as pH, mobile phase , its composition and proportion, detection wavelength and other factors were exhaustively studied

SELECTION OF CHROMATOGRAPHIC METJHOD:

- Proper selection of method depends upon the nature o the sample , molecular weight, and solubility.
- Non polar compounds can be separate by either normal phase or reverse phase chromatography.

SELECTION OF DILUENT:

- The nature of the drug reveals certain information about the drug such as solubility, pKa. Based o the solubility of the drug, the diluent is selected. Amlodipine and Telmisartan are soluble in methanol, so methanol is used as diluent.

SELECTION OF THE DETECTION WAVELENGTH:

- Standard solution of amlodipine and telmisartan were injected separately as all as in combination into HPLC system and then scanned over entire UV range (234-238).
- The spectrum of Amlodipine and Telmisartan was recorded for determination of λmx.

- The λmx of Amlodipine and Telmisartan were detected at 234nm.

SELECTION OF COLUMN:

- In reverse phase chromatography non polar stationary phase is used for separation.
- C8, C18 are the commonly used columns in reverse phase chromatography.
- Aegispak C_{18} – F, 5 µm, 120 A, 4.6 X 250 mm, is used of the separation.

SELECTION OF MOBILE PHASE:

A number of trails were made to fine out the mobile phase for eluting the drug. The mobile phase selected are

Acetonirile:waer:methanol (50:30:20) which contains HPLC grad methanol+3ml acetic acid + HPLC acetonitrile + HPLC grade water.

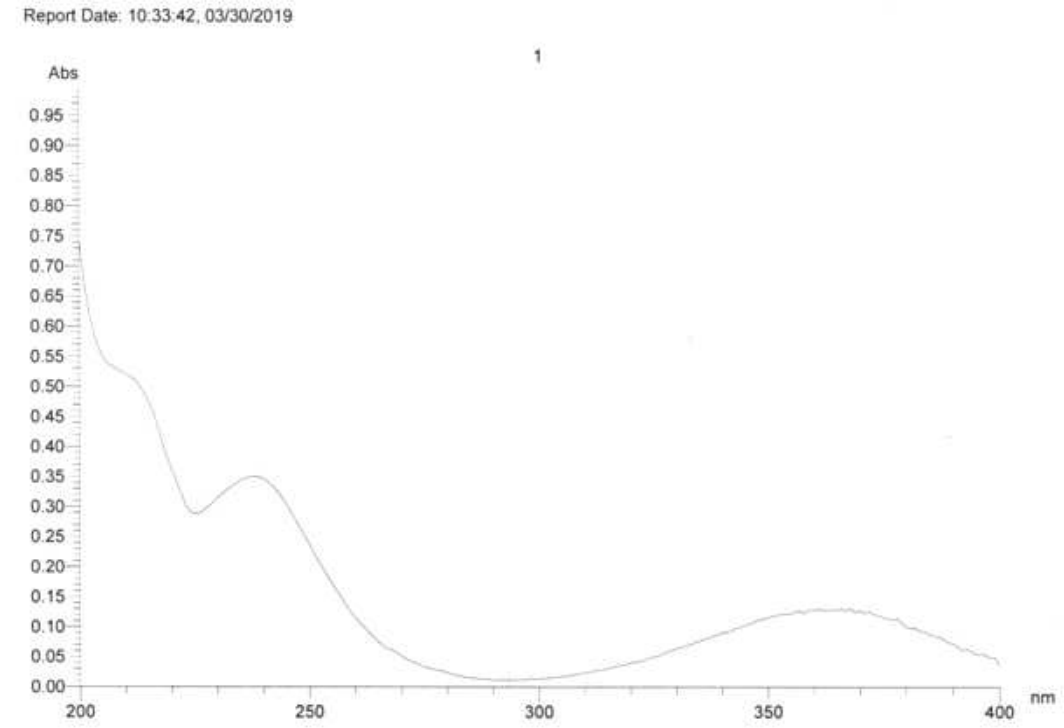
PREPARAION OF STANDARD SOLUTION:

Accurately weigh and transfer 13.63mg of Amlodipine and 80.33mg of Telmisartan working standard in to separate 10ml clear dry volumetric flask containing methanol. The solution was sonicated for about 10mins and then made upto volume with methanol.

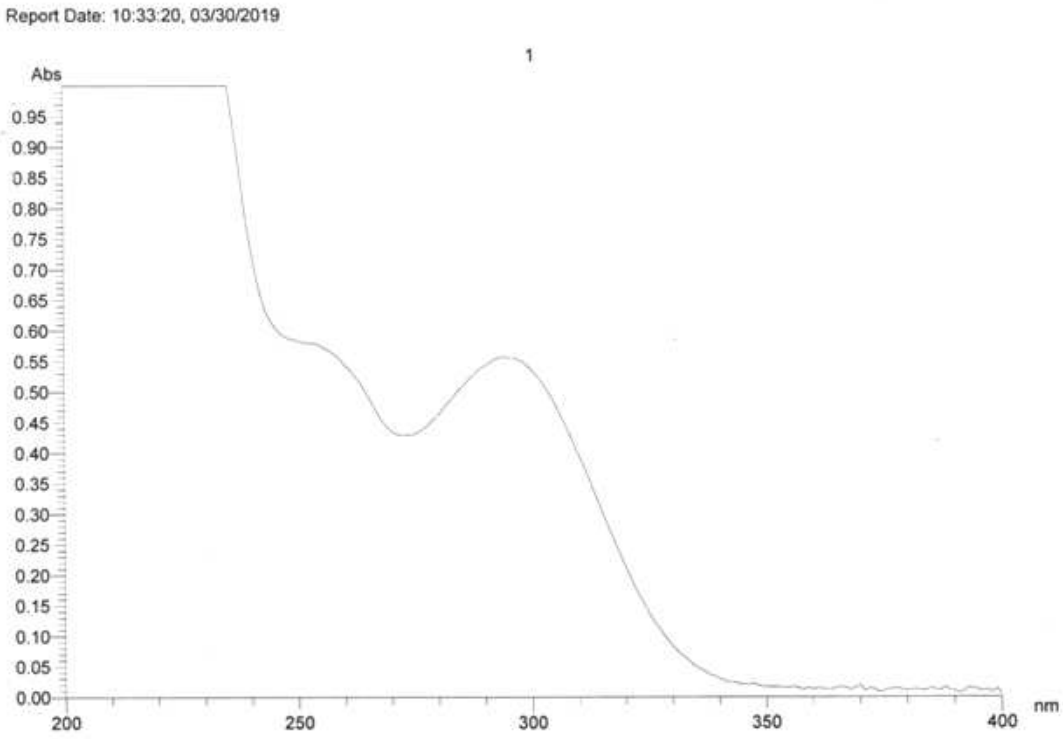
PREPARATION OF SAMPLE STOCK SOLUTION:

10 tablets were taken, powdered and weighted. Average weight was taken. From this amount of powder equivalent to 13.63mg of Amlodipine and 80.33mg of Telmisartanws as taken into 10ml volumetric flask containing mobile phase. The solution was sonicated for 10mins and made upto volume with mobile phase.

UV SPECTRA OF AMLODPINE AND TELMISARTAN:



Amlodipine UV Spectra



Sample: 1
File name: TELMISARTAN.UDS
Run Date: 17:33:12, 03/28/2019
Operator: Administrator
Comment: TELMISARTAN

Peak Integration
Method: Rectangular
Sensitivity: 1
Threshold: 0.0100

Peaks

Peak #	Apex (nm)	Height (Abs)	Area (Abs*nm)
1	294.0	0.557	24.477

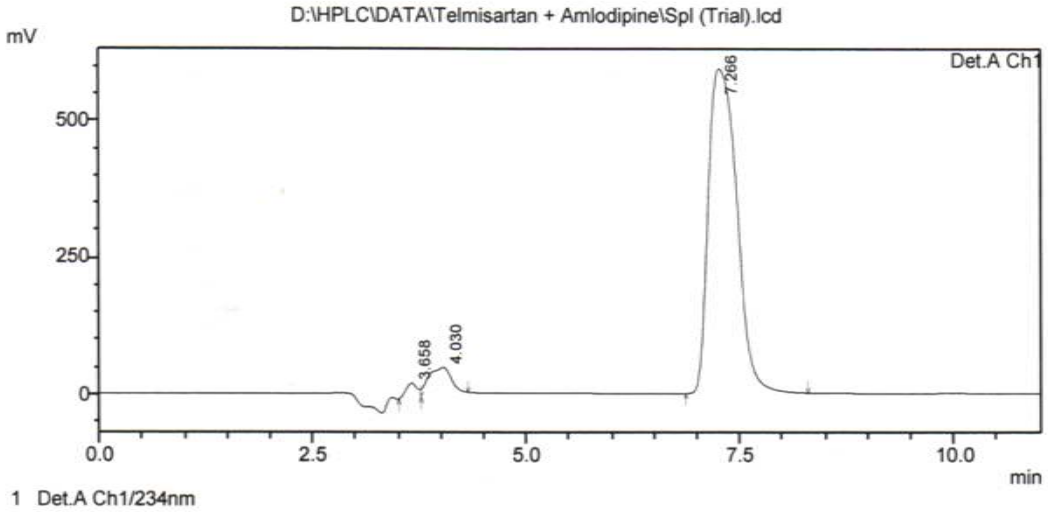
Telmisartan UV Spectra

TRAILS METHODS:

The following trails were conducted.

TRAIL-1

Mobile phase	:	Methanol :formate buffer (70:30)
Column	:	Aegispak C_{18} – F , 5 µm, 120 A, 4.6 X 250 mm
Detection wavelength	:	234nm
Flow rate	:	2.0 min/ml
Injection volume	:	20µl/min
Column temperature	:	room temperature
Run time	:	30min
Observation	:	**peak shape is not good**



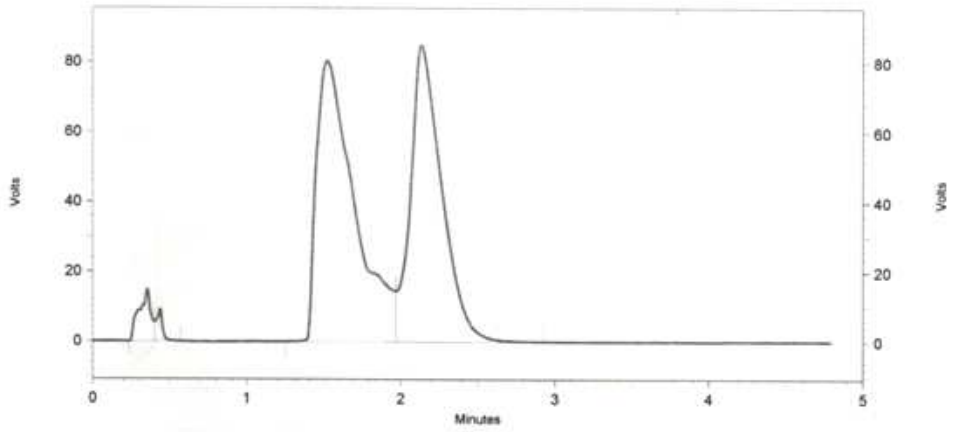
PeakTable

Detector A Ch1 234nm

Name	Ret. Time	Area	Area %	Theoretical Plates/mete	Tailing Facto
	3.658	245739	1.639	17964	0.00
Amlodipine	4.030	929474	6.198	7791	0.00
Telmisartan	7.266	13821279	92.163	17174	1.43
		14996493	100.000		

TRAIL - II

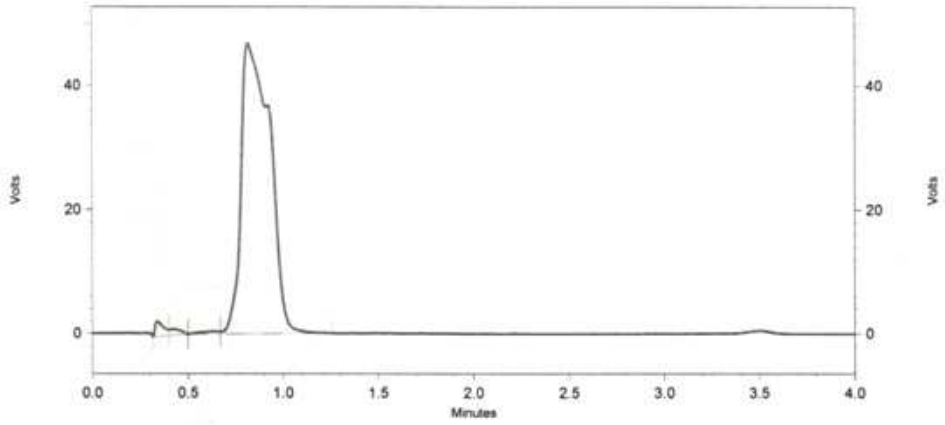
Mobile phase	:	Methanol : acetate buffer (85:15)
Column	:	Aegispak C_{18} – F , 5 µm, 120 A, 4.6 X 250 mm
Detection wavelength	:	234nm
Flow rate	:	1.5min/ml
Injection volume	:	20µl/min
Column temperature	:	room temperature
Run time	:	30 min
Observation	:	**peak shape is not good**



Name	Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
	0.355	78671	2.94	84	0.00	0.00
	0.438	25312	0.95	583	0.73	0.00
	1.522	1380796	51.62	184	4.16	0.00
	2.132	1190146	44.49	502	1.47	0.00
Totals		2674925	100.00			

TRAIL - III

Mobile phase	:	Methanol :Acetonitrile : Water (50:30:20)
Column	:	Aegispak C_{18} – F , 5 µm, 120 A, 4.6 X 250 mm
Detection wavelength	:	234nm
Flow rate	:	1.5min/ml
Injection volume	:	20µl/min
Column temperature	:	room temperature
Run time	:	30 min
Observation	:	**Amlodipine and Telmisartan peaks was observed**



Name	Retention Time	Area	Area %	Theoretical plates (USP)	Resolution (USP)	Asymmetry
	0.338	7568	1.48	263	0.00	0.00
	0.428	3847	0.75	0	0.00	0.00
	0.630	2992	0.59	113	0.00	0.00
	0.815	496652	97.18	173	0.76	1.56
Totals		511059	100.00			

OPTIMIZED METHOD:

PREPARATION OF BUFFER AND MOBILE PHASE :

Prepare 300ml water +500ml acetonitrile +200ml methanol +3ml acetic acid , pH 5.5 are mixed and filtered.

HPLC CHROMATOGRAPHIC CONDITIONS FOR OPTIMIZATION:

Instrument	:	LC –Zolocht, Shimadzu
Column	:	Aegispak C18 – F , 5 µm, 120 A, 4.6 X 250 mm
Detection wavelength	:	234nm
Flow rate	:	1.5min/ml
Injection volume	:	10µl/min
Column temperature	:	room temperature
Run time	:	5 min
Mobile phase	:	Methanol :Acetonitrile : Water (50:30:20)

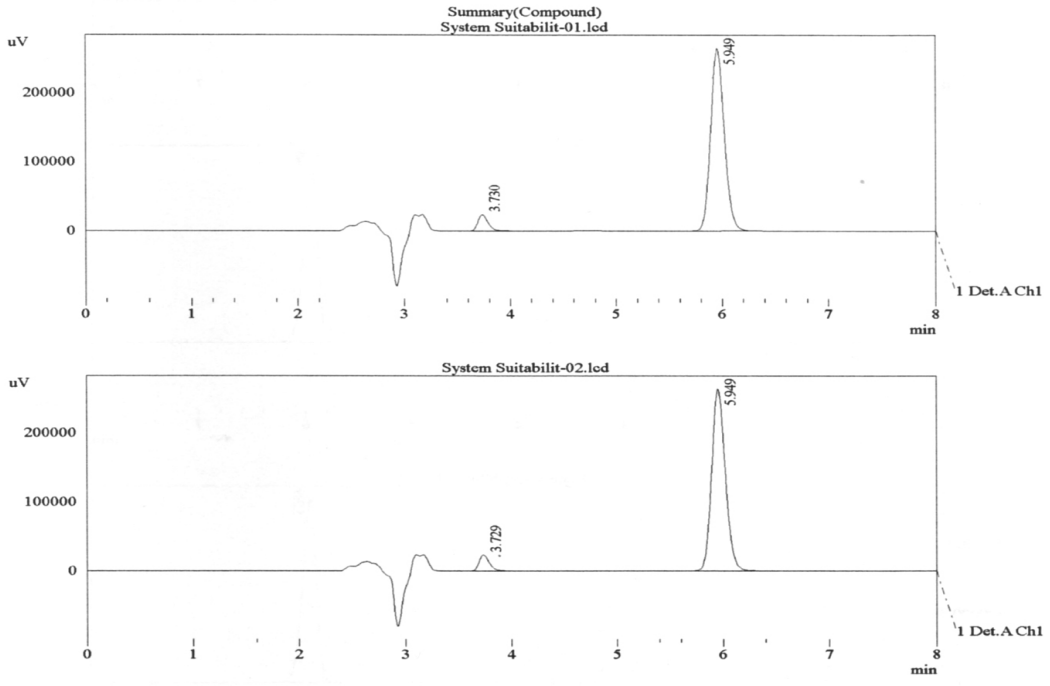
VALIDATION

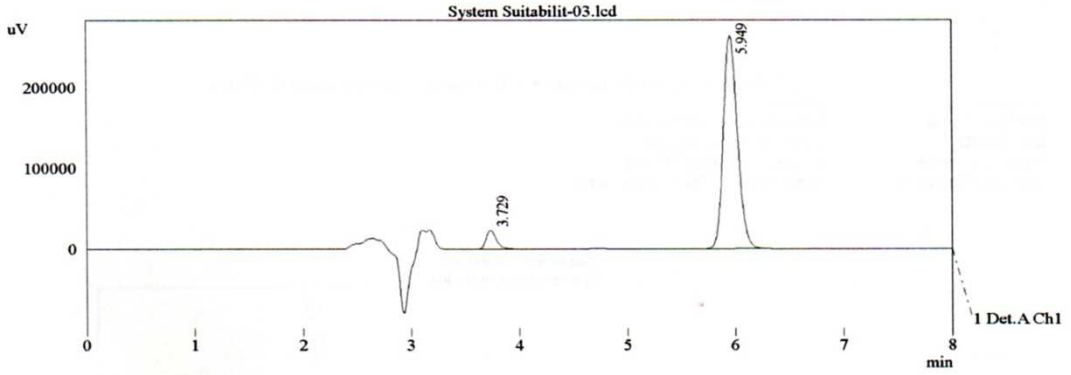
SYSTEM SUITABILITY:

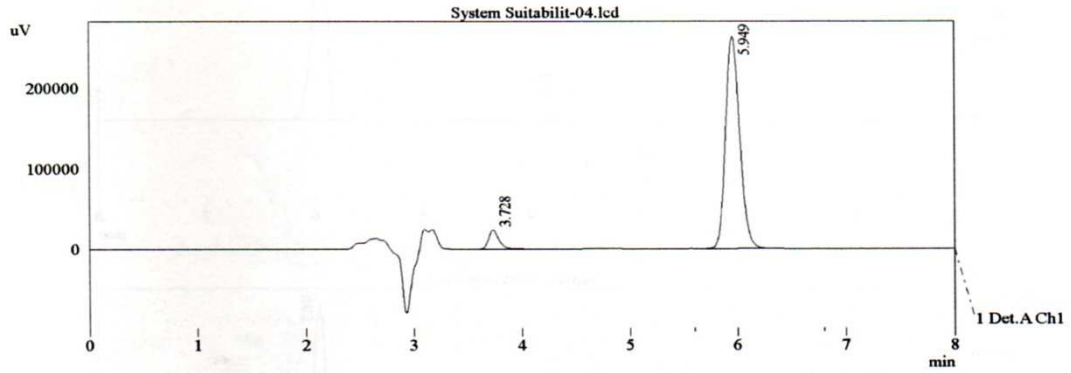
- Mixed working standard solutions were injected and chromatograms were recorded.
- The system suitability studies were carried out as specified in USP.
- These parameters include column efficiency, Resolution, capacity factor , theoretical plates and tailing factor.

