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Stability Indicating HPTLC Method Development And Validation For Tablet Formulation Contaning Losartan Potassium And Hydrochlorothiazide

[1]Mansi P. Rana, [2]Hasnain M. Shah, [3]Dhruvi R. Patel, [4]Vikram M. Pandya
Assistant Professor[1], PG students[2,3,], Principal[4]
[1]Department of Quality Assurance, [2,3,4,]Department of Pharmaceutics
Tathya Pharmacy College, Thala, Chikhli,;Gujarat.

ABSTRACT: The present work describes the development and validation of a stability-indicating High-Performance Thin Layer Chromatography (HPTLC) method for the simultaneous estimation of losartan potassium and hydrochlorothiazide in tablet dosage form. Losartan potassium, an angiotensin II receptor antagonist, and hydrochlorothiazide, a thiazide diuretic, are commonly used in combination for the treatment of hypertension. Considering the importance of quality control and stability assessment of combined pharmaceutical formulations, a simple, precise, accurate, and reliable HPTLC method was developed.Chromatographic separation was achieved on precoated silica gel 60 F254 plates using a mobile phase consisting of chloroform: methanol: formic acid: acetone (7.5:1.3:0.2:0.5 v/v). Densitometric scanning was performed at 232 nm. The developed method showed good resolution with Rf values of 0.72 for losartan potassium and 0.43 for hydrochlorothiazide. The method was validated as per ICH Q2 (R1) guidelines for parameters such as linearity, accuracy, precision, specificity, limit of detection, and limit of quantitation. Forced degradation studies demonstrated that the method is stability indicating. The proposed HPTLC method was found to be suitable for routin analysis and stability testing of losartan potassium and hydrochlorothiazide in combined tablet dosage form.

KEYWORDS: Stability -indicating method, HPTLC, Losartan potassium, Hydrochlorothiazide, Hypertension, Method development, Method validation, forced degradation, Tablet formulation.

INTRODUCTION: Hypertension is a major cardiovascular disorder characterized by persistently elevated blood pressure, where the force of blood pushing against the arterial walls exceeds normal limits. Blood pressure reflects the resistance encountered by blood as it flows through arteries, which are responsible for carrying oxygenated blood from the heart to body tissues. Hypertension is broadly classified into primary (essential) hypertension and secondary hypertension. Primary hypertension develops gradually over many years and has no identifiable cause in most adults. In contrast, secondary hypertension appears suddenly and is associated with underlying conditions such as kidney disorders, adrenal gland tumours, thyroid abnormalities, obstructive sleep apnea, congenital blood vessel defects, or the use of certain medications and illegal drugs. Most individuals with hypertension remain asymptomatic until the disease reaches severe or life-threatening stages, although some may experience headaches, shortness of breath, or nosebleeds.

Losartan potassium and hydrochlorothiazide are widely used in combination therapy for the effective management of hypertension. Losartan potassium is an angiotensin II receptor type 1 (AT1) antagonist that reduces peripheral resistance and cardiac preload by blocking the effects of angiotensin II, including aldosterone release. This action ultimately leads to decreased sodium retention and reduced blood pressure. Hydrochlorothiazide is a thiazide diuretic that acts on the distal renal tubules to increase the excretion of sodium and chloride, thereby reducing plasma volume. The combination of these two drugs provides a synergistic antihypertensive effect and is commonly formulated into tablet dosage forms.

Quality control and stability assessment of such combined pharmaceutical formulations require reliable and validated analytical techniques. High-Performance Thin Layer Chromatography (HPTLC) is an advanced form of thin layer chromatography that offers high separation efficiency, precise sample application, reproducible chromatographic development, and software-controlled evaluation. HPTLC is a standardized, validated, and scientifically established technique suitable for both qualitative and quantitative analysis. It meets the stringent quality requirements of modern analytical laboratories, even in regulated environments.

HPTLC offers several advantages, including rapid development time, minimal risk of contamination, simplicity of operation, reusability of TLC plates, and flexible detection options. The chromatogram produced is visually observable, enabling easy identification of separated components. HPTLC operates as a three-phase system involving solid (stationary), liquid (mobile), and vapor phases, where separation is governed by complex physicochemical interactions. Variations in adsorption behaviour and equilibrium conditions influence the retardation factor (Rf) values of compounds, especially when analysed in mixtures.

Method development in HPTLC is a critical step involving careful selection of stationary phase, optimization of mobile phase composition, sample application technique, detection, and quantitation. Mobile phase selection is often achieved through trial-and-error or literature guidance, with solvent polarity playing a key role in compound migration. Detection is commonly performed using fluorescence indicators under UV light, while densitometric scanning converts spot intensity into quantitative chromatographic data.

Analytical method validation is an integral part of good analytical practice and provides documented evidence that a method consistently produces reliable results. According to USP and ICH guidelines, validation parameters include accuracy, precision, specificity, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), ruggedness, and robustness. These parameters ensure that the analytical procedure is suitable for its intended purpose.

Forced degradation studies, as described in ICH Q1A(R2) guidelines, are essential for developing stability-indicating methods. Such studies evaluate how drug substances and products respond to environmental stress conditions, ensuring that degradation products do not interfere with the quantification of active ingredients.

Although several analytical methods such as RP-HPLC, HPLC, TLC, UV, and HPTLC have been reported for individual drugs and combinations, no stability-indicating HPTLC method has been reported for the combined tablet formulation of losartan potassium and hydrochlorothiazide. Therefore, the present work aims to develop and validate a stability-indicating HPTLC method for the simultaneous estimation of losartan potassium and hydrochlorothiazide in tablet dosage form, ensuring accuracy, precision, specificity, and compliance with regulatory guidelines.

HYPERTENSION: Hypertension is high blood pressure. Blood pressure is the force of blood pushing against the walls of arteries as it flows through them. Arteries are the blood vessels that carry oxygenated blood from the heart to the body's tissues.

- **Causes: Primary(essential) hypertension:** For most adults, there is no identifiable cause of high blood pressure. This type of high blood pressure, called primary (essential) hypertension, tends to develop gradually over many years.

SECONDERY HYPERTENSION: Some people have high blood pressure caused by an underlying condition. This type of high blood pressure, called secondary hypertension, tends to appear suddenly and causes higher blood pressure than primary hypertension. Various conditions and medications can lead to secondary hypertension, including.

- Obstructive sleep apnea Kidney problems
- Adrenal gland tumors
- Thyroid problems
- Certain defects you are born with (congenital) in blood vessels.
- Illegal drugs, such as cocaine and amphetamines.

- **Symptoms:** Most people with high blood pressure have no signs or symptoms, even if blood pressure readings reach dangerously important levels. A few people with high blood pressure may have headaches, shortness of breath or nosebleeds, but these signs and symptoms are not specific and usually do not occur until high blood pressure has reached a severe or life-threatening stage.

INTRODUCTION TO HPTLC: High-Performance Thin layer Chromatography (HPTLC) is the most advanced form of TLC and comprises the use of chromatographic layers of most separation efficiency and the employment of state-of-the-art instrumentation for all layer steps in the procedure: precise sample application, standardized, reproducible chromatogram development and software control evaluation.

- HPTLC is an entire concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis.

- HPