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"DEVELOPMENT & VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF ATENOLOL & LOSARTAN POTASSIUM IN BULK & TABLET DOSAGE FORM"

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ABSTRACT

A simple, accurate, and precise dual wavelength UV spectrophotometric method was developed for simultaneous determination of Atenolol and Losartan potassium in combined pharmaceutical dosage forms. The absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest. During the selection of two wavelengths the interfering component shows same absorbance while the component of interest shows important difference in absorbance with concentration. The literature review shows that there is no dual wavelength method was developed for this combination of drugs, hence this method was developed. The wavelengths selected for determination of atenolol were 275 nm & 253 nm, whereas, the wavelengths selected for determination of losartan potassium were 235 nm and 242 nm. Ethanol and distilled water were taken as the solvents. Correlation coefficient was found was vary from 0.974 to 0.989 for atenolol and Losartan potassium, for dual wavelength method. Accuracy of method was found between 99-101%. The precision (intra-day, interday and analyst to analyst) of method was found within limits (%CV<2). LOD was found to be 0.198 µg/ml and 1.155 µg/ml for Atenolol and 0.132 µg/ml and 0.143 µg/ml for losartan potassium for respective wavelength and LOQ was found to be 0.6 µg/ml and 3.5 µg/ml for Atenolol and 0.4 µg/ml and 0.43 µg/ml for Losartan potassium at respective wavelengths. The proposed method was successfully applied to determination of these drugs in laboratory-prepared mixtures and commercial tablets.

KEYWORDS: losartan, atenolol, UV spectrophotometry, method validation, simultaneous estimation.

1- INTRODUCTION

1.1 General Introduction

Analytical chemistry may be defined or outlined as the "science and art of determining the composition of materials in terms of the element of compound contained''. In analytical chemistry it is of prime importance to gain information about the quantitative constitution of substance & chemical species, that is, to find out what a substance is made up of and exactly how much. In quantitative analysis the query is how much is present? The research work in thesis is based on this and other related criteria. Analytical method is a particular application of a technique to resolve an analytical problem. The use of instrumentation is an exciting and very interested part of chemical analysis. That interacts with all areas of chemistry and with many other areas of pure and applied science. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection provides the lower detection limits required to assure safe foods, drugs, water and air. The manufacture of materials, whose composition must be known preciously such as substance used in integrated circuit chips, is monitored by analytical instruments.[1]

Instruments or physicochemical methods are based on the theory of relation between the content and the corresponding physicochemical and physical properties of the chemical system being analyzed. The changes in the system properties are either detected or recorded through the measurement of electrode potential, electrical conductivity,current, refractive index,optical density etc. with suitable and sensitive instruments. In instrument analysis physical property of a substance is measured to determine its chemical composition. The instrument is only one compound of the total analysis. Often it is necessary to use several instrumental techniques to obtain the information required to solve the analytical problem. Instrumental methods may be used by analytical chemists to save time, to avoid chemical separation or to obtain increased accuracy. The time saving feature can be realized in routine analysis, or where a considerable number of determinations are to be made.[2]

Most important techniques fit into one of the three principle areas: spectroscopy, electrochemistry, and chromatography.

1.1.1Spectroscopic techniques

- Ultraviolet & Visible Spectrophotometry
- Fluorescence & Phosphorescence Spectrophotometry
- Atomic Spectrometry (emission & absorption)
- Infrared Spectrophotometry
- Raman Spectroscopy
- α – ray Spectroscopy
- Radiochemical Technique including activation analysis
- Nuclear Magnetic Resonance Spectroscopy
- Electron Spin Resonance Spectroscopy.

1.1.2Electrochemical Techniques

- Potentiometry
- Voltametry
- Voltametric Technique
- Amperometric Technique
- Coulometry
- Electrogravimetry
- Conductance Technique.

1.1.3 Chromatographic Technique

- Gas Chromatography
- High Performance Liquid Chromatography
- High performance thin-layer chromatography
- Electrophoresis.

1.1.4Miscellaneous Technique

- Thermal analysis
- Mass Spectrometry
- Kinetic Technique.

1.1.5Hyphenated or combined techniques

- GC-MS (Gas Chromatography-Mass spectrometry)
- ICP-MS (Inductively coupled plasma Mass spectrometry)

- GC-IR (Gas Chromatography- Infrared Spectroscopy)
- MS-MS (Mass Spectroscopy – Mass Spectroscopy).[2]

1.2 Method Development

Today the development of a method for analysis is usually based on prior art or existing literature, using the same or quite similar instrumentation. The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirement of the method. Method development commonly requires selecting the method necessity and deciding on what type of instrumentation to utilize and why ? In the development stage, decisions regarding choice column, mobile phase, detectors and method of quantitation must be addressed. In this way development considers all the parameters pertaining to any method.

There are several valid reasons for developing new method for analysis.

- There may not be a appropriate method for a specific analyte in the specific sample matrix.
- Existing method may be inaccurate, artifact, and/ or contamination prone, or they may be unreliable (have poor accuracy or precision)
- Existing method may be too expensive or costly, time consuming or energy intensive, or they may not be easily automated.
- Existing method may not provide sufficient sensitivity or analyte selectivity in samples of interest.
- Newer instrumentation and techniques may have evolved that supply opportunities for improved methods, including improved analyte recognition or detection limits, greater accuracy or precision, or better return on investment.
- There may be a need for an alternative method to confirm for legal or scientific reasons analytical data originally obtained by existing methods.

Once the instrumentation has been selected, based on the criteria suggested above, it is important to determine, "analyte parameters" of interest. To develop a method it is necessary to consider the properties of the analytes of interest that may be advantageous to establish optimal ranges of analyte parameter values. It is important that method development be performed using only analytical standards that have been well identified and characterized, and whose purity is already known. Such precautions will prevent problems in the future and

will remove variables when one is trying to optimize or improve initial conditions during method development.[3]

1.3 Method Development Approaches

In the validation stage, an attempt should be made to demonstrate that the method works with samples of the given analyte, at the expected concentration in the matrix, with a high degree of accuracy and precision. Other validation criteria exist; is as defined and elaborated upon later, but complete method validation can occur only after the method is developed and optimized.In validation studies, suitability of the final method for the given analyte and a select sample matrix is demonstrated, using specified instrumentation, samples and data handling, ultimately, the method can be transferred from one laboratory to another that is suitably equipped and staffed. A method that provides all or most of the original method requirements is deemed optimized and becomes ready for validation.

1.3.1 Validation

Validation is defined as followed by different agencies.

- **FDA-Guidelines:** Validation is establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.
- **World Health Organization(WHO):** Action of providing that any procedure, process, equipment, material, activity or system actually leads to the expected results.
- **United States Pharmacopoeia:**Validation is process by which it is established that the performance characteristic of the method or system meet the requirements for the intended analytical application.

Method validation is the process that the analytical procedure employed for a specific text is suitable for its intended use. Method needs to be validated or revalidated.

- Before their introduction into routine use.
- Whenever the conditions change for which the method has been validated. (e.g., instrument with different condition.)
- Whenever the method is changed and the change is outside the original scope of the method.
- When quality control indicates an established method is changing with time.

- In order to demonstrate the equivalence between two methods. (e.g., a new and standard method).

Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies. The international standard ISO/IEC requires validation of non-standard methods; laboratory designed / developed methods, and standard methods used outside their intended scope, and amplifications and modification of standards methods to confirm that the methods are suitable for intended use.

Steps involved in method validation are

- Development of validation protocol or operating procedure for validation
- Define the application, purpose and scope of the method.
- Define the performance parameter and acceptance criteria.
- Define validation experiments.
- Verify relevant performance parameter and acceptance criteria.
- Define validation experiments
- Verify relevant performance characteristics of equipment
- Qualify material (e.g. standard and reagents)
- Perform Pre-validation experiments
- Adjust method parameters or / and acceptance criteria if necessary
- Perform full internal and external validation experiments
- Develop standard operating procedure (SOP) for executing the methods in the routine
- Define the criteria for re-validation
- Define type and frequency of system suitability tests or analytical quality control (AQC) checks for the routine
- Document validation experiments and results in the validation report.[3]

1.3.2 Types Of Validation

1.3.2.1 Prospective Validation: - This is execute for all new equipments, products and processes. It is a proactive approach of documenting the design, specifications and execution before the system is operational. This is most accusable type of validation.