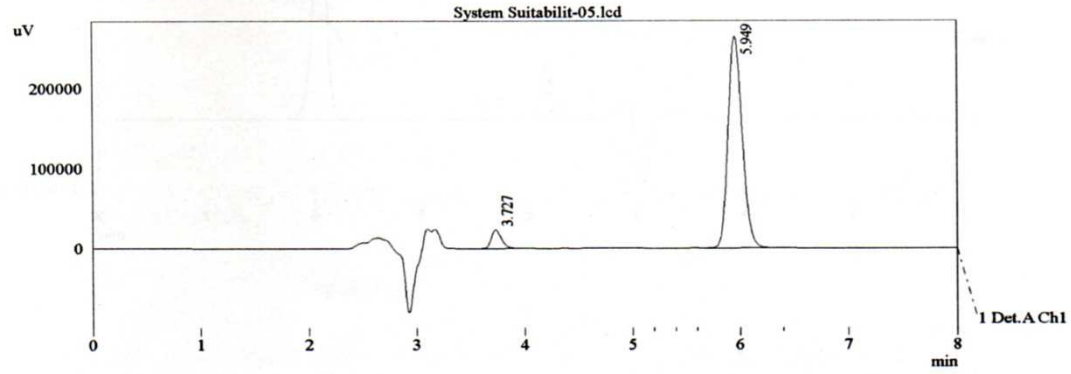
Acceptance Criteria:

- The % RSD for the retention time of principle peak from 5 replicate injections of each standard solution should be not more than 2.0%
- The number of theoretical plates (N)for the drug peak is not less than 2500.
- The tailing factor (T)for the drug peak is not more than 2.0.









<< Detector A >>
ID#1 Compound Name: Amlodipine

Title	Sample Name	Ret. Time	Area	Area %	ieoretical Plat	Tailing Factor
System Suitabilit-01.lcd	Amlodipine + Te	3.730	155713	6.063	6551.440	1.386
System Suitabilit-02.lcd	Amlodipine + Te	3.729	154819	6.066	6563.787	1.380
System Suitabilit-03.lcd	Amlodipine + Te	3.729	155642	6.065	6575.730	1.385
System Suitabilit-04.lcd	Amlodipine + Te	3.728	155542	6.065	6563.934	1.387
System Suitabilit-05.lcd	Amlodipine + Te	3.727	155722	6.068	6560.237	1.388
Average		3.729	155488	6.065	6563.026	1.385
%RSD		0.032	0.245	0.030	0.133	0.223
Maximum		3.730	155722	6.068	6575.730	1.388

Title	Sample Name	Ret. Time	Area	Area %	ieoretical Plat	Tailing Factor
Minimum		3.727	154819	6.063	6551.440	1.380
Standard Deviation		0.001	381	0.002	8.730	0.003

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	ieoretical Plat	Tailing Factor
System Suitabilit-01.lcd	Amlodipine + Te	5.949	2412628	93.937	9173.134	1.161
System Suitabilit-02.lcd	Amlodipine + Te	5.949	2397627	93.934	9184.836	1.158
System Suitabilit-03.lcd	Amlodipine + Te	5.949	2410622	93.935	9183.196	1.161
System Suitabilit-04.lcd	Amlodipine + Te	5.949	2409065	93.935	9170.214	1.162
System Suitabilit-05.lcd	Amlodipine + Te	5.949	2410615	93.932	9192.653	1.162
Average		5.949	2408111	93.935	9180.807	1.161
%RSD		0.005	0.249	0.002	0.099	0.146
Maximum		5.949	2412628	93.937	9192.653	1.162
Minimum		5.949	2397627	93.932	9170.214	1.158
Standard Deviation		0.000	5996	0.002	9.129	0.002

LINEARITY:

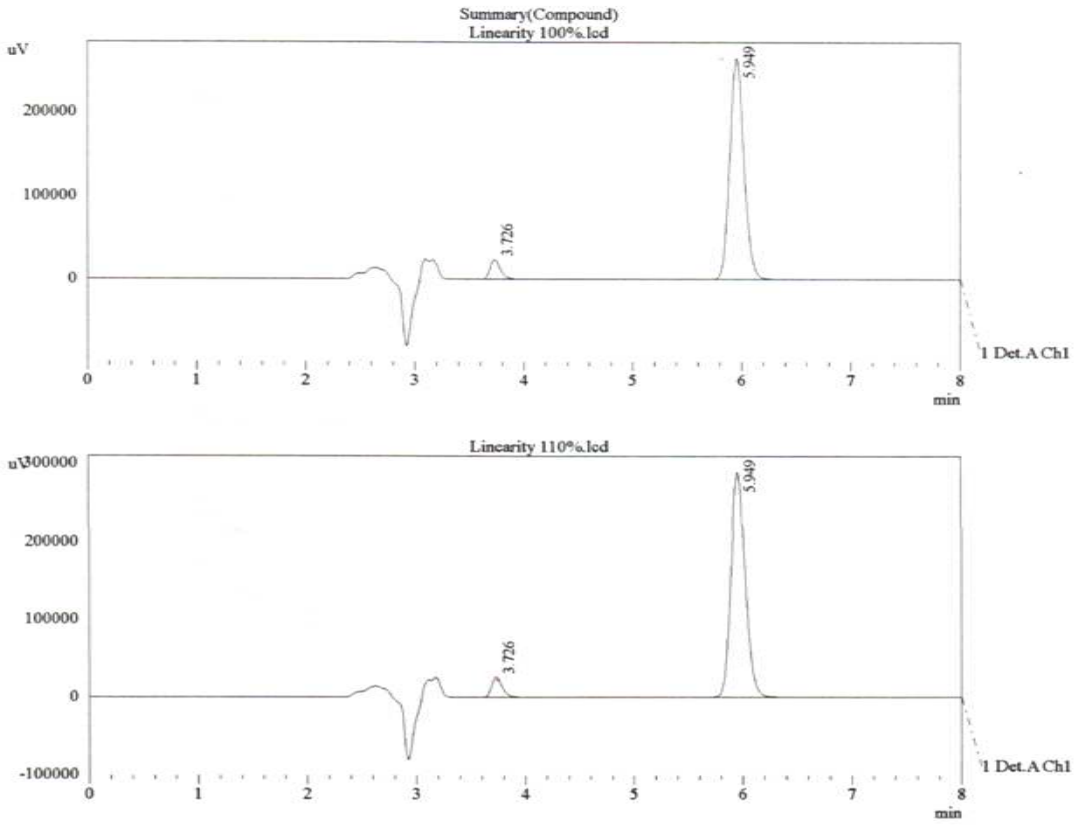
PREPRATION OF STANDARD STOCK SOLUTON :

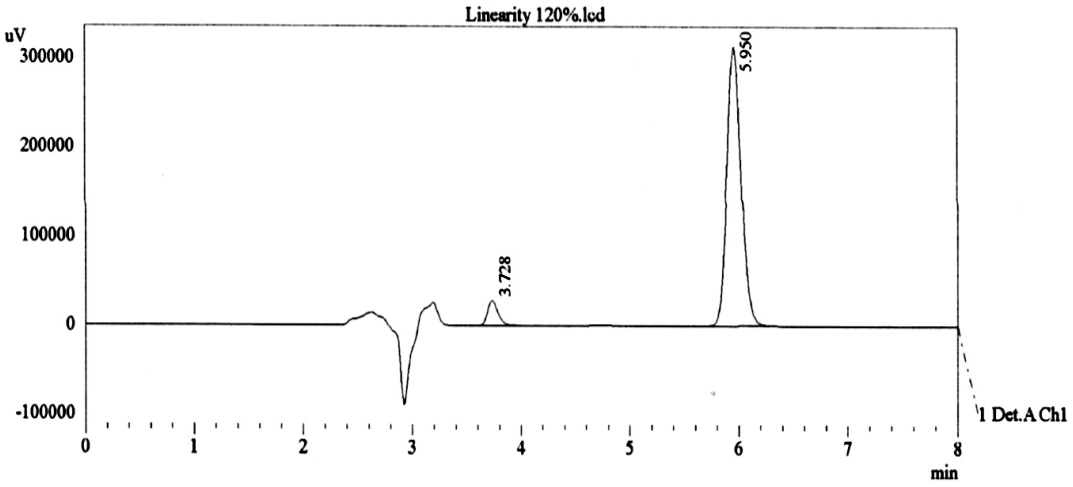
Accurately weigh and transfer 13.63mg of Amlodipine and 80.33mg of Telmisartan working standard in to separate 10ml clear dry volumetric flask containing methanol . the solution was sonicated for about 10mins and then made upto volume with methanol.

PREPARATION OF INTERMEDIATE DILUTION:

2ml of Amlodipine solution was taken from the preparation standard stock solution in a 10ml volumetric flask and made up to volume with mobile phase .

1.30ml ofTelmisartan solution was taken from the preparation standard stock solution in a 10ml volumetric flask and made up to volume with mobile phase.

