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A VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE AND ATENOLOL IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

Ms.G.Bhavani*,Mrs.K.Srivani[1],Mrs.N.Sandhya Rani[1]

Assistant Professors,Department of Pharmaceutical Analysis,Smt.Sarojini Ramulamma College of Pharmacy,Sesadri Nagar,Mahabub Nagar-5009001,Telangana,India

Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Amlodipine and Atenolol, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5 (35:65) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240 nm. The retention time of the Amlodipine and Atenolol was 2.256, 5.427 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Amlodipine and 50-250µg/ml of Atenolol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords *Amlodipine, Atenolol, RP-HPLC, validation.*

Corresponding author:

Ms.G.Bhavani,

Department of Pharmaceutical Analysis,
Smt.Sarojini Ramulamma College of Pharmacy,
Sesadri Nagar,Mahabub Nagar-5009001,
Telangana,India.
Email-yadavbhavani199801@gmail.com,
Mobile-+916301406837.

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INTRODUCTION:

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

Qualitative analysis is the identification of elements, species and/or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (QC) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies the levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays the commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research the chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

Analytical techniques there are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are

related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various analytical techniques. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic*, *molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2.

Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds.

Electrophoresis's another separation technique with similarities to chromatography that is particularly useful for this parathion of charged species. The principal separation techniques and their applications are listed in Table 1.

Analytical methods

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

The purpose of the analysis, the required time scale and any cost constraints;
The level of Analyte(s) expected and the detection limit required;
The nature of the sample, the amount available and the necessary sample preparation procedure;

The accuracy required for a quantitative analysis;
The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;

Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;

The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
Quality control and safety factors.

Table 1 Analytical techniques and principal applications

Technique	Property measured	Principal areas of application
Gravimetry	Weight of pure analyte or compound of known as stoichiometry	Quantitative for major or minor components
Titrimetry	Volume of standard reagent solution reacting with the analyte	Quantitative for major or minor Component
Atomic molecular spectrometry	Wavelength and intensity of electromagnetic radiation emitted/ absorbed by the analyte	Qualitative, quantitative or structural or for major down to trace level components
Mass spectrometry	Mass of analyte or fragments of it	Qualitative or structural for major down to trace level components isotope ratios
Chromatography and electrophoresis	Various physicochemical properties of separated analytes	Qualitative and quantitative separations of mixtures at major to trace levels
Thermal analysis	Chemical/physical changes in the analyte when heated or cooled	Characterization of single or mixed major/minor compounds
Electrochemical analysis	Electrical properties of the analyte in solution	Qualitative and quantitative for major to trace level components
Radiochemical analysis	Characteristic ionizing nuclear radiation emitted by the analyte	Qualitative and quantitative at major to trace levels

Table 2 Spectrometric Techniques and Principal Applications

Technique	Basis	Principal applications
Plasma emission spectrometry	Atomic emission after excitation in high temperature gas plasma	Determination of metals and some non-metals mainly at trace levels
Flame emission spectrometry	Atomic emission after flame excitation	Determination of alkali and alkaline earth metals
Atomic absorption spectrometry	Atomic absorption after atomization by flame or electro thermal means	Determination of trace metals and some non-metals
Atomic fluorescence spectrometry	Atomic fluorescence emission after flame excitation	Determination of mercury and hydrides of non-metals at trace levels
X-ray emission spectrometry	Atomic or atomic fluorescence emission after excitation by electrons or radiation	Determination of major and minor elemental components of metallurgical and geological samples
γ-spectrometry	γ-ray emission after nuclear excitation	Monitoring of radioactive elements in environmental samples
Ultraviolet/visible spectrometry	Electronic molecular absorption in solution	Quantitative determination of unsaturated organic
Infrared spectrometry	Vibrational molecular absorption	Identification of organic compounds

Nuclear magnetic resonance spectrometry	Nuclear absorption (change of spin states)	Identification and structural analysis of organic compounds
Mass spectrometry	Ionization and fragmentation of molecules	Identification and structural analysis of organic compounds

Table3 Separation techniques and principal applications

Technique	Basis	Principal applications
Thin-layer chromatography	Differential rates of migration of analytes through a stationary phase by movement of a liquid or gaseous mobile phase	Qualitative analysis of mixtures
Gas chromatography	-Do-	Quantitative and qualitative determination of volatile compounds
High-performance liquid chromatography	-Do-	Quantitative and qualitative determination of non-volatile compounds
Electrophoresis	Differential rates of migration of analytes through a buffered medium	Quantitative and qualitative determination of ionic compounds

Chromatography [2]

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

" *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient.