1.3.2.2 Concurrent Validation: - This is performed in two instances i.e. for existing equipment and verification of proper installation along with specific operational tests. In case

of an existing, infrequently made product, data is collected from at least three successful trials.

1.3.2.3 Retrospective Validation: - This is establishing documented evidence that the process is performed satisfactorily and consistently over time, based on review and analysis of historical data. The source of such data is from production and QA/QC records. The issues to be mentioned here are changes to equipment, process, specifications and other suitable changes in the past.[3]

1.3.3 Analytical Method Validation Parameters

According to ICH, typical analytical performance characteristics that should be considered in the validation of all types of methods are.

- Accuracy
- Precision
- Specificity
- Limit of detection
- Limit of quantification
- Linearity
- Range
- Robustness
- Ruggedness
- Sensitivity
- System suitability.

1.3.3.1 Accuracy

The accuracy of an analytical method is the closeness of test results acquired by that method to the true value. The accuracy of an analytical method should be constituted across its range. In the case of assay of a drug in the formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product contents to which known amount of analyte have been added within the range of the method. If it is not possible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product (that is to spike) or compare results with those of a second well characterized method, the accuracy of which has been stated or designed.

1.3.3.2 Precision

The precision of an analytical method express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of an analytical method is usually directed as the **variance, standard deviation or coefficient of variance** of a series of measurements.

Precision may be considered as given below.

1.3.3.2.1 **Repeatability** expresses the precision under the same operating conditions over a short interval of time. Repeatability is also known as Intra-assay precision.

1.3.3.2.2 Reproducibility directed the precision between laboratories.

1.3.3.2.3 Intermediate precision expresses within laboratories variations, different days, different analysts, different equipment etc.

1.3.3.3 Specificity

The ICH documents define specificity as the efficiency to assess directly the analyte in the presence of components that may be expected to be present, such as degradation products, impurities and matrix components. Lack of specificity of an individual analytical procedure may be satisfied by other supporting analytical procedures.

In case of assay, determination of specificity requires to be shown that the procedure is unmoved by the affection of impurities or excipients. In practice this can be done by spiking the drug substance or product with suitable levels of impurities or excipients and presenting that the assay result is unmoved by the presence of this extraneous material.

1.3.3.4 Limit Of Detection

The detection limit is a specific characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental condition.

The detection limit is normally determined by the analysis of samples with known concentration of analyte to demonstrate the minimum level at which the analyte can be detected.

1.3.3.5 Limit Of Quantification

The quantitation limit is a characteristic of a quantitative assay for low–level compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. It is the lowest quantity of analyte in a sample that can be find out with acceptable precision and accuracy below the stated experimental situation. The quantitation limit is explained as the concentration of analyte. (Ex. Percentage, parts per billion) in the sample.

1.3.3.6 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by means of well specified mathematical transformation, proportional to the concentration of analytes in sample within a given range.

1.3.3.7 Range

The specified range is generally obtained from linearity studies and mostly depends on the intended application of the selected procedure. It is demonstrated by confirming or establishing that the analytical procedure gives an acceptable degree of accuracy, linearity and precision when applied to samples containing amounts of analyte within or at the terminals of the specified range of the analytical procedure.

1.3.3.8 Robustness

Robustnessis the measure of the analytical method to remain unaffected by small, but deliberate variations in method parameters. It provides an evidence of its reliability during a normal usage.

1.3.3.9 Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under variety of condition, such as different laboratories analysts, instruments, reagents, elapsed assay time, assay temperature etc. Ruggedness is normally expressed as the lack of influence on first test results of operational and environmental conditions that differ but are still within the specified parameters of the assay. The grade of reproducibility of test results is then obtained as a function of the all assay variables.

1.3.3.10 Sensitivity

Capacity of test procedure to record small variations to record small variations in concentration. There is a list of variable validation characteristics regarded as a most important for the analytical procedures cited but occasionally exception should be dealt on a case-by-case basis.

1.3.3.11 System Suitability

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, electronic, analytical operation and sample constituent an integral system that can be evaluated as a whole.

1.4 **Ultraviolet Spectroscopy**

UV light can be absorbed by molecules to excite higher energy (mostloosely bound) electrons from lower energy states to higher states.Such transitions can be studied extensively to understand the binding energy of the corresponding electrons undergoing transition.Since π-electrons are most loosely bound in an organic molecule, UVspectroscopy yields a lot of information about the degree of unsaturation in a molecule.When the wavelength of the transition exceeds the UV range, based on the same principle, even the colours of molecules can be explained on the basis of absorption of visible light.

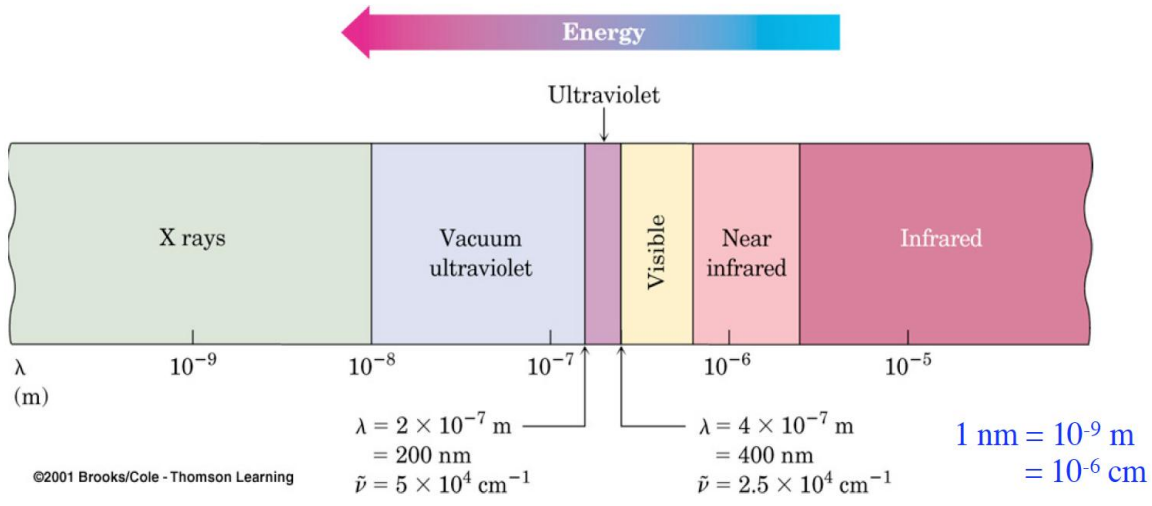


Figure 1.1: Electromagnetic Spectrum.