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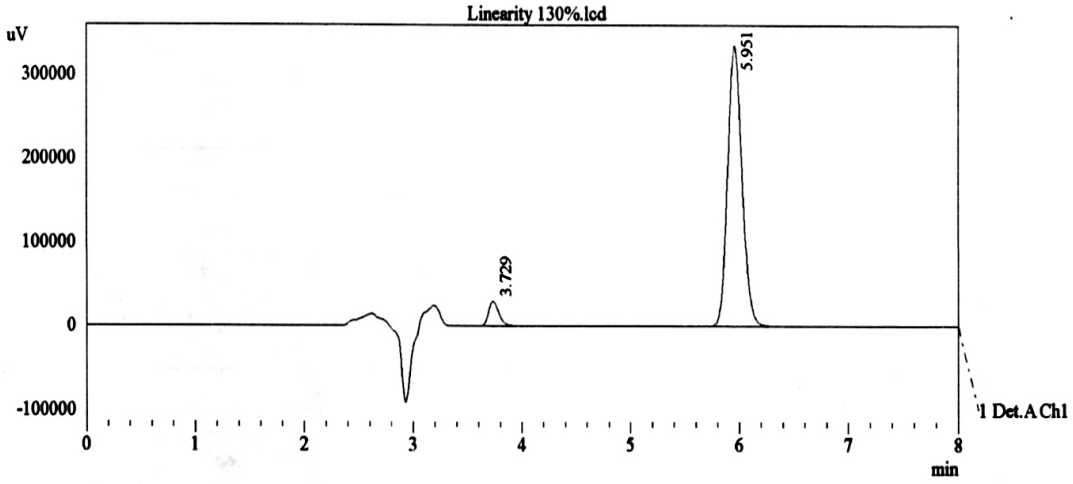
1 Det.A Ch1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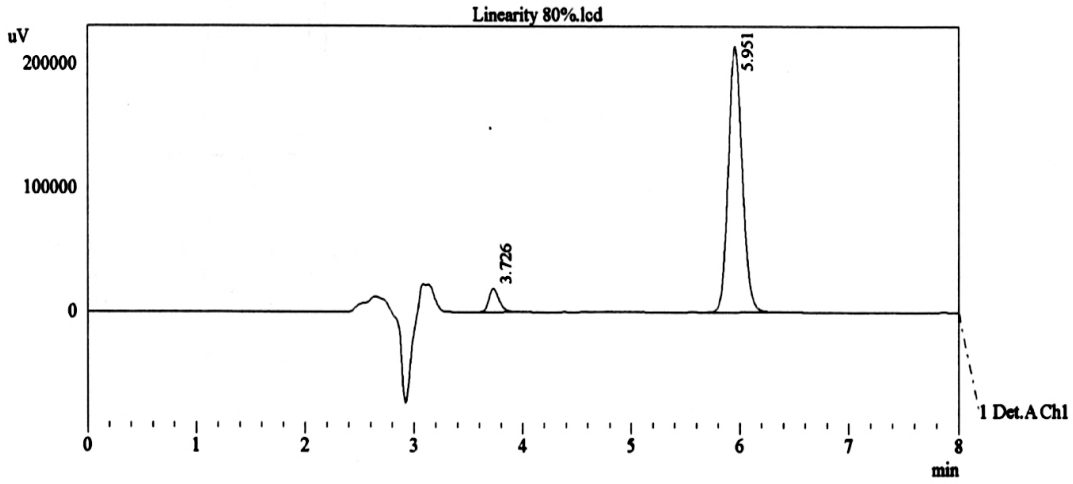
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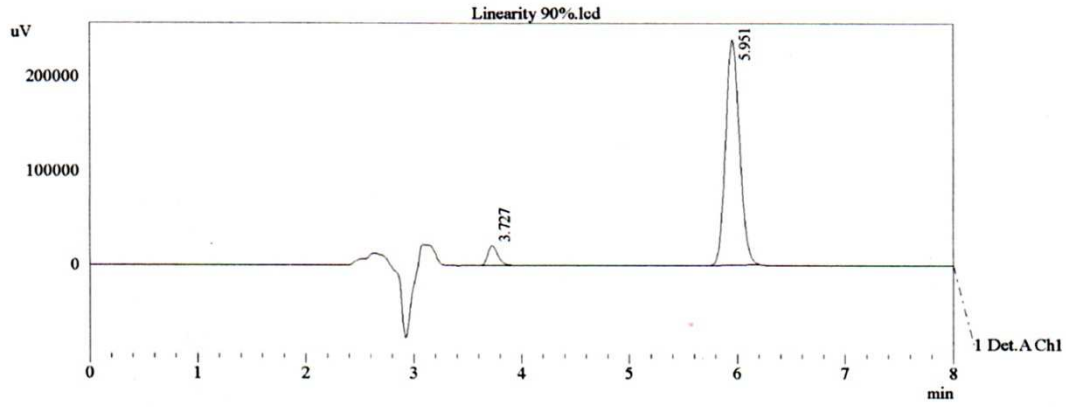


<< Detector A >>
ID#1 Compound Name: Amlodipine

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Linearity 100%.lcd	Amlodipine + Te	3.726	155781	6.065	6548.017	1.395
Linearity 110%.lcd	Amlodipine + Te	3.726	171535	6.071	6511.538	1.391
Linearity 120%.lcd	Amlodipine + Te	3.728	187411	6.072	6462.605	1.385
Linearity 130%.lcd	Amlodipine + Te	3.729	203013	6.078	6417.943	1.382
Linearity 80%.lcd	Amlodipine + Te	3.726	124688	6.051	6632.939	1.397
Linearity 90%.lcd	Amlodipine + Te	3.727	140110	6.110	6597.435	1.394
Average		3.727	163756	6.074	6528.413	1.391
%RSD		0.036	17.929	0.326	1.242	0.429
Maximum		3.729	203013	6.110	6632.939	1.397
Minimum		3.726	124688	6.051	6417.943	1.382
Standard Deviation		0.001	29361	0.020	81.109	0.006

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Linearity 100%.lcd	Amlodipine + Te	5.949	2412949	93.935	9164.580	1.163
Linearity 110%.lcd	Amlodipine + Te	5.949	2653871	93.929	9048.686	1.163
Linearity 120%.lcd	Amlodipine + Te	5.950	2899121	93.928	8900.677	1.163
Linearity 130%.lcd	Amlodipine + Te	5.951	3137223	93.922	8734.677	1.163
Linearity 80%.lcd	Amlodipine + Te	5.951	1935891	93.949	9402.678	1.163
Linearity 90%.lcd	Amlodipine + Te	5.951	2152898	93.890	9341.898	1.157
Average		5.950	2531992	93.926	9098.866	1.162
%RSD		0.012	17.918	0.021	2.824	0.220
Maximum		5.951	3137223	93.949	9402.678	1.163
Minimum		5.949	1935891	93.890	8734.677	1.157
Standard Deviation		0.001	453688	0.020	256.949	0.003

ACCURACY:

The closeness of agreement between the true value which is accepted either conventional new value or an accepted reference value and the value found.

PREPRATION OF STANDARD STOCK SOLUTON :

Accurately weigh and transfer 13.63mg of Amlodipine and 80.33mg of Telmisartan working standard in to separate 10ml clear dry volumetric flask containing methanol . the solution was sonicated for about 10mins and then made upto volume with methanol.

PREPARATION OF INTERMEDIATE DILUTION:

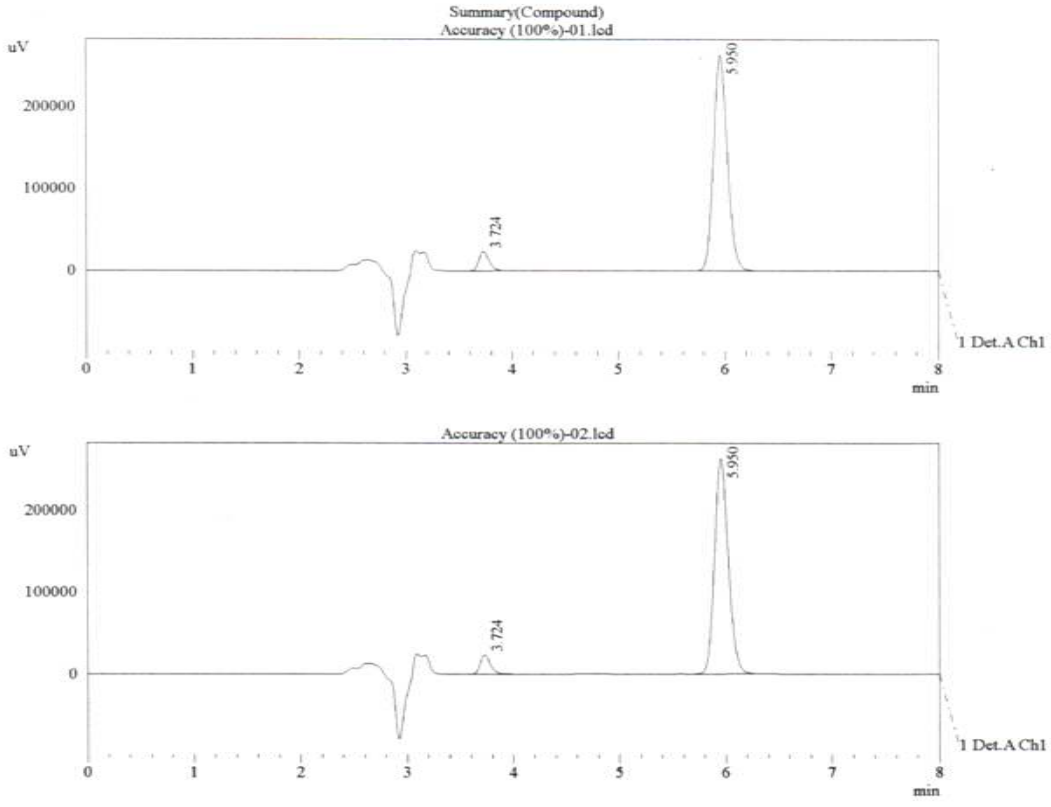
2ml of Amlodipine solution was taken from the preparation standard stock solution in a 10ml volumetric flask and made up to volume with mobile phase . 1.30ml of Telmisartan solution was taken from the preparation standard stock solution in a 10ml volumetric flask and made up to volume with mobile phase .