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Chromatographic Process [4]

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at

the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

Types of HPLC techniques[5]

1. Based on modes of separation

Normal phase chromatography
Reversed phase chromatography

2. Based on principal of separation

Adsorption chromatography
Partition chromatography
Ion exchange chromatography
Ion pair chromatography
Size exclusion or Gel permeation chromatography
Affinity chromatography
Chiral phase chromatography

3. Based on elution technique

Isocratic elution
Gradient elution

4. Based on scale of operation

Analytical HPLC
Preparative HPLC

5. Based on types of analysis

Qualitative analysis
Quantitative analysis

Working Principle of HPLC [6]

The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound bands as they elute from the HPLC column. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography. The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb Ultra Violet light, a UV-absorbance detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an Evaporative-Light-Scattering Detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a Mass Spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

Components of HPLC[9]

Solvent
Solvent Delivery System (Pumps)
Injector
Column
Detectors
Recorder (Data Collection)

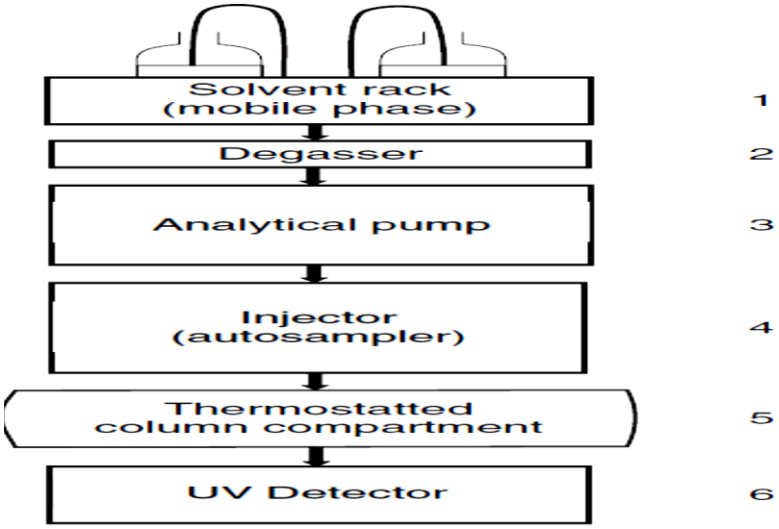


Figure 1 Schematic of a modular HPLC instrument

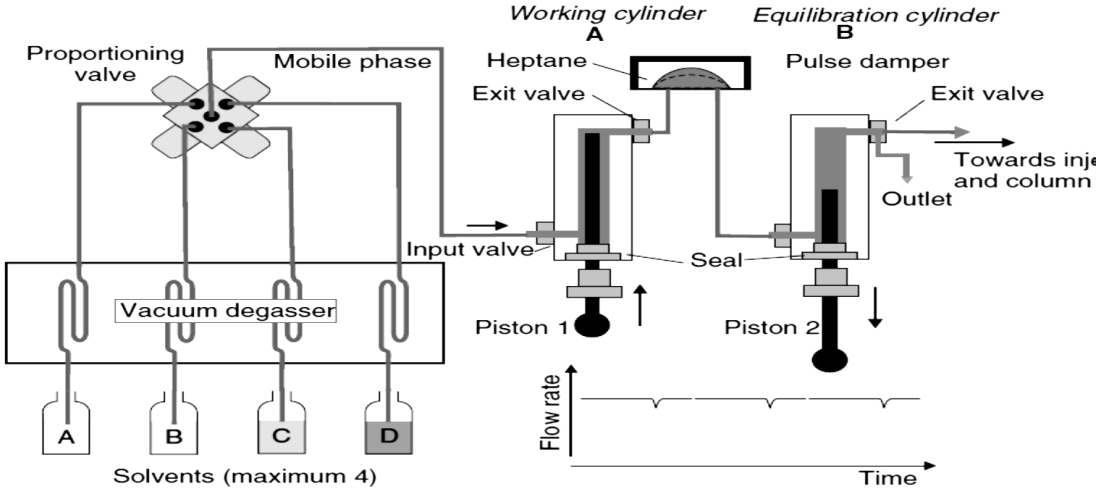


Figure 2 Schematic of the dual-headed reciprocating pump

The most simplified way of explaining the cycle of operation, without taking into account the compressibility of the solvents, is as follows. From the moment when the outlet valve of cylinder a closes and its entrance valve open, the piston in A, moving backwards, sucks the eluent through the inlet check valve and the chamber fills. Meanwhile cylinder B is open and its piston moves forward to force the mobile phase towards the injector and the column. The volume displaced by piston B is half of that available in the chamber of piston A. With chamber A full, the entrance valve of a closes and the corresponding outlet valve opens. Piston a now advances and pushes out the contents of the chamber. Half of this volume is expelled directly towards the column, the other half serves to fill cylinder B as piston B retracts. A pulse absorber is located between the two cylinders (diagram courtesy of Agilent Technologies).

Solvent

Compatible with the instrument (pumps, seals, fittings, detector etc).
Compatible with the stationary phase.
Readily available (often use liters/day) and of adequate purity.
Spectroscopic and trace-composition usually.
Not too compressible (causes pump/flow problems).
Free of gases (which cause compressibility problems).

The polarity index is a measure of the relative polarity of a solvent. It is used for identifying suitable mobile phase solvents.