Energy is proportional to frequency

Frequency is inversely proportional wavelength

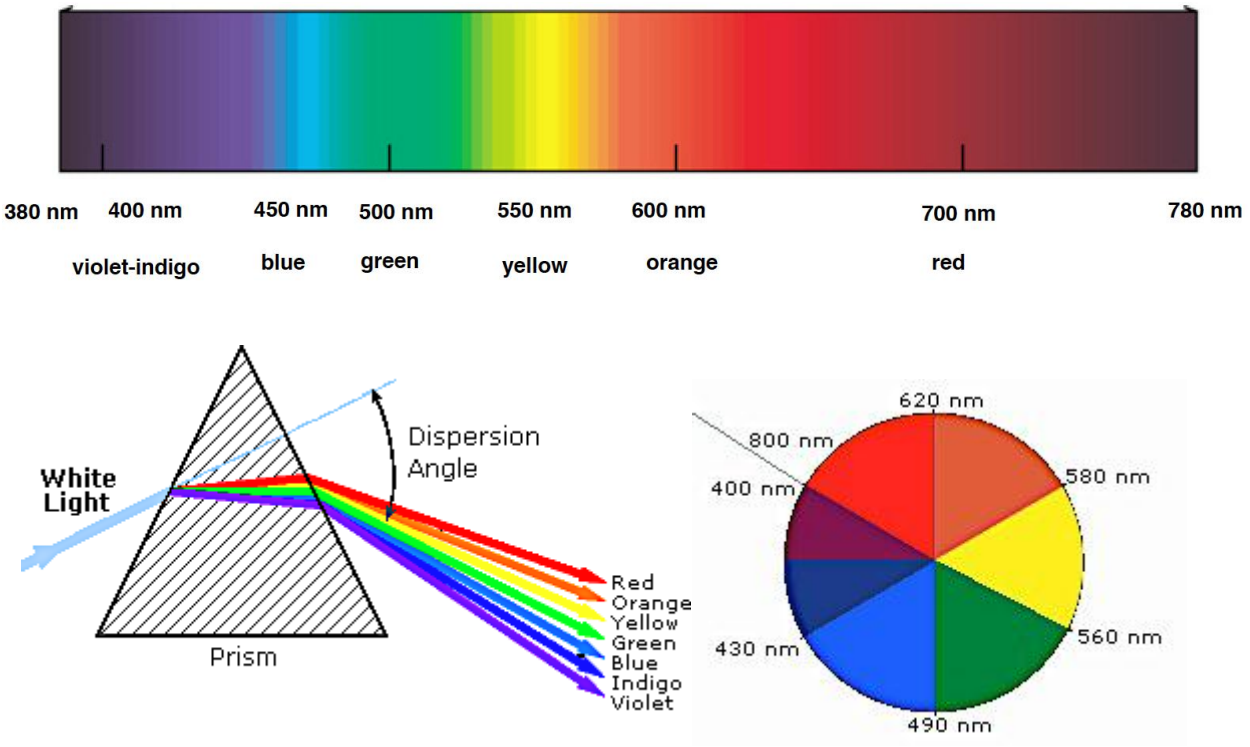


Figure 1.2 The Visible Spectrum.

1.4.1 Instrumentation

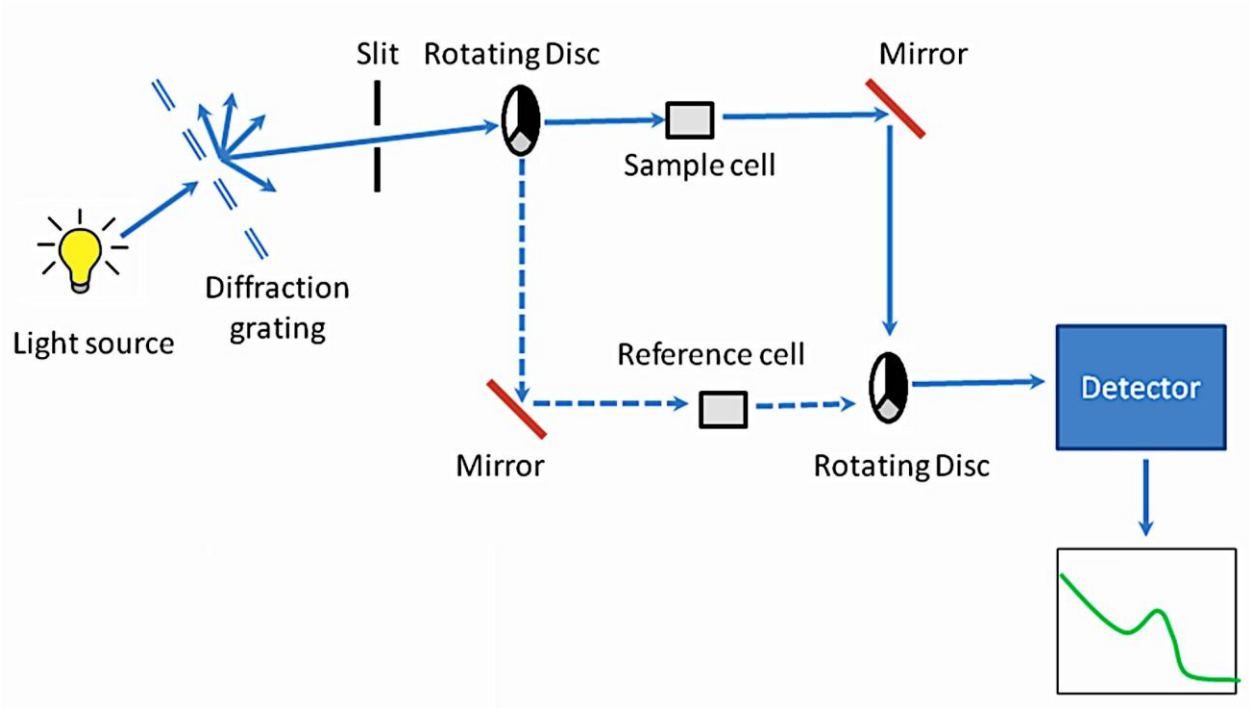


Figure 1.3 Double Beam UV Spectrometer.

1.4.1.2 Light Source

- Combined sources used to cover a range of 200-800 nm
- Deuterium lamp for UV range
- Tungsten/halogen for visible range

1.4.1.3 Diffraction Grating & The Slit

- Diffraction grating splits lights to its componentcolors like a prism
- Slit allows to pass only a narrow range ofwavelengths to the rotating disk

1.4.1.4 Rotating Disks

- Rotating disks are made of different numberof segments
- If light hits the mirrored section, it bounces back to a mirror. The reflected light meets the transparent section of the second disk and passes through it to the detector.
- If light hits the transparent section, it will pass through and bounced by a mirror onto a second rotating disk. Light meets the mirrored section of the second disk and bounces onto the detector.

1.4.1.5 Sample & Reference Cells

Small rectangular glass/quartz containersdesigned in such a way that light has to travel 1 cmthrough the contents.

1.4.1.6 Detector & Computer

- Detector converts light to current. The greater is the intensity of light, the higher is the current.
- Intensity of light passing through reference cell = Io
- Intensity of light passing through sample = I

An absorbance (A) could be written as

$A=\log_{10}\frac{Io}{I}$ ……..(1)

1.4.2 Principle Of Uv Spectrometers

1.4.2.1 Lambert-Beer's Law.

Molar absorptivity = Absorbance of 1 mol dm-3 solution if cell length = 1 cm.

$$A=\log_{10}\frac{Io}{I}=\epsilon lc \quad \text{.....(2)}$$

The intensity of an absorption band in UV is expressed as the "molar absorptivity" at maximum absorption ϵ_{max}.

1.4.2.2 Relative Energies of Various Orbitals

- These energy gaps are different in different compounds.
- The difference in energy between two orbitals-

$$\Delta E = h\nu = \frac{hc}{\lambda} = h\bar{\nu}c$$

- When light passes through a compound, some of its energy promotes an electron from one of the bonding or non-bondingorbitals to one of the anti-bonding orbitals.
- The frequency (or wavelength) of absorption depends on theenergy gaps between those two energy levels.

1.4.2.3 Electronic Transitions

- The higher is the energy gap, the loweris the wavelength of the light absorbed.
- Bigger jumps requires more energy, soabsorb light with a shorter wavelength.
- Some but not all electronic transitions are allowed. Certain limitations should be judged for electronic transitions, called "selection rules"
- The spin quantum number of an electron is remains constant during the electronictransition.
- The transition between two orbitals should be symmetry permitted or allowed.
- Any transition that infringe these rules are called "forbiddentransition". Most common "forbidden transition" is n-π*.

1.4.2.4 SOLVENTS & SOLUTIONS

- Solvents should not absorb UV-radiation within same range as the substance.

SOLVENT	λ_{max}	SOLVENT	λ_{max}
Chloroform	240 nm	Methanol	205 nm
Cyclohexane	195 nm	Isooctane	195 nm
1,4-Dioxane	215 nm	Water	190 nm
95% Ethanol	205 nm	Trimethyl phosphate	210 nm

- A strong absorbing solvent allows very little amount of light to pass through the sample.
- Non-polar solvents do not form H bond with solute, so "fine structure"is often observed.
- Polar solvents form solute-solvent complexes through H-bonding, hence, "fine structure" may disappear

1.4.2.5 Solvent Effects

- The position and intensity of an absorption band may shift if the spectrum was recorded in different solvents.
- Conjugated dienes and aromatic hydrocarbons undergoes very less "solvent effect".
- α,β-Unsaturated carbonyls show two different shifts in bands for changing solvents from non-polar to a polar protic one.
- ππ*⇨band moves to longer wavelength, n π* ⇨band moves to shorter wavelength.
- π* orbitals get stabilized (due to more polarity) by solvation than π orbitals. n orbitals get stabilized mainly by H-bonding.[4]

1.5 Hypertension

Hypertension, also termed to as high blood pressure, is a situation in which the arteries have consistently elevated blood pressure. At all time the human heart beats, it pumps blood to the complete body through the arteries. Blood pressure is the force or pressure of blood pushing up against the blood vessel walls. The higher or maximum the pressure the harder the heart has to pump. Hypertension can cause to damaged organs, also several illnesses, such as renal failure (kidney failure), heart failure, aneurysm, heart attack or stroke.