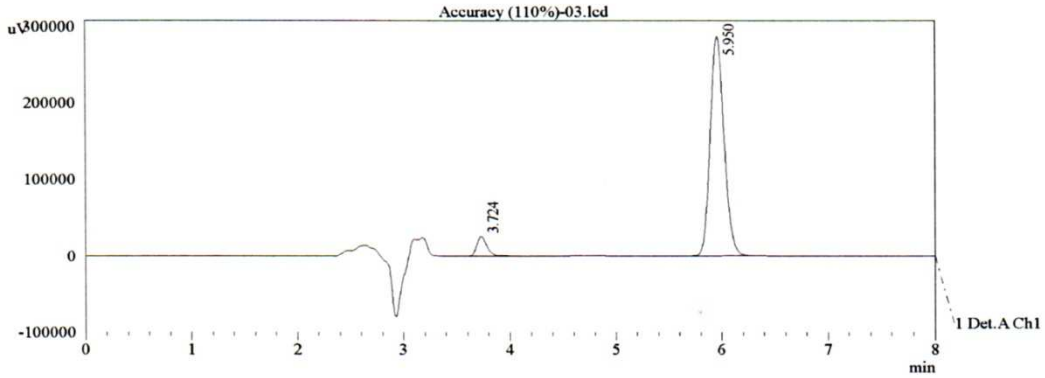
PROCEDURE:

Inject the standard solution , accuracy 110% , accuracy 120%, accuracy 130% solution . calculate the amount of amlodipine and telmisartan added and calculate the individual recovery and mean recovery values.

ACCEPTAENCE CRITERIA:

The recovery for each level should be between 98.0 to 102.0





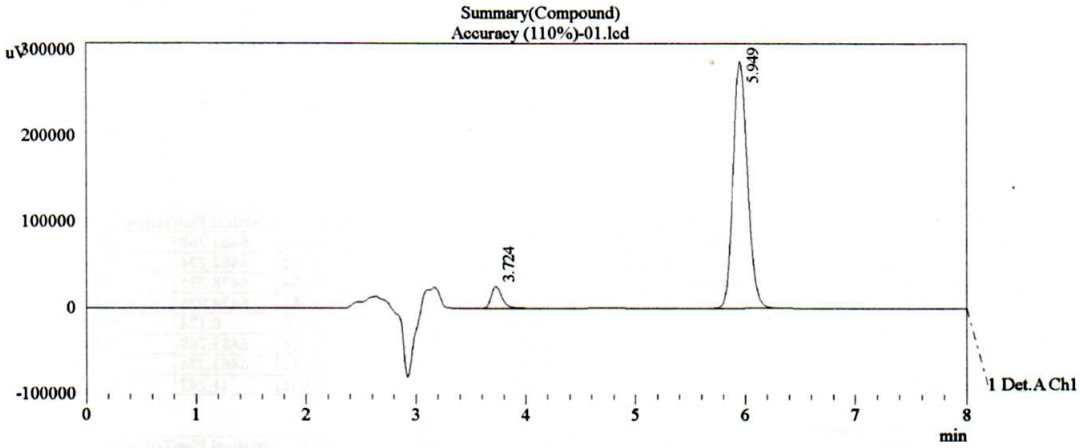
<< Detector A >>

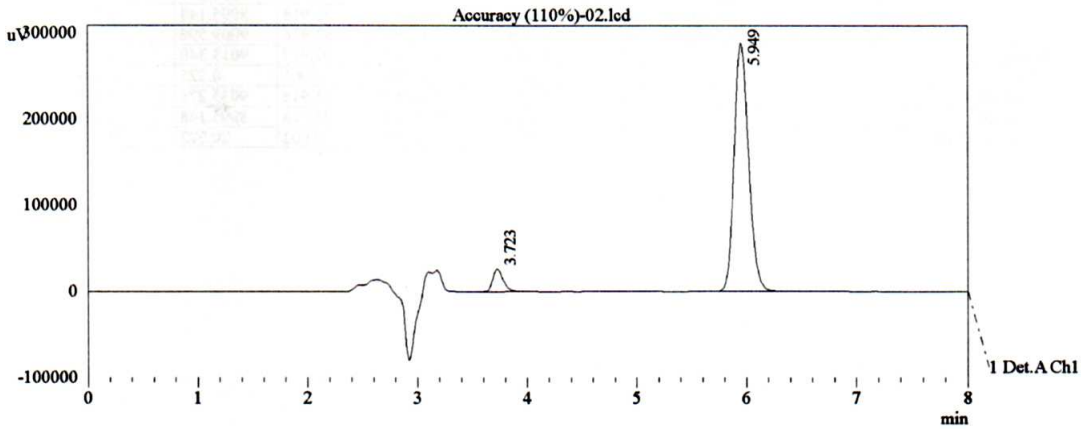
ID#1 Compound Name: Amlodipine

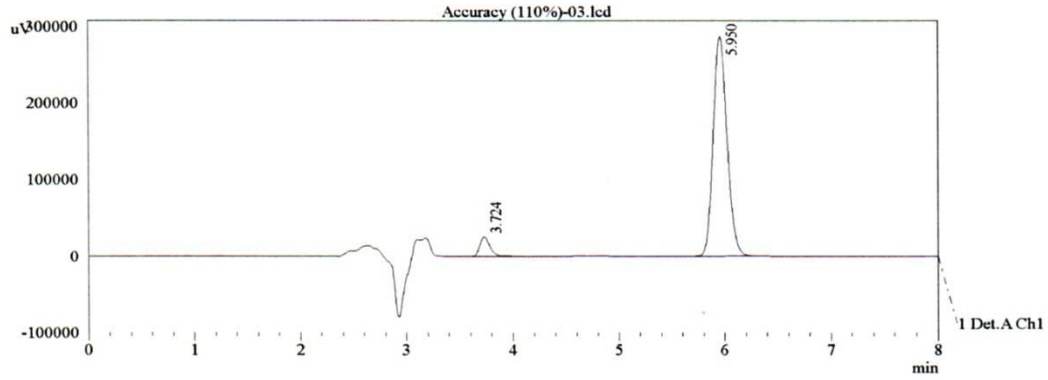
Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (110%)-01.lcd	Amlodipine + Te	3.724	170891	6.084	6483.768	1.402
Accuracy (110%)-02.lcd	Amlodipine + Te	3.723	171496	6.082	6462.274	1.402
Accuracy (110%)-03.lcd	Amlodipine + Te	3.724	171367	6.084	6478.735	1.404
Average		3.724	171251	6.083	6474.926	1.403
%RSD		0.014	0.186	0.018	0.174	0.093
Maximum		3.724	171496	6.084	6483.768	1.404
Minimum		3.723	170891	6.082	6462.274	1.402
Standard Deviation		0.001	318	0.001	11.242	0.001

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (110%)-01.lcd	Amlodipine + Te	5.949	2638039	93.916	9035.275	1.166
Accuracy (110%)-02.lcd	Amlodipine + Te	5.949	2648281	93.918	8995.148	1.168
Accuracy (110%)-03.lcd	Amlodipine + Te	5.950	2645504	93.916	9009.598	1.168
Average		5.949	2643941	93.917	9013.340	1.168
%RSD		0.011	0.200	0.001	0.225	0.094
Maximum		5.950	2648281	93.918	9035.275	1.168
Minimum		5.949	2638039	93.916	8995.148	1.166
Standard Deviation		0.001	5297	0.001	20.323	0.001