Increasing eluent strength or polarity index values mean increasing solvent polarity, and
The analyte(s) and samples must be mobile phase and stationary phase compatible.

Pumps

It drives the mobile phase from the reservoir to the column.
Because of the small particles used in modern HPLC, modern pumps need to operate reliably and precisely at pressures of 10,000 p.s.i. or at least 6,000 p.s.i.

To operate at these pressures and remain sensibly inert to the wide variety of solvents used, HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats.

For analytical purposes HPLC pumps should have flow rates that range from 0 to 10 ml/min., but for preparative HPLC, flow rates in excess of 100 ml/min may be required.

The level of constancy is required because most HPLC detectors are flow sensitive and errors in quantization will result from changes in flow rate.

General HPLC column care [9]

The correct use of an HPLC column is extremely important for the life time of a column and therefore for the benefit of your HPLC analysis. The following lines will give us some guidelines for the use, cleaning and storage of HPLC columns. These guidelines depend on the one hand on the nature of the chromatographic support (silica, polymers or others) and on the other hand on the surface chemistry of the corresponding stationary phase.

1.4.6 Detectors of HPLC [7-11]

A chromatography detector is a device used liquid chromatography (LC) to visualize components of the mixture being eluted off the chromatography column.

There are two general types of detectors: destructive and non-destructive.

The destructive detectors perform continuous transformation of the column effluent (burning, evaporation or mixing with reagents) with subsequent measurement of some physical property of the resulting material (plasma, aerosol or reaction mixture).

The non-destructive detectors are directly measuring some property of the column effluent (for example UV absorption) and thus affords for the further analyte recovery.

The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column.

Analytical Method Development[12-13]

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

Careful sampling and sample preparation.

Appropriate choice of the column.

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

Reliable performance of the recording and data handling systems.

Suitable integration/peak height measurement technique.

The mode of calculation best suited for the purpose.

Validation of the developed method.

ICH Method validation parameters[14-15]

For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

They are

Selectivity,Specificity (*) , Precision (*), Repeatability (*), Intermediate precision (*),Reproducibility (**) (*),Accuracy (*),Trueness,Bias, Linearity (*), Range (*), Limit of detection (*), Limit of quantitation (*), Robustness (**),Ruggedness, Included in ICH publications (**),Terminology included in ICH but are not part of required parlament.