Hypertension may be classified as essential or secondary. Essential hypertension term is express for high blood pressure with unrecognized cause. It measures for about 95% of cases. Secondary hypertension is the term used for high blood pressure with a known direct cause, such astumors, kidney disease, or birth control pills. About 70 million adults are affected by hypertensionin the United States. The same condition also affects about two million teens and children.

The ACE inhibitors are now first line drugs in all grades of hypertension. About 50% patients of essential hypertension responds to themonotherapy with ACE inhibitors and majority of the rest patientsresponds to their combination with diuretics or β blockers. The hypotensive effect of lower doses develops constantly over 2-3 weeks. They offer the following advantages:

• Lack of postural hypotension, electrolyte disturbances, feeling of weakness and CNS effects.

• Safety in diabetics, asthmatics, and peripheral vascular disease patients.

• Recent evidence indicates that long-term ACE inhibitor therapy has the potential to reduce Incidence of type 2 diabetes in high risk subjects.

• Prevention of the secondary hyperaldosteronism and alsoK+ ionsloss due to diuretics.

• Renal blood flow is well maintained.

• They reverse left ventricular hypertrophy and the increased wall-to-lumen ratio of blood vessels that occurs in hypertensive patients.

• No hyperuricaemia, no destructive effect on plasma lipid profile.

• No rebound hypertension on withdrawal.

• Minimum worsening of quality of life parameters like general wellbeing, work performance, sleep, sexual performance, etc. Large multicentric trials have confirmed that ACE inhibitors reduce cardiovascular morbidity and increase life expectancy of hypertensive patients. It appears that by their specific effect on myocardial and vascular cell growth/remodeling, they have greater protective potential than other classes of antihypertensive drugs. ACE inhibitors are highly effective and first choice drugs in renovascular and resistant hypertension. They are particularly suitable for diabetic hypertensive's in which they reduce cardiovascular complications more than other antihypertensive drugs, probably by improving endothelial function.

1.	Normal	< 120 and < 80
2.	Pre-hypertension	120- 139 or 80-89
3.	Hypertension Stage I	140-159 or 90-99
4.	.Hypertension Stage II	≥160 or ≥100

1.5.1 Causes of Hypertension

The exact causes or reasons of hypertension are generally unknown, there are various factors that have been highly collaborated with the condition. These include.

- Smoking
- Obesity or being overweight
- Diabetes
- Sedentary lifestyle
- Lack of physical activity
- High levels of salt intake (sodium sensitivity)

- High levels of alcohol consumption
- Stress
- Aging
- Medicines such as birth control pills
- Genetics and a family history of hypertension - Chronic kidney disease
- Adrenal and thyroid problems or tumour.

1.5.2 Therapy For Hypertension

The main objective of treatment for hypertension is to decrease blood pressure to lower than 140/90 - or even lower in few groups such as people with diabetes, and people with chronic kidney diseases. Treating hypertension is most important for decreasing the risk of stroke, heart attack, and heart failure. High blood pressure may be treated medically, by doing changes in the lifestyle factors, or a combination of the two. Significant lifestyle changes contain losing weight, smoking,quitting, eating a healthful diet, exercising regularly, reducing sodium intake and limiting alcohol consumption. Pharmacological options to treat hypertension contains several classes of drugs.Beta-blockers, ARB drugs, ACE inhibitors, diuretics, alpha-blockers,calcium channel blockers and peripheral vasodilators are the primary drugs used in treatment therapy. These drugs may be used alone or in combination, and specific are only used in combination. In addition, some of these drugs are preferred to others depending on the characteristics or personality of the patient (diabetic, pregnant, etc.). If blood pressure is successfully lowered, it is rational to have frequent checkups and to take preventive measures to abandon a relapse of hypertension.

1.5.3 Classification

1 . Diuretics

Thiazides: Hydrochlorothiazide, Chlorthalidone, Indapamide

High ceiling: Furosemide, etc.

K+ Sparing: Spironolactone, Amiloride.

2. ACE inhibitors

Captopril, Enalapril, Lisinopril, Perindopril, Ramipril, Fosinopril, etc.

3. Angiotensin (A T1 receptor) blockers

Losartan, Candesartan, Irbesartan, Valsartan, Telmisartan.

4. Calcium channel blockers

Verapamil, Diltiazem, Nifedipine, Felodipine, Amlodipine, Nitrendipine, Lacidipine, etc.

5. β Adrenergic blockers

Propranolol, Metoprolol, Atenolol, etc.

6. β+ α Adrenergic blockers

Labetalol, Carvedilol.

7. αAdrenergic blockers

Prazosin, Terazosin, Doxazosin, Phentolamine, Phenoxybenzamine.

8. Central sympatholytics

Clonidine, Methyldopa.

9. Vasodilators

Arteriolar: Hydralazine, Minoxidil, Diazoxide.

Arteriolar + venous: Sodium nitroprusside.

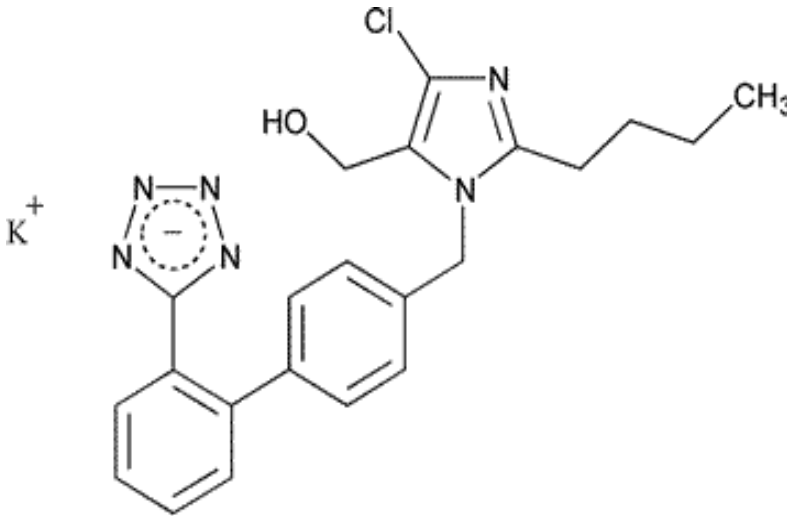
Adrenergic neuron blockers (Reserpine, Guanethidine, etc.) and ganglion blockers (Pentolinium, etc.) are only of historical impor.

1. 6- Drug Profile

1.6.1 Nature Of Drugs

The Drug may define as any substance which is used fortreatment, diagnosis, cure, mitigation of a disease. The drug molecule combines with a specific molecule in the biologic system which plays an important regulatory role. This molecule is known as receptor. The useful drug must have necessary properties to be transported from its site of administration to its site of action & the practical drug should be inactivated or excreted from the human body at a fair rate so that its action will be of appropriate time. Drugs may be solid, liquid, or gaseous at room temperature. These factors help to determine the best route of administration. The molecular size of drug was changes from very small (lithium ion, MW 7) to very large. The maximum majority of drugs have its molecular weights between 100 and 1000.