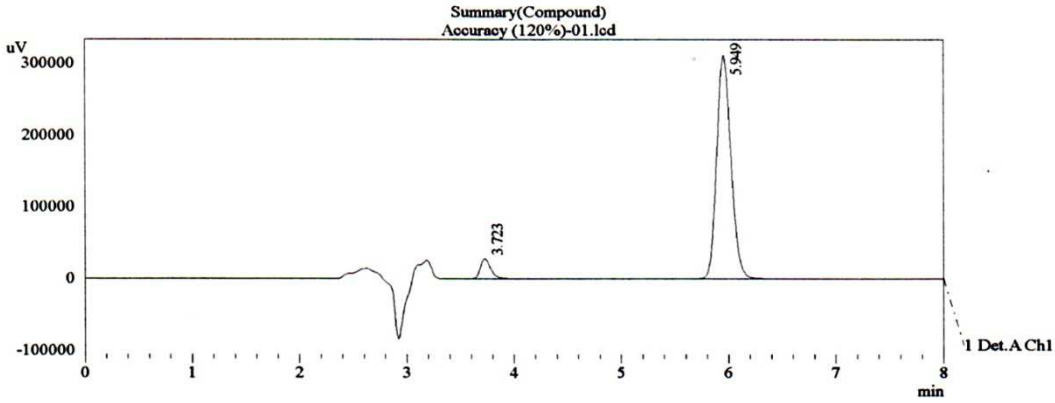
<< Detector A >>

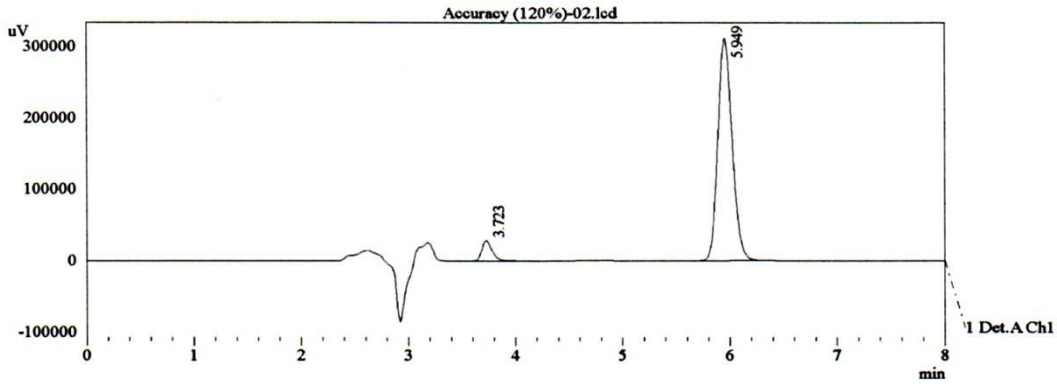
ID#1 Compound Name: Amlodipine

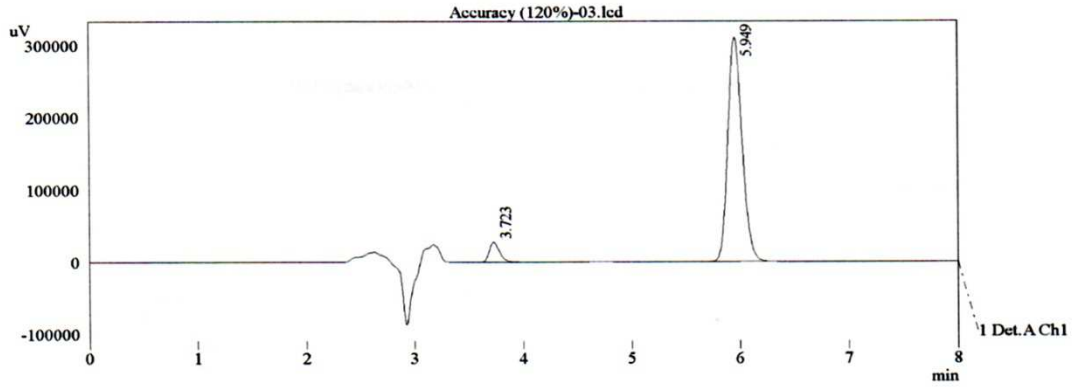
Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (110%)-01.lcd	Amlodipine + Te	3.724	170891	6.084	6483.768	1.402
Accuracy (110%)-02.lcd	Amlodipine + Te	3.723	171496	6.082	6462.274	1.402
Accuracy (110%)-03.lcd	Amlodipine + Te	3.724	171367	6.084	6478.735	1.404
Average		3.724	171251	6.083	6474.926	1.403
%RSD		0.014	0.186	0.018	0.174	0.093
Maximum		3.724	171496	6.084	6483.768	1.404
Minimum		3.723	170891	6.082	6462.274	1.402
Standard Deviation		0.001	318	0.001	11.242	0.001

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (110%)-01.lcd	Amlodipine + Te	5.949	2638039	93.916	9035.275	1.166
Accuracy (110%)-02.lcd	Amlodipine + Te	5.949	2648281	93.918	8995.148	1.168
Accuracy (110%)-03.lcd	Amlodipine + Te	5.950	2645504	93.916	9009.598	1.168
Average		5.949	2643941	93.917	9013.340	1.168
%RSD		0.011	0.200	0.001	0.225	0.094
Maximum		5.950	2648281	93.918	9035.275	1.168
Minimum		5.949	2638039	93.916	8995.148	1.166
Standard Deviation		0.001	5297	0.001	20.323	0.001







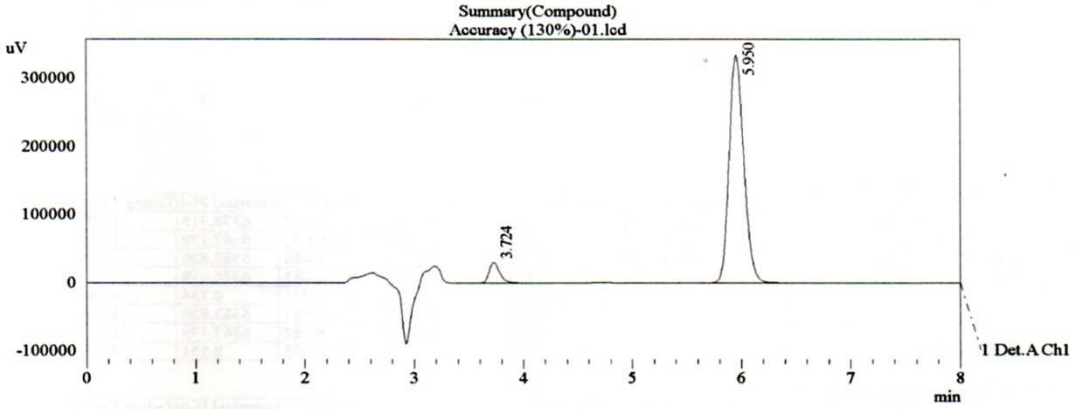
<< Detector A >>

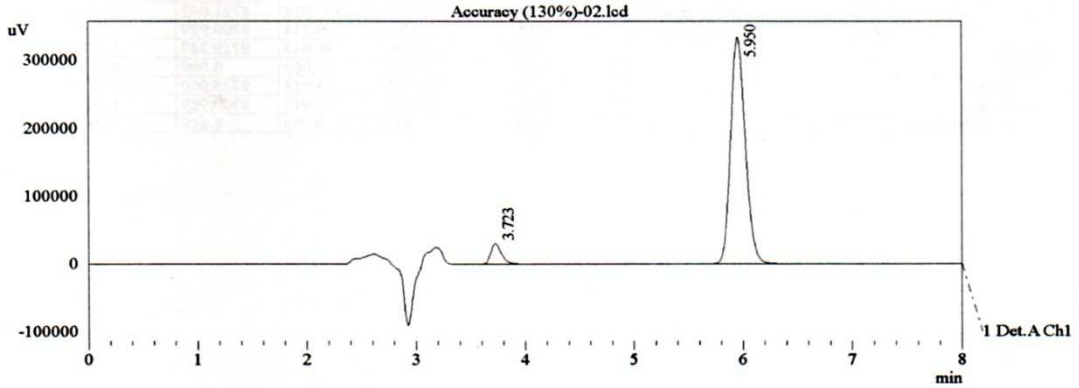
ID#1 Compound Name: Amlodipine

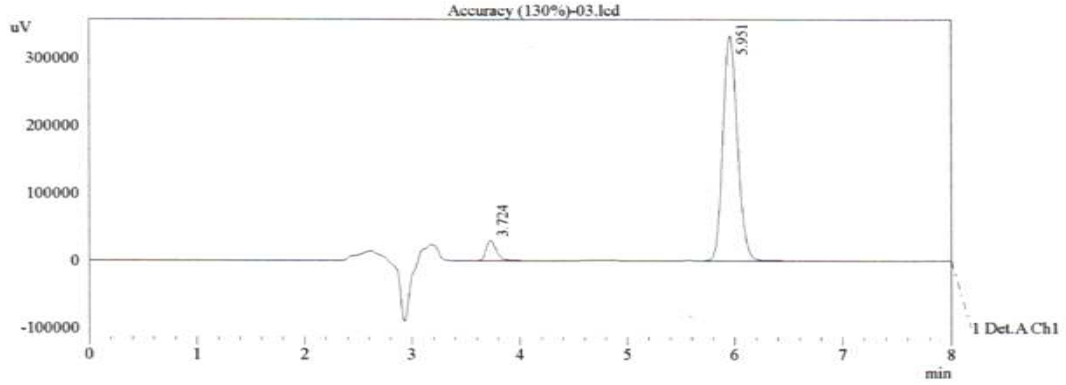
Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (120%)-01.lcd	Amlodipine + Te	3.723	187101	6.087	6425.630	1.398
Accuracy (120%)-02.lcd	Amlodipine + Te	3.723	187162	6.087	6428.917	1.399
Accuracy (120%)-03.lcd	Amlodipine + Te	3.723	187328	6.089	6425.228	1.400
Average		3.723	187197	6.088	6426.592	1.399
%RSD		0.002	0.063	0.017	0.031	0.066
Maximum		3.723	187328	6.089	6428.917	1.400
Minimum		3.723	187101	6.087	6425.228	1.398
Standard Deviation		0.000	117	0.001	2.024	0.001

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (120%)-01.lcd	Amlodipine + Te	5.949	2886856	93.913	8876.539	1.168
Accuracy (120%)-02.lcd	Amlodipine + Te	5.949	2887472	93.913	8877.811	1.168
Accuracy (120%)-03.lcd	Amlodipine + Te	5.949	2889354	93.911	8878.955	1.169
Average		5.949	2887894	93.912	8877.768	1.168
%RSD		0.004	0.045	0.001	0.014	0.066
Maximum		5.949	2889354	93.913	8878.955	1.169
Minimum		5.949	2886856	93.911	8876.539	1.168
Standard Deviation		0.000	1301	0.001	1.208	0.001







<< Detector A >>

ID#1 Compound Name: Amlodipine

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (130%)-01.lcd	Amlodipine + Te	3.724	202741	6.087	6378.915	1.397
Accuracy (130%)-02.lcd	Amlodipine + Te	3.723	202669	6.082	6367.179	1.395
Accuracy (130%)-03.lcd	Amlodipine + Te	3.724	202816	6.086	6383.820	1.399
Average		3.724	202742	6.085	6376.638	1.397
%RSD		0.018	0.036	0.041	0.134	0.140
Maximum		3.724	202816	6.087	6383.820	1.399
Minimum		3.723	202669	6.082	6367.179	1.395
Standard Deviation		0.001	73	0.003	8.551	0.002

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Accuracy (130%)-01.lcd	Amlodipine + Te	5.950	3128227	93.913	8726.960	1.170
Accuracy (130%)-02.lcd	Amlodipine + Te	5.950	3129643	93.918	8721.092	1.169
Accuracy (130%)-03.lcd	Amlodipine + Te	5.951	3129701	93.914	8709.989	1.170
Average		5.950	3129190	93.915	8719.347	1.170
%RSD		0.011	0.027	0.003	0.099	0.025
Maximum		5.951	3129701	93.918	8726.960	1.170
Minimum		5.950	3128227	93.913	8709.989	1.169
Standard Deviation		0.001	835	0.003	8.619	0.000

PRECISION:

PREPRATION OF STANDARD STOCK SOLUTION :

Accurately weigh and transfer 13.63mg of Amlodipine and 80.33mg of Telmisartan working standard in to separate 10ml clear dry volumetric flask containing methanol . the solution was sonicated for about 10mins and then made upto volume with methanol.