Material and Methods

Instruments Used

Chemicals Used

Table2 chemicals used

Sl.No	Chemical	Brand names
1	Amlodipine	Sura labs
2	Atenolol	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethylamine	Sura labs

HPLC Method Development

Preparation of standard solution

Accurately weigh and transfer 10 mg of Amlodipine and Atenolol working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of the Amlodipine and 1.5ml of the Atenolol stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Blank

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Amlodipine and 10mg of Atenolol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.15ml of the Amlodipine and 1.5ml of the Atenolol stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Results and Discussion

Validation

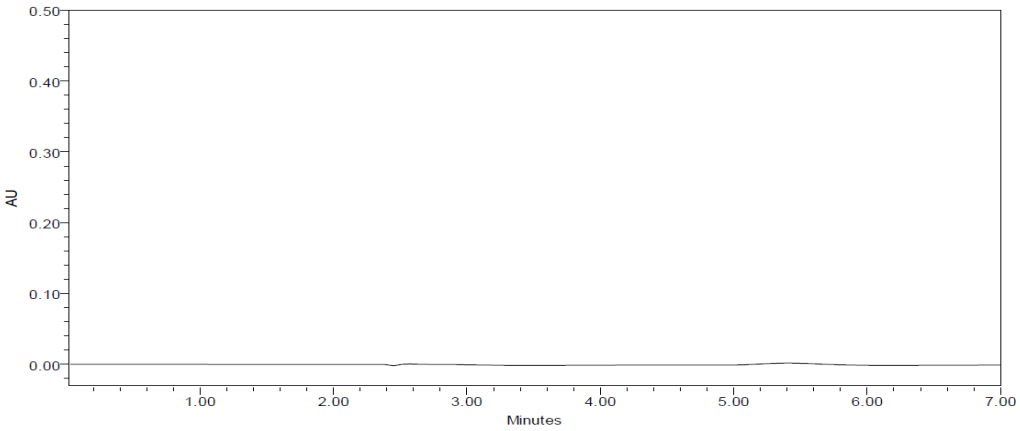


Figure 3 Chromatogram showing blank (mobile phase preparation)

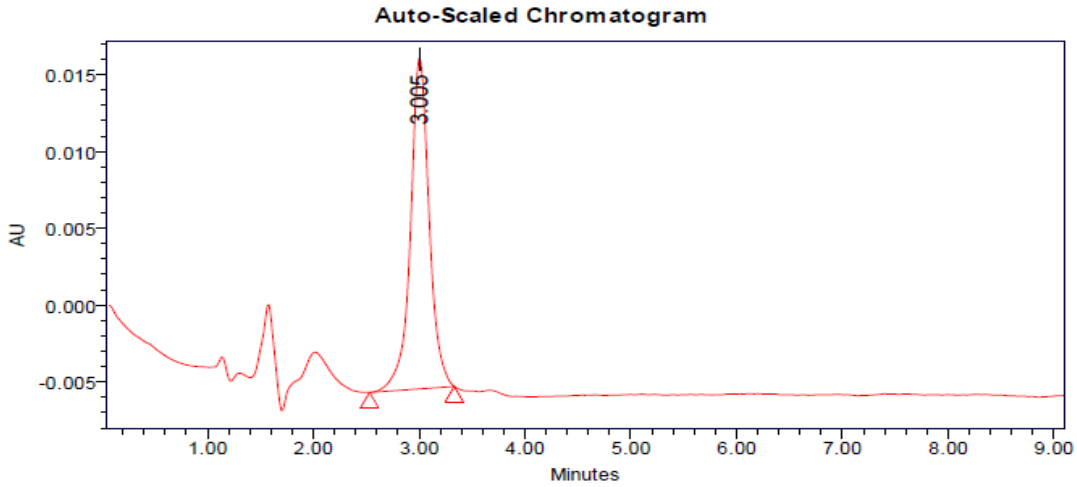


Figure 4 chromatogram for trail 1

Table 3 peak results for trail 1

S.No	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP Plate count
1		3.005	23432	1234		5.1	675

Observation

In a separation of Amlodipine and Atenolol peak was obtained only for one compound because there may be less solubility. So, we go for further trails.