1.6.2 Structure Of Losartan Potassium



Structure of Losartan Potassium

IUPAC name	***(2-butyl-4-chloro-1-{[2'-(1H-tetrazole-5-yl)biphenyl]methyl} -1H-imidazole-5-yl)methanolmonopotassium salt.***
Molecular formula	$C_{22}H_{23}ClKN_6O$
Molecular weight	461.91g/ml
Solubility	Freely soluble in water
Category	Angiotensin II receptor antagonist
Bioavailability	25-35%
Half life	1.5 to 2hours
Excretion	Renal 13-25%
PKa	---
Routes	Oral

Losartan is a competitive antagonist and inverse agonist of A-11, 10 thousand fold more selective for AT1 than AT2 receptor; does not freeze any other receptor or ion channel, exclude thromboxane A2 receptor (has just about platelet antiaggregatory property). It blocks all open actions of A-11, namely vasoconstriction, central and peripheral sympathetic stimulation, departure of aldosterone and Adr from adrenals, renal activity boost salt and water resorption, central actions like starve, vasopressin discharge and growth-promoting actions on heart and blood vessels. No suppression of ACE has been noted. Pharmacologically, AT1 receptor antagonists disagree from ACE inhibitors in the following ways:They do not interpose with deterioration ofbradykinin and other ACE substrates: no rise in level or potentiation of bradykinin occurs. Accordingly, ACE inhibitor related cough is rare.

They resultant in more complete prohibition of AT 1 receptor activation, because alternate pathway of A-II generation and consequent AT 1 receptor activation remain intact with ACEinhibitors.

They result in indirect AT 2 receptor activation. Due to block up of AT1 receptor mediated feedback inhibition-more A-II is produced which acts on AT2 receptors that remain unfreezed. ACE inhibitors result in depression of both AT 1 and AT 2 activation. The impact of these deviations on clinical efficacy and therapeutic value of the two classes of RAS inhibitors is not known.

Losartan results fall in BP in hypertensive patients which lasts for 24 hours, while HR remains unchanged and cardiovascular reflexes, are not interfered. No significant effect on plasma lipid profile, carbohydrate tolerance, insulin sensibility has been noted. It is also a mild uricosuric.

1.6.2.1 Pharmacokinetics

Oral absorption of losartan is not affected by food, but bioavailability is only 33% due to first pass metabolic process. It is partly carboxylated in liver to active metabolites(E3174) which is a 10-30 times more stronger noncompetitive AT1 receptor antagonist. Afteroral consumption peak plasma levels are attained at oral intake peak plasma levels are attained at 1 hr for losartan and at 3-4hours for E3174. & both compounds are 98% plasma protein bound do not enter brain and are excreted by the kidney.The plasma $t^{1/2}$ of losartan is 2 hr, but that of E3174 is 6-9 hr. No dose adjustment is required in renal insufficiency, but dose should be reduced in presence of hepatic dysfunction.

1.6.2.2 Adverse Effects

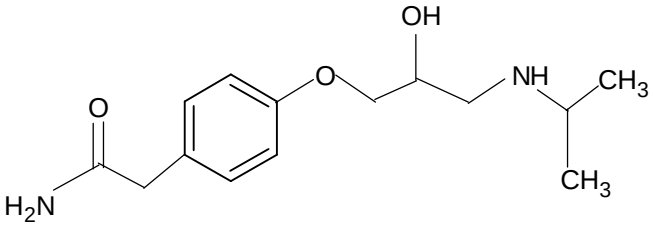
Losartan is well tolerated has side effect profile similar to placebo. Like ACE inhibitors it can induce hypotension and hyperkalemia, but first dose hypotension is rarethough, a few reports of dry cough have appeared, losartan is considered to be free of cough and dysgeusia inducement potential patients with a history of ACE inhibitor related cough have taken losartan without return. Angiodema is reported in very less cases. Headache, dizziness weakness and upper that is side effects are mild &occasional. However, losartan has fetopathie potential like ACE inhibitors not to be dispensed during pregnancy.

Dose: 50 mg OD, rarely BD; in liver disease.

LOSACAR, ALSARTAN 25, 50 mg.

1.6.3 Atenolol

Atenolol is a beta-adrenergic receptor antagonist, or a more usually known as a beta-blocker.



Structure of Atenolol

IUPAC Name	**(**RS)-4-2(2-hydroxy-3-isopropylamino propoxy)phenylacetamide
Molecular formula	$C_{14}H_{22}N_2O_3$
Category	Anti-anginal, Anti-arrhythmic, Anti-hypertensive
Solubility	Soluble in ethanol and methanol, sparingly soluble in water,slightly soluble in dichloromethane, and practically insoluble in ether.
Physical appearance	A white or almost white powder
Melting point	152-155 ^{0}C
Optical rotation	+0.10^0 to -0.10^0
Dissociation constant	9.6 @ 24 ^{o}C

Atenolol is a selective beta-1 adrenergic receptor antagonist. It is used in the management ofcardiovascular diseases such as angina, hypertension, cardiac arrhythmias and myocardial infarctions. Atenolol competitively blocks beta-adrenergic receptors in the heart and juxtoglomerular apparatus.

Beta-blockers are competitive inhibitors and interpose with the action of stimulating hormones on beta-adrenergic receptor in the nervous system.

1.6.3.1 Pharmacological Effects

Atenolol has a fundamental action on various organs; mainly contain the effect on heart, smooth muscles, central nervous system, etc. Cardiovascular effects include decrease in the heart rate, force of contraction and cardiac output and its effect on the bronchiolar smooth muscle is vasodilatation there by it aid in the management of cardiovascular disease such as angina, hypertension, cardiac arrhythmias, an myocardial infractions.

1.6.3.2 Mechanism Of Action

Atenolol works by competing for receptor sites on β_1 receptor, which is situated in the heart (cardiac muscle) and arterioles (smooth muscles). The β blockers repress blood pressure primarily by decreasing cardiac output. They also decrease sympathetic out flow from the rennin from kidney This diminish the formation of Angiotensin II and secretion of aldosterone.

1.6.3.3 Pharmacokinetic

Atenolol is incompletely absorbed of about 50 % but most of the absorbed dose passes the systemic circulation. It has no inherent sympathomimetic activity. Atenolol is having very low lipid solvability, it is having protein binding activity of about 5-16 % and half-life of this drug is about 6-9 hours. Its first pass metabolite is not significant hence has longer duration of action. It is excreted unaltered in feces.

Category to which Atenolol belongs

- Anti-adrenergic
- Anti-anginal
- Anti-anxiety therapy adjunct
- Anti-arrhythmic
- Anti-hypertensive
- Anti-tremor agent
- Hypertrophic cardiomyopathy (therapy adjunct)
- Myocardial infraction (prophylactic)
- Myocardial infraction therapy
- Vascular headache prophylactic

1.6.3.4 Therapeutic Uses Of Atenolol

- Hypertension
- Cardiac disorder
- Angina pectoris
- Cardiac arrhythmias
- Congestive heart failure
- Myocardial infraction
- Alcohol withdrawal

- Hyperthyroidism
- Migraine
- Tremor

1.6.3.5 Adverse Effects

- Cardiovascular effects – bradycardia and hypotension
- Bronchospasm
- CNS Effects – include depression, hallucinations, confusion and sleep disturbance
- Fatigue
- Gastro-Intestinal effects – Nausea and vomiting, diarrhea, constipation
- Integumentary system Effects – skin rash, pruritus.
- Ocular Effects – Decreased tears production, blurred vision and soreness
- Hematological Effects

1.6.3.6 Dosage And Administration

- For High Blood Pressure
  - Adults – 25-100 mg once/twice a day

Administration can be by oral administration as tablets, capsules, syrup and also by i.v. as injection. It is also available as Antihypertensive in the form of compound preparations with diuretics, cal.

2- MATERIAL AND METHODS

2.1 Chemicals And Reagents

Atenolol and Losartan potassium were procured from Unichem Laboratories Ltd. (Goa). Commercial pharmaceutical preparation Losar beta tablets, manufactured by Unichem lab. Ltd., containing 50 mg of ATN and 50 mg of LOS was collected from local market. Acetonitrile, ethanol and water used were of analytical grade (LobaChemie, Mumbai, India). A 0.45 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

2.2 Instrumentation

The proposed work was carried out on a Shimadzu UV-visible spectrophotometer (model UV-1800 series), which possesses a double beam double detector configuration with a 1 cm

quartz matched cell. All weighing was done on electronic balance (Sansui-vibra DJ-150S-S). A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase.