PREPARATION OF INTERMEDIATE DILUTION:

2ml of Amlodipine solution was taken from the preparation standard stock solution in a 10ml volumetric flask and made up to volume with mobile phase.

1.30ml of Telmisartan solution was taken from the preparation standard stock solution in a 10ml volumetric flask and made up to volume with mobile phase.

PROCEDURE:

The standard solution was injected six times and measure the area for all six injection in HPLC.

The % RSD for the area of six replicates injections was found to be within the specified limits.

Standard deviation $$SD = \sqrt{\frac{\sum (r_i - r_{avg})^2}{n-1}}$$

Where , x= sample

x^i=mean value of sample

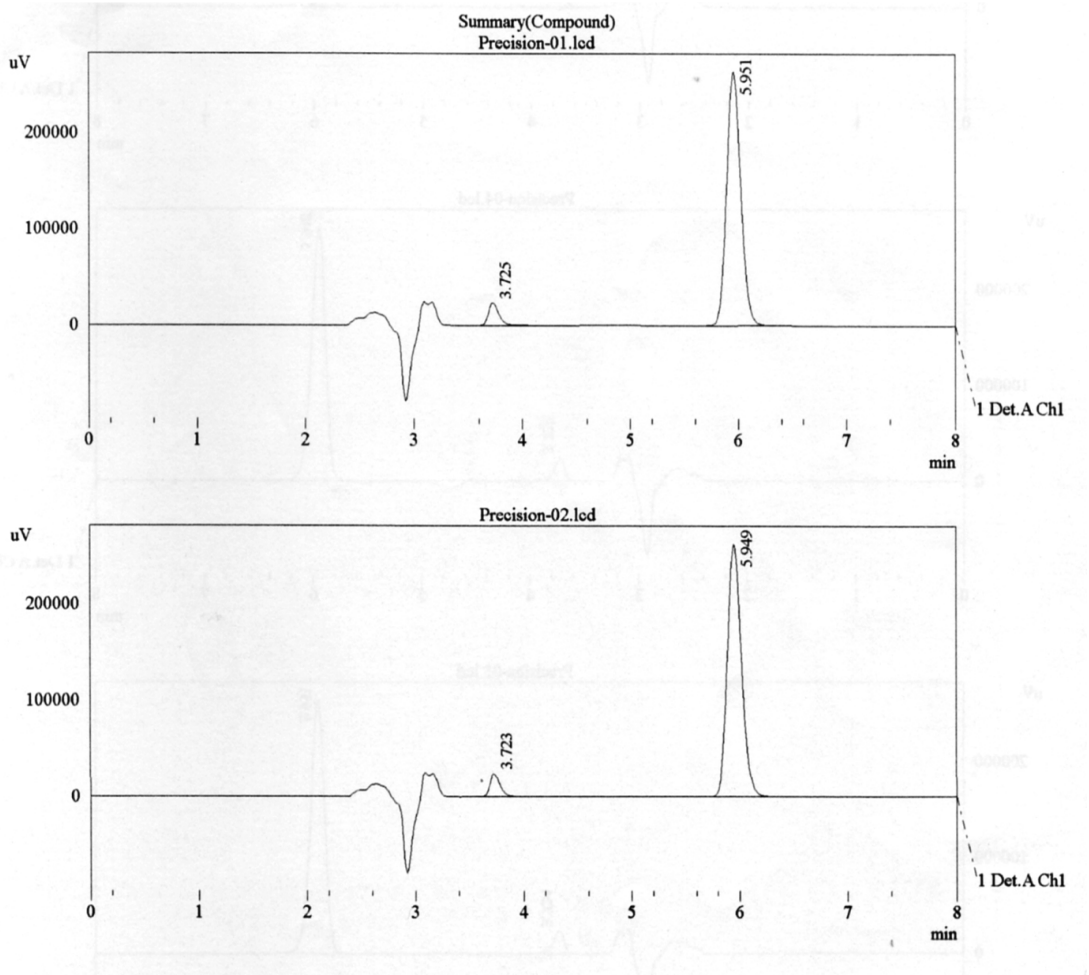
N= number of sample

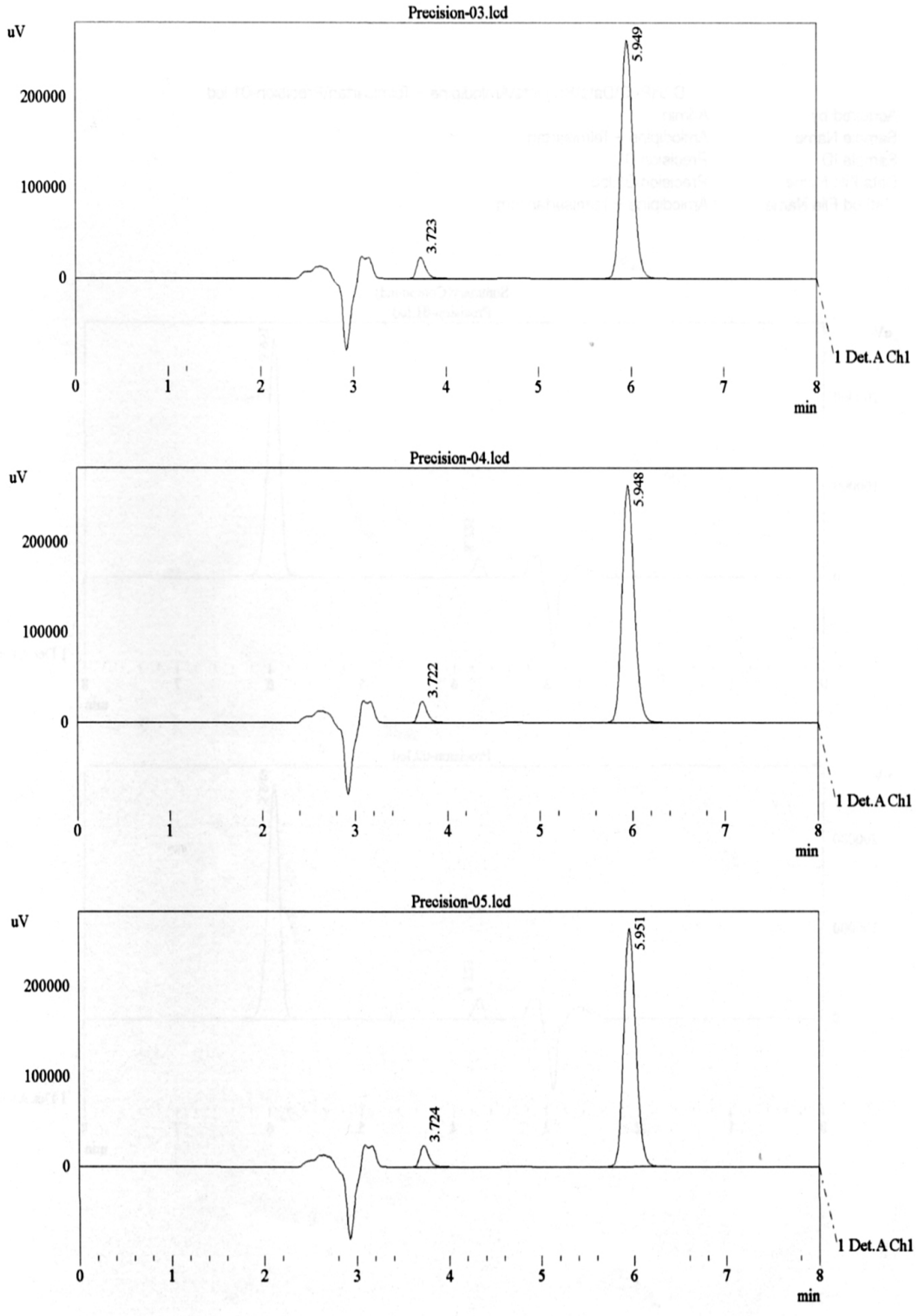
Coefficient of variance / Relative deviation :

$$CV\ (\%) = \left(\frac{Standard\ deviation}{Mean}\right) \times 100$$

Acceptance criteria:

The % RSD for the area of six standard injection results should not be more than 2%.