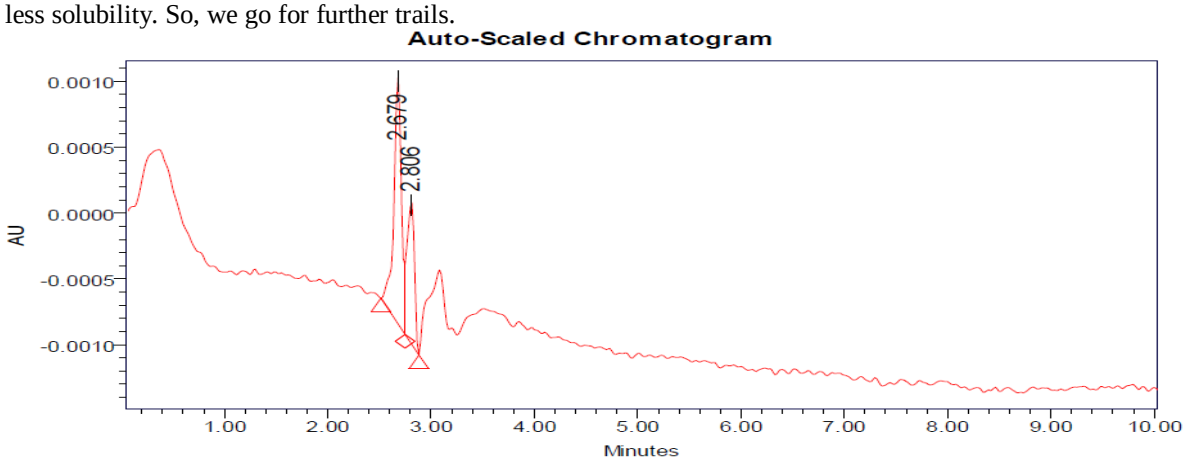


Figure 5 chromatogram for trail 2

Table 4 peak results for trail 2

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amlodipine	2.679	9605	1833			
2	Valsartan	2.806	5320	1084			

Observation

From the above chromatogram it was observed that the void peaks are obtained and sample peaks are not separated and show less plate count in the chromatogram. So it's required more trials to obtain well peaks.

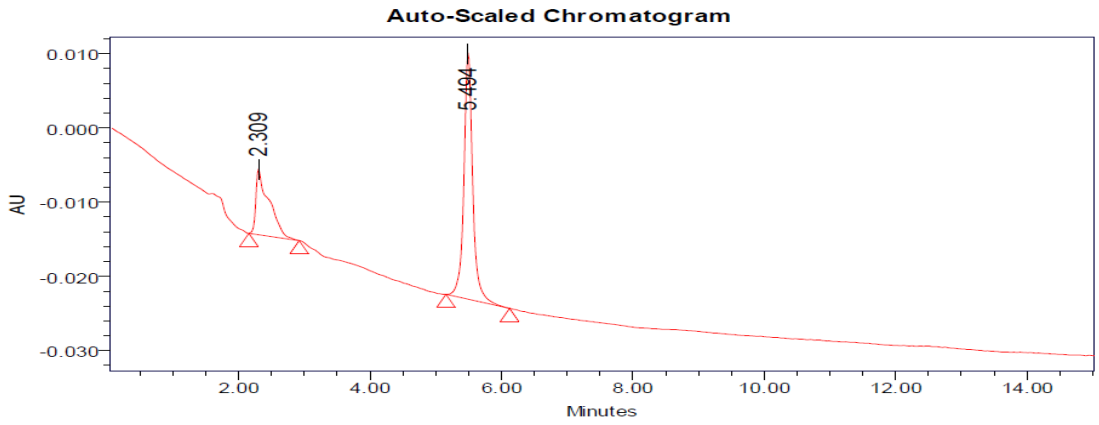


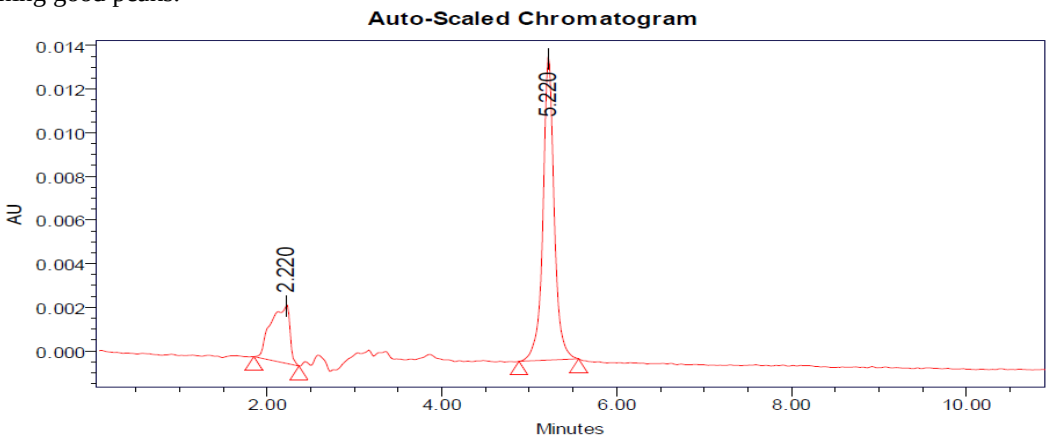
Figure 6 chromatogram for trail 3

Table 5 peak results for trail 3

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amlodipine	2.309	123459	8872		2.72	154
2	Atenolol	5.494	310282	33205	8.62	1.25	9682