2.3 Selection Of Solvents

On the basis of solubility study ethanol was selected as the solvent for dissolving ATN and LOS.[7]

2.4 Preparation Of Standard Stock Solutions Of ATN And LOS

2.4.1 Atenolol Stock Solution

An accurately weighed quantity of ATN (50 mg) was taken in 50 mL volumetric flask and dissolved in ethanol (20 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using ethanol to get Atenolol standard stock solution (1 mg / mL).[8]

2.4.2 Atenolol Working Standard Solution

Atenolol standard stock solution 5 mL was diluted to 50 mL using ethanol to get working standard solution 100 µg / mL.

2.4.3 Losartan Stock Solution

An accurately weighed quantity of LOS (50 mg) was taken in 50 mL volumetric flask and dissolved in ethanol (20 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using ethanol to get Losartan standard stock solution (1 mg / mL).[9]

2.4.4 Losartan Working Standard Solution

Losartan standard stock solution 5 ml was diluted to 50 mL using ethanol to get working standard solution 100 µg / ml.[9]

2.5 Determination Of ƛ Max Of Individual Component

An appropriate aliquot portion of ATN (0.8 mL) and LOS (0.2 mL) were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using ethanol to obtain ATN (80 µg/mL) and LOS (20 µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm. ATN shows λ max at 275 nm while LOS at 235 nm.[10]

2.6 Estimation Of Laboratory Mixture By Proposed Method

In order to see the feasibility of proposed method for simultaneous estimation of ATN and LOS in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed ATN (50 mg) and LOS (50 mg) were taken in 100 mL volumetric flask, dissolved in ethanol (60 mL) with the help of ultrasonication for about 10 min and the volume was made up to mark using the same. Appropriate aliquot portion (1 mL) was transferred to 10 mL volumetric flask and further diluted using ethanol to get ATN (50 µg/ mL) and LOS (50 µg/ mL). The absorbance was recorded at 275 nm and 235 nm against solvent as blank.

Amount of each drug was estimated using following equations,

$$C_x = \frac{A_2 \times ay_1 - A_1 \times ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad (1)$$

$$Cy = \frac{A_1 \times ax_2 - A2 \times ax_1}{ax_2\, ay_1 - ax_1\, ay_2} \quad (2)$$

Where;

A1 and A2 are the absorbance of diluted mixture at λ_1 and λ_2

Cx and Cy are the concentration of X and Y respectively

ax1 and ax2 are absorptivities of X at λ_1 and λ_2 respectively

ay1 and ay2 are absorptivities of Y at λ_1 and λ_2 respectively

2.7 Application Of The Proposed Method For Estimation Of Drugs In Tablets

Twenty 'Losar Beta' Tablets containing ATN (50 mg) and LOS (50 mg) were weighed and ground to fine powder. A quantity of sample equivalent to ATN (50 mg) and LOS (50 mg) was transferred into 100 mL volumetric flask containing ethanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbance of the solutions was measured at 275 nm and 235 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations.[11,12,13]

2.8 Validation Of Proposed Method

The Proposed method was validated as per the ICH guidelines.

2.8.1 Accuracy [Recovery Study]

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula[12]

% Recovery = [A - B/ C] X 100 ……..(3)

Where,

A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

2.8.2 Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing ATN (19.2, 25.6, and 32 µg/mL) and LOS (19.2, 25.6, and 32 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week.[13]

2.8.3 RUBUSTNESS

Rubustness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions.[13]

2.8.4 Limit Of Detection (Lod) And Limit Of Quantitation (Loq): LOD was determined usingthe relation 3.3 σ/s where ‘σ’ is the standard deviation of the response and ‘s’ is the slope ofthe calibration curve. The standard deviation of the response can be obtained either bymeasuring the standard deviation of the blank response or by calculating the residual standarddeviation of the regression line or by calculating the standard deviation of the y-intercept ofthe regression line, i.e. the standard error of the estimate. Similarly, LOQ was determinedusing the relation 10 σ/s.[13]

3 – RESULT AND DISCUSSION

3.1 Determination Of Wavelength For Dual Wavelength Method

The standard solution of ATN and LOS were scanned separately in the UV range of 200-400 nm. At ant two wavelengths one drug shows same absorbance so the absorbance different of

these wavelengths were selected for estimation of second drug, while second drug show same absorbance at any two wavelength so the absorbance differences of these wavelengths were selected for estimation of first drug. In dual wavelength method, the diluted solution were scanned over the wavelength range of 200-400 nm. The absorbance difference of 253nm and 275nm were selected for quantitation of ATN where LOS shows same absorbance and the absorbance difference of 235nm and 242nm were selected for quantitation of ATN where LOS shows same absorbance. By this the estimation of one drug, without interference of another drug is possible.

3.2 Calibration Curve For Atenolol

Preparation of calibration curve

The stock solutions of 10 µg/mL concentration werepreparedin ethanol. For Atenolol three absorption maxima were observed at value of 225nm, 275nm and 322nm. The λmax 275 nm was used forthis study. For losartan potassium an absorption maxima observed at 235nm was used.Working standard solution (100 µg/mL) was made fromstock solution (S) by suitably diluting with ethanol. Aliquots were taken from this working standard solution and suitably diluted withethanol to give a concentration range of 2 to 14µg/mL. For atenolol and losartan the absorbance were recorded at275 nm and 232 nm respectively against a reagent blankand calibration curve was plotted as shown in Fig. 8.1, 8.2,8.3, 8.4.

Table 3.1: Absorbance of Atenolol.

Sr No	Concentration µg/ml	Absorbance at 275 nm	Absorbance at 253 nm
1	2	0.01548	0.01512
2	4	0.0254	0.0234
3	6	0.02848	0.02841
4	8	0.04528	0.04522
5	10	0.05420	0.0547
6	12	0.06196	0.06181
7	14	0.07272	0.07211

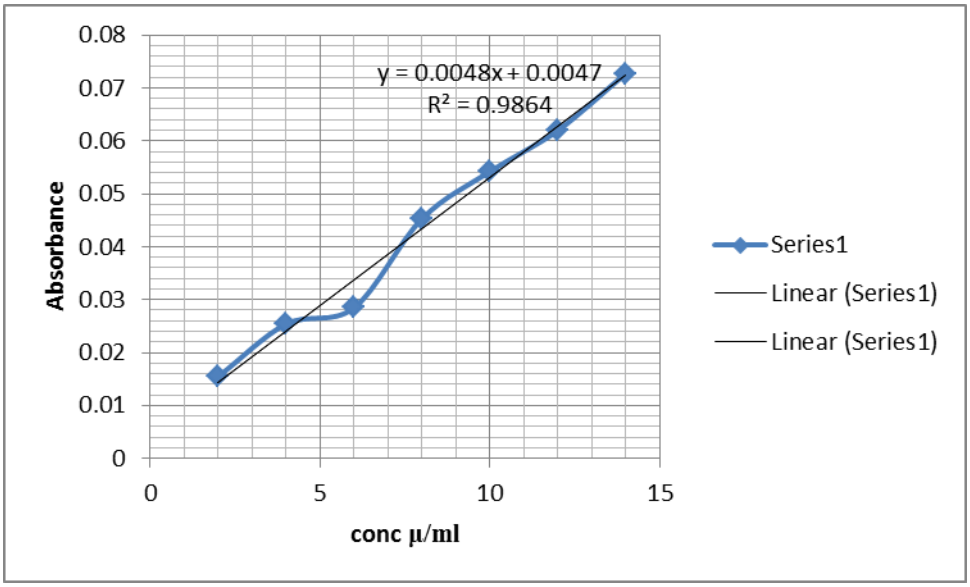


Figure 3.1 – Calibration curve for Atenolol at 275 nm.