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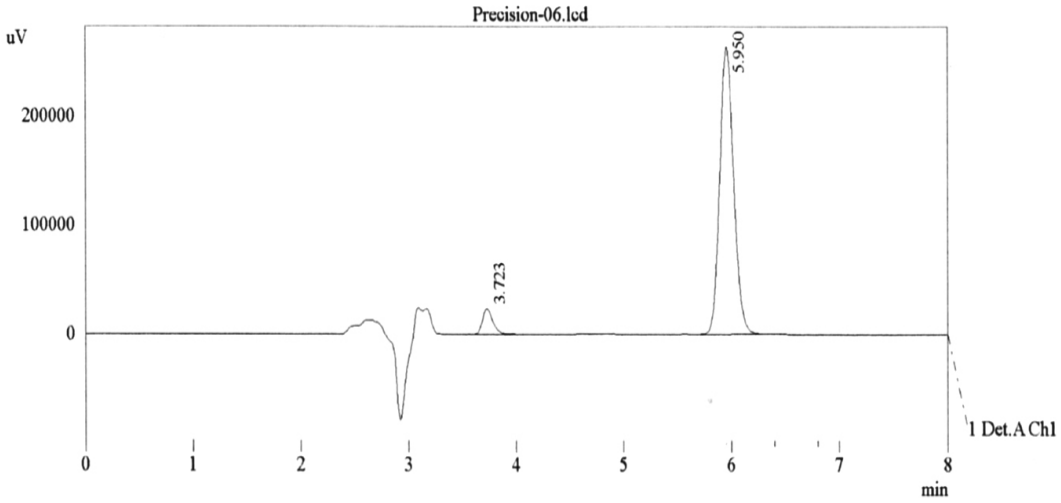


<< Detector A >>

ID#1 Compound Name: Amlodipine

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Precision-01.lcd	Amlodipine + Te	3.725	155262	6.077	6587.733	1.381
Precision-02.lcd	Amlodipine + Te	3.723	154993	6.077	6544.438	1.400
Precision-03.lcd	Amlodipine + Te	3.723	155435	6.079	6526.361	1.402
Precision-04.lcd	Amlodipine + Te	3.722	155364	6.080	6504.441	1.397
Precision-05.lcd	Amlodipine + Te	3.724	155521	6.077	6551.102	1.402
Precision-06.lcd	Amlodipine + Te	3.723	155567	6.078	6540.228	1.405
Average		3.724	155357	6.078	6542.384	1.398
%RSD		0.034	0.135	0.018	0.423	0.605
Maximum		3.725	155567	6.080	6587.733	1.405
Minimum		3.722	154993	6.077	6504.441	1.381
Standard Deviation		0.001	209	0.001	27.705	0.008

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	heoretical Plat	Tailing Factor
Precision-01.lcd	Amlodipine + Te	5.951	2399609	93.923	9231.662	1.154
Precision-02.lcd	Amlodipine + Te	5.949	2395482	93.923	9182.628	1.164
Precision-03.lcd	Amlodipine + Te	5.949	2401435	93.921	9156.899	1.166
Precision-04.lcd	Amlodipine + Te	5.948	2400154	93.920	9160.832	1.166
Precision-05.lcd	Amlodipine + Te	5.951	2403591	93.923	9171.559	1.164
Precision-06.lcd	Amlodipine + Te	5.950	2403823	93.922	9169.093	1.166
Average		5.950	2400682	93.922	9178.779	1.163
%RSD		0.019	0.128	0.001	0.299	0.412
Maximum		5.951	2403823	93.923	9231.662	1.166
Minimum		5.948	2395482	93.920	9156.899	1.154
Standard Deviation		0.001	3078	0.001	27.416	0.005

ASSAY:

Assay of different formulation available in market was carried out by injection sample corresponding to equivalent weight in to HPLC system and percentage pure was found out by following formula. Recovery studies were carried out.

Calculate the purity of Amlodipine and Telmisartan present in the tablet using the formula.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt.}{LC} \times 100$$

Where,

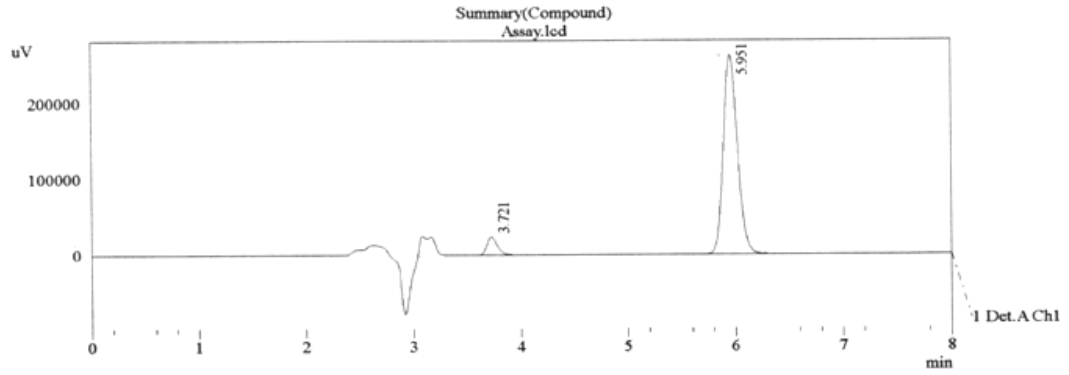
AT = Average are counts of sample preparation

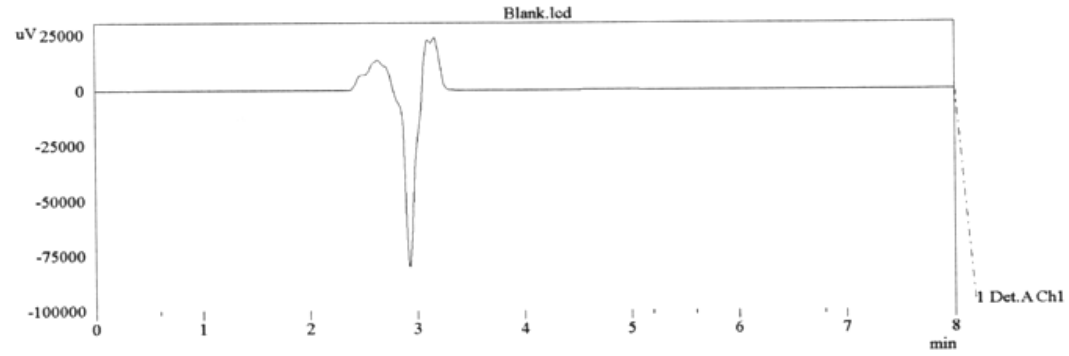
AS = Average area counts of standard preparation

WS = Weight of working standard taken in mg

P = Percentage purity of working standard

LC = Label claim





<< Detector A >>

ID#1 Compound Name: Amlodipine

Title	Sample Name	Ret. Time	Area	Area %	eoretical Plat	Tailing Factor
Assay.lcd	Amlodipine + Te	3.721	155743	6.080	6491.896	1.407
Blank.lcd	Amlodipine + Te	0.000	0	0.000	0.000	0.000
Average		3.721	155743	6.080	6491.896	1.407
%RSD		0.000	0.000	0.000	0.000	0.000
Maximum		3.721	155743	6.080	6491.896	1.407
Minimum		3.721	155743	6.080	6491.896	1.407
Standard Deviation		0.000	0	0.000	0.000	0.000

ID#2 Compound Name: Telmisartan

Title	Sample Name	Ret. Time	Area	Area %	eoretical Plat	Tailing Factor
Assay.lcd	Amlodipine + Te	5.951	2405848	93.920	9136.402	1.170
Blank.lcd	Amlodipine + Te	0.000	0	0.000	0.000	0.000
Average		5.951	2405848	93.920	9136.402	1.170
%RSD		0.000	0.000	0.000	0.000	0.000
Maximum		5.951	2405848	93.920	9136.402	1.170
Minimum		5.951	2405848	93.920	9136.402	1.170

RESULTS AND DISCUSSION

Estimation of Amlodipine and Telmisartan by HPLC method:

An exertion has been made, rapid, accurate, and precise stability indicating analytical method based on HPLC using PDA detection was developed and validated for assay determination of amlodipine and telmisartan in pure form and in tablet dosage form. The development trials were taken using different mobile phase with different composition. The column has been selected based on back pressure, peak shape, theoretical plates and retention time after evaluated all these factors, Aegispak C_{18} – F(5 µm, 120 A, 4.6 X 250 mm) column was found to be giving satisfactory result. Best results were obtained with acetonitrile : water : methanol (50:30:20). The selection of mobile phase acetonitril : water : methanol (50:30:20) was chosen to reduce longer retention time and to attain good peak shape.

Hence, the separation was carried out Aegispak C18 – F(5 µm, 120 A, 4.6 X 250 mm) column using mobile phase consisting acetonitrile : water : methanol (50:30:20). The flow rate 1.0ml/min and the injection volume was 10µL. the detection was carried out at 234 nm. The peak retention time of amlodipine and telmisartan were found to be 3.731 min and 5.944 min respectively. So this method was finalized as optimized method for the estimation of amlodipine and telmisartan .

The system suitability parameters like number of theoretical plates, tailing factor were calculated and all the parameters are within the limits. five injections were injected for the system suitability by using amlodipine and telmisartan, conducted concentration range 0.5-2.5 µg/mL. five independent determination were

performed as each concentration, and standard deviation were 0.003 and 0.002 for amlodipine and temisartan respectively.

Commercial formulation containing amlodipine and telmisartan was analysed by proposed method. Six replicate analysis of formulation were carried out and the mean percentage of amlodipine and telmisaratn were 99.66 and 99.19 respectively. Precision of the method was confirmed by repeatability studies. The system precision study was performed by analysis of the standard solution was repeated six times. The %RSD was found to be 0.223 and 0.146 for amlodipine and telmisartan respectively. The low percentage RSD value (%2) indicating that the method has good precision.

The accuracy of the method was confirmed by recovery studies. Amlodipine and telmisartanwere added to pre analysed formulation at the different levels viz.., 100%, 110%, 120% and 130%. Three replicated analysis were carried out for each level. The low %RSD value indicated that there is no interference due to excipients used in formulation. So the accuracy of the method was confirmed.

From the literature it was found that the HPLC method for the estimation of amlodipine and telmisartan but no stability indicating method was reported. So an attempt was made to develop a stability indicating analytical method.

The retention times in all HPLC method were more when compared to the developed methods can be applied for the estimation of amlodipine and telmisartan.

SUMMARY AND CONCLUSION

The developed chromatographic method or the simultaneous determination of amlodipine and telmisartan in dosage form is simple, reliable, sensitive and less time consuming. This method advantage is dose not require complicated mobile phase and it is simple method. This can be used or routine and quality control analysis of amlodipine and telmisartan in bulk and pharmaceutical dosage form.

The present work shows a validated, sensitive and selected method for the determination of amlodipine and telmisartan in pharmaceutical dosage form. Accuracy and precision are major parameters of control of the all validation procedures are within the acceptable limits.

This developed method has a valuable data for the validation of amlodipine and telmisartan combination method in bulk and pharmaceutical dosage form.

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