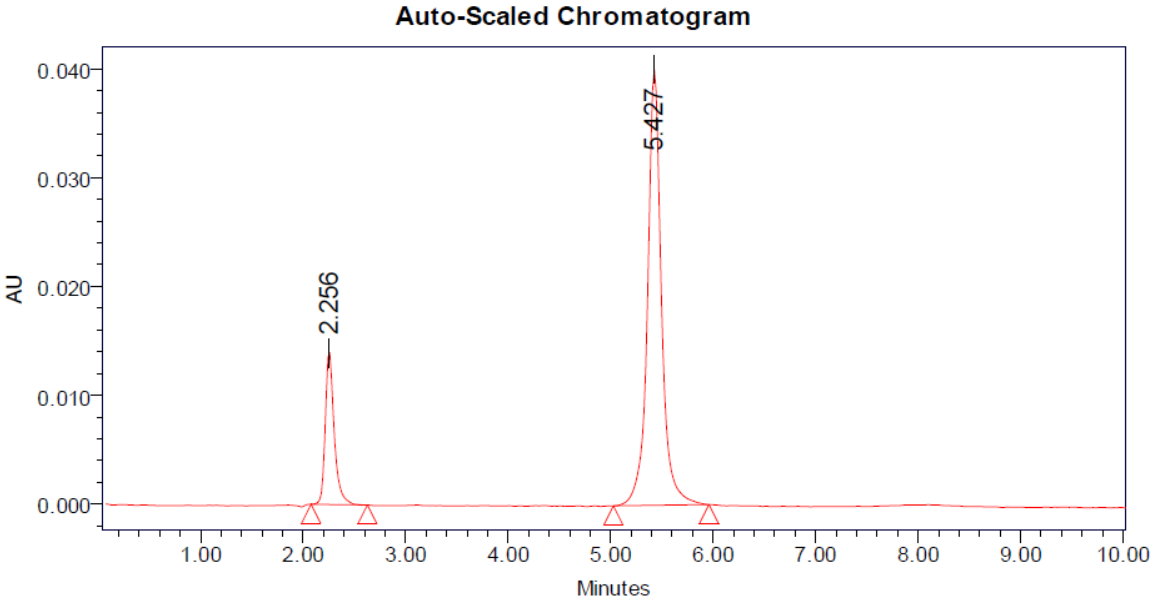
Observation

This trial show very less plate count and sample peaks are not well separated, so more trials were required for obtaining good peaks.



Observation

The chromatogram shows void peaks, and broad peak is obtained. The trial shows tailing effect so go for further trials to obtain good separation of the peak.



Optimized Chromatogram

Table 6 peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amlodipine	2.256	84994	13905		1.32	5535
2	Atenolol	5.427	377906	39948	16.27	1.03	9101

Observation

From the above chromatogram it was observed that the Amlodipine and Atenolol peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