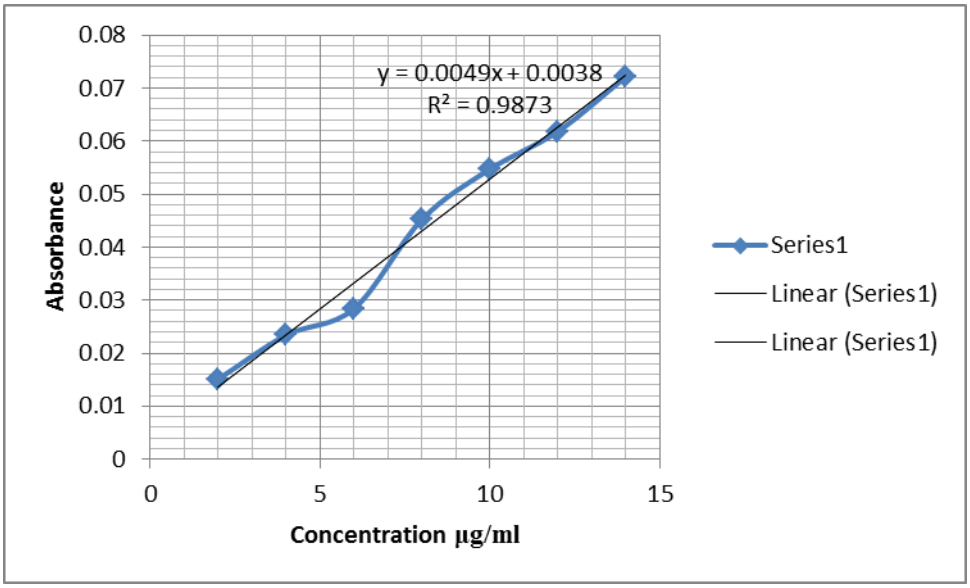


Figure 3.2 – Calibration curve for Atenolol at 253nm.

3.3 Calibration Curve For Losartan Potassium

Table 3.2: Absorbance of Losartan Potassium.

Sr No.	Concentration µg/ml	Absorbance at 235 nm	Absorbance At 242nm
1	2	0.1471	0.1478
2	4	0.2070	0.2071
3	6	0.2874	0.2879
4	8	0.3358	0.3751
5	10	0.4742	0.4844
6	12	0.5566	0.5769
7	14	0.7068	0.7071

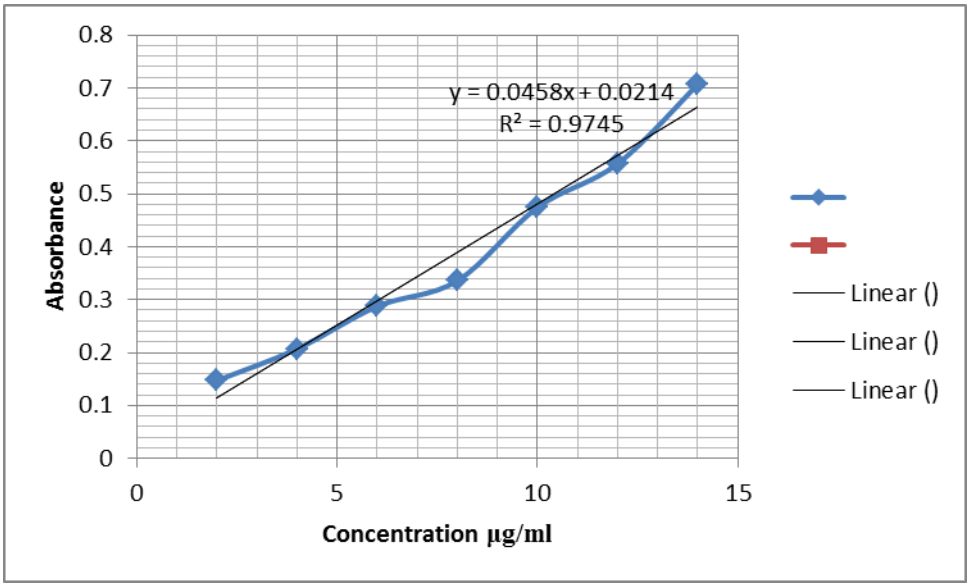


Figure 3.3 Calibration curve of LOS at 235 nm.

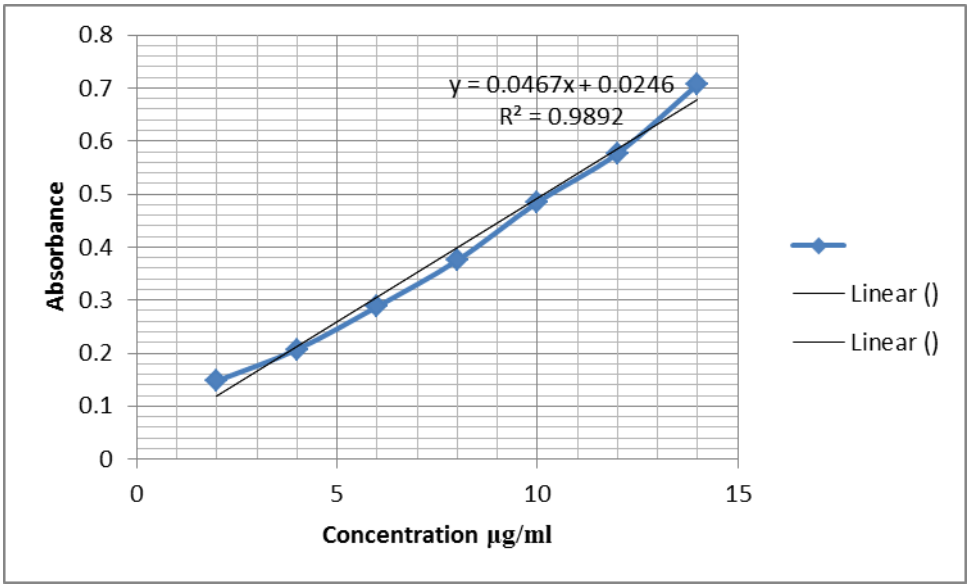
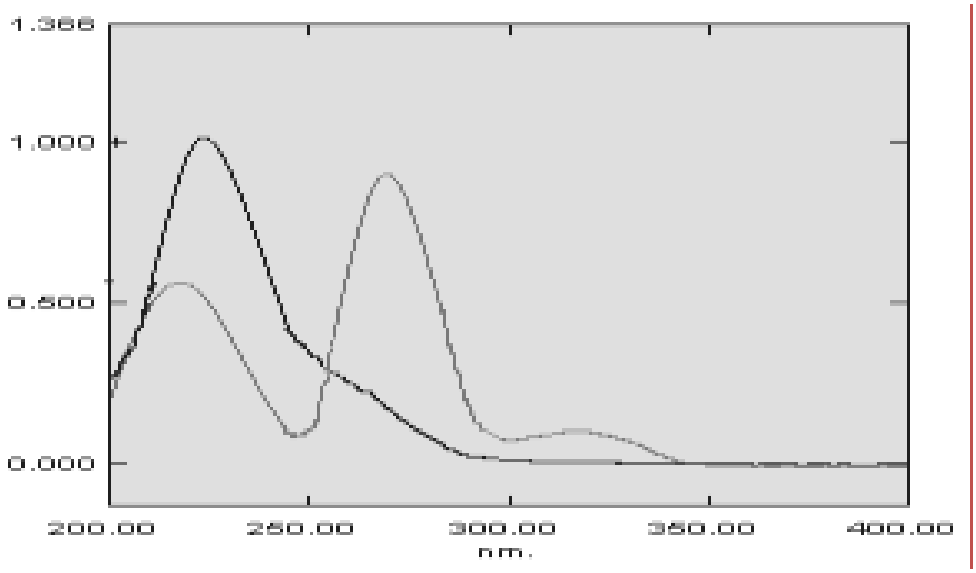


Figure 3.4 Calibration curve of LOS at 242 nm.

3.4 Overlay Spectra Of Atenolol And Losartan Potassium

The overlain spectrum of both drugs was recorded and two wavelengths 275.0 nm (λ max of ATN) and 235.0 nm (λ max of LOS) were selected for further study.



3.5 Estimation Of Laboratory Mixture

In order to see the feasibility of proposed method for simultaneous estimation of ATN and LOS in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed ATN (50 mg) and LOS (50 mg) were taken in 100 mL volumetric flask, dissolved in ethanol (60 mL) with the help of ultrasonication for about 10 min and the volume was made up to mark using the same. Appropriate aliquot portion (1 mL) was transferred to 10 mL volumetric flask and further diluted using 95% v/v ethanol to get ATN (50 µg/ mL) and LOS (50 µg/ mL). The result was found to be.

Table 3.3: Results of Estimation of Atenolol and Losartan Standard Laboratory Mixture.