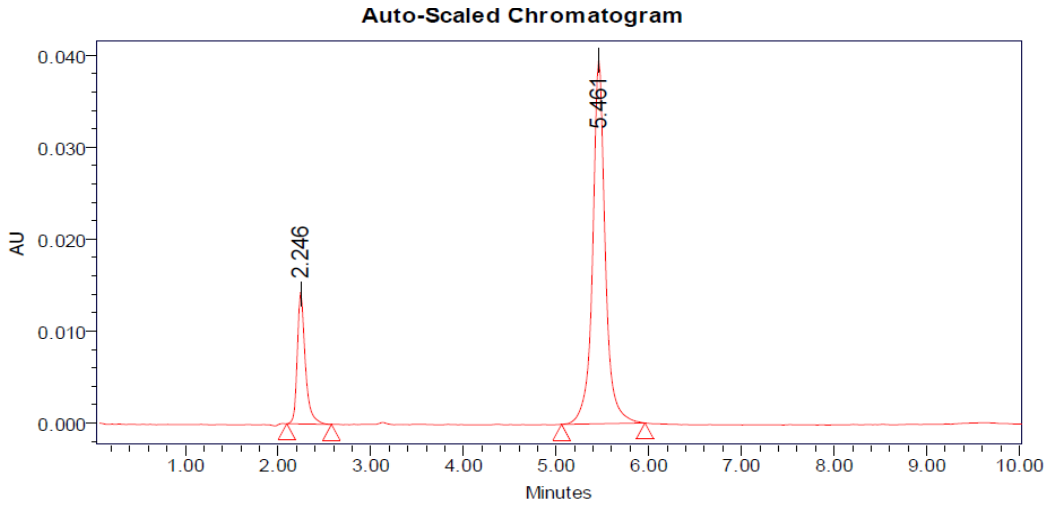


Figure 7 Optimized Chromatogram (Sample)

Table 7 Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Amlodipine	2.246	86052	33061		1.32	5506
2	Atenolol	5.461	364678	39373	16.42	1.02	9146

Acceptance criteria

Resolution between two drugs must be not less than 2,Theoretical plates must be not less than 2000, Tailing factor must be not less than 0.9 and not more than 2.

It was found from above data that all the system suitability parameters for developed method were within the limit.

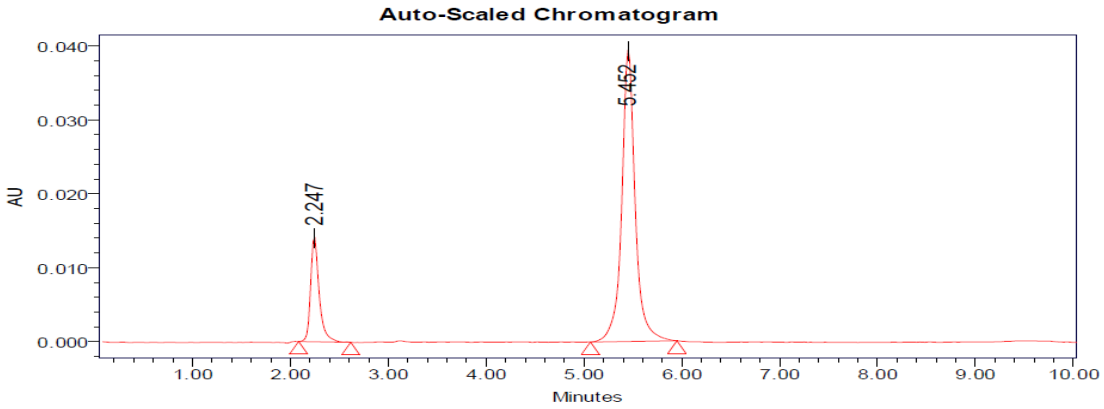


Figure 8 Chromatogram showing injection -1

Table 7 Results of system suitability for Amlodipine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Amlodipine	2.247	86092	14051	5506	1.36
2	Amlodipine	2.246	85626	14025	5674	1.2
3	Amlodipine	2.248	85557	14132	5298	1.2
4	Amlodipine	2.252	86141	14306	5032	1.0
5	Amlodipine	2.248	86557	14152	5812	1.33
Mean			85994.6			
Std. Dev			410.662			
% RSD			0.4			

Acceptance criteria

%RSD of five different sample solutions should not more than 2

The %RSD obtained is within the limit, hence the method is suitable.

Table 8 Results of system suitability for Atenolol

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atenolol	5.452	376065	39373	9146	1.04	15.0
2	Atenolol	5.484	373325	39429	9024	1.5	15.5
3	Atenolol	5.491	373435	39403	9167	1.2	15.3
4	Atenolol	5.482	375113	39745	9076	1.1	15.1
5	Atenolol	5.491	373435	39403	9327	1.2	15.2
Mean			374274.6				
Std. Dev			1247.001				
% RSD			0.3				

Acceptance criteria
%RSD for sample should be NMT 2

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Amlodipine and Atenolol in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Amlodipine and Atenolol was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5 (35:65) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Amlodipine and Atenolol in bulk drug and in Pharmaceutical dosage forms.

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