Analyte	% Concentration estimated (Mean ±S.D.)	%R.S.D.
ATN	99.63 ± 0.15270	0.153264
LOS	99.84 ± 0.39820	0.398815

*Average of five determinations; R.S.D. = Relative Standard Deviation

3.6 Application Of The Proposed Method For Estimation Of Drugs In Tablets

Twenty 'Losar beta' Tablets containing ATN (50 mg) and LOS (50 mg) were weighed and ground to fine powder. A quantity of sample equivalent to ATN (50 mg) and LOS (50 mg) was transferred into 100 mL volumetric flask containing ethanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume

was adjusted to the mark. The absorbance of the solutions was measured at 275 nm and 235 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations. The results was found to be.

Table 3.4: Results of Estimation of Atenolol and Losartan in Tablets.

Analyte	Label claim (mg/tab)	% Label claim estimated(Mean ±S.D.)	%R.S.D.
ATN	50	99.94 ± 0.676225	0.676604
LOS	50	100.12 ± 0.863018	0.869922

*Average of five determinations; S.D. =Standard Deviation

3.7 Validation

3.7.1 Accuracy [Recovery Study]

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

% Recovery = [A - B/ C] X 100

Where,

A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

Accuracy for Atenolol and Losartan Potassium was found to be

Table 3.5 Accuracy of Atenolol and Losartan Potassium.

Sr.No.	Drug	Absorbance at	% Recovery
1	Atenolol	275 nm	99.92 ±0.0072
2	Atenolol	253 nm	99.73±0.0042
3	Losartan Potassium	235 nm	99.68±0.0054
4	Losartan Potassium	242 nm	99.54±0.0060

3.7.2 Precision Study

3.7.2.1 Intra-Day Precision

The Intraday precision, it was carried or agreed out by prepare three replicates of three different concentrations, inside the linearity range and the measure the absorbance of each of the solutions on the same day same way. The % RSD (% relative standard deviation) it was calculated. The absorbance at 253 nm (Isoabsorptive point) and 275 nm (λ-max of Atenolol)

were measured and absorptive coefficients were calculated by using calibration curve it was found to be following result.

Table No.3.6 Intraday Precision study of ATENOLOL.

Sr.No	Concentration (µg/ml)	Absorbance at 275 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.02848	0.02846	0.O2845	0.2846	0.002

Sr.No	Concentration (µg/ml)	Absorbance at 253 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.O2843	0.02840	0.02845	0.02842	0.005

Table No.3.7 Intraday Precision study of Losartan Potassium.

Sr.No	Concentration (µg/ml)	Absorbance at 235 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.2871	0.2874	0.2879	0.2874	0.08

Sr.No	Concentration (µg/ml)	Absorbance at 242 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.2889	0.2883	0.2879	0.1092	0.1

3.7.2.2 Interday Precision

The Interday precision, it was carried or agreed out by prepare three replicates of three different concentrations, inside the linearity range and measure the absorbance of each solution on the three different days. The % RSD (% relative standard deviation) was calculated. The absorbance at 253 nm (Isoabsorptive point) and 275 nm (λ-max ofAtenolol) were measured and absorptive coefficients were calculated by using calibration curve it was found to be following result.

Table No.3.8 Interday Precision study of ATENOLOL.

Sr.No	Concentration (µg/ml)	Absorbance at 275 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.02842	0.02851	0.O2848	0.02847	0.009

Sr.No	Concentration (µg/ml)	Absorbance at 253 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.O2844	0.02840	0.02846	0.02843	0.006

Table No.3.9 Interday Precision study Losartan Potassium.

Sr.No	Concentration (µg/ml)	Absorbance at 235 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.2872	0.2881	0.2878	0.2874	0.09

Sr.No	Concentration (µg/ml)	Absorbance at 242 nm			% RSD	SD
		Absorbance-1	Absorbance-2	Absorbance-3		
1.	6	0.2881	0.2889	0.2882	0.2881	0.08

3.8.1The Limit Of Detection (L.O.D.):-

The L.O.D. were estimated or projected from the set of three calibration curves is a used to determine and demonstrated method linearity the L.O.Q. may be calculated as per following points,

$LOD = 3.3 \times (\sigma/S)$……(1)

Where it was,

σ = Standard deviation (SD) of the Y-intercepts of the three calibration curves.

σ S = the mean slope of the three Calibration curves.

Table No. 3.10 Detemination L.O.D.

Sr. No.	Drug	Absorbance at	Value of L.O.D
1	Atenolol	275 nm	0.198 µg/ml
2	Atenolol	253 nm	1.155 µg/ml
3	Losartan Potassium	235 nm	0.132 µg/ml
4	Losartan Potassium	242 nm	0.143 µg/ml

3.8.2.. The Limit of Quantification (L.O.Q.)

The L.O.Q. was estimated or calibrated from the set of three calibration curves used to the determination method linearity, the L.O.Q. may be calculated as

$LOQ = 10 \times (\sigma/S)$……(2)

Where it was,

σ = The Standard deviation of the Y-intercepts of the three calibration curves under done.

S = the mean slope of the three calibration curves.

Table No.3.11 Determination L.O.Q.

Sr.No.	Drug	Absorbance at	Value of L.O.D
1	Atenolol	275 nm	0.6 µg/ml
2	Atenolol	253 nm	3.5 µg/ml
3	Losartan Potassium	235 nm	0.4 µg/ml
4	Losartan Potassium	242 nm	0.43 µg/ml

3.9. Robustness

Definition:-The robustness is an analytical procedure is assessed or measure of its capacity or strength to remain unaffected by small, but conscious variations in method parameters and provide an indication of its reliability at some stage in normal usage form.

Recommendation

The robustness should be measured early in the development of a method. If the results of a method or other measurements are susceptible to variations in method parameters, these parameters should be adequately controlled and a precautionary statement included in the method documentation.

Expression/calculation

The effect of these changed parameters on system suitability parameters.

Acceptance criteria

The method must be robust enough to withstand slight changes and allow routine analysis of sample.

Table No. 3.12 Robustness study of ATENOLOL,

ANALYST:-I.

Sr.No	Concentartion	Absorbance 275 nm	% RSD	Absorbance 275 nm	% RSD
1	6	0.02840	0.02902	0.02842	0.02905
2	6	0.02948		0.02955	
3	6	0.02920		0.02918	

ANALYST:-II

Sr.No	Concentartion	Absorbance 253 nm	% RSD	Absorbance 253 nm	% RSD
1	6	0.02844	0.02890	0.02847	0.02891
2	6	0.02927		0.02923	
3	6	0.02901		0.02903	

Table No. 3.13 Robustness study of LOSARTAN POTASSIUM

ANALYST:-I

Sr.No	Concentartion	Absorbance 235 nm	% RSD	Absorbance 235 nm	% RSD
1	6	0.2878	0.2930	0.2874	0.2931
2	6	0.2969		0.2971	
3	6	0.2944		0.2949	

ANALYST:-II

Sr.No	Concentartion	Absorbance 242 nm	% RSD	Absorbance 242 nm	% RSD
1	6	0.2883	0.2935	0.2877	0.2926
2	6	0.2973		0.2970	
3	6	0.2951		0.2933	

4- SUMMARY AND CONCLUSION

Summary

A convenient and rapid UV method has been developed for simultaneous estimation ofAtenolol and Losartan Potassium in bulk and tablet dosage form. The assay provides a linearresponse across a wide range of concentrations. The Correlation coefficient of these drugswas found to be close to 1.00, indicating good linearity. Intra-day, inter-day and % R.S.D.values were found to be less than 2 % as required by ICH guidelines, which indicates thevalidity of methods; hence, this method can be easily and conveniently adopted for routineanalysis of Atenolol and Losartan Potassium in pure form and its tablet dosage forms. It can also be used for theanalysis of these drugs in biological fluids and in qualitycontrol laboratories.

CONCLUSION

A rapid, easy and convenient method used for simultaneous estimation of Atenolol and Losartan Potassium in bulk and tablet dosage form. The statistical analysis was carried out by proposed method. The values of standard deviation and coefficient of variation value were satisfactory match within a limit. The proposed simultaneous estimation and dual wavelength method was simple, time consuming, low cost and found that one of the best analytical technique. This method is further used for analysis of drugs.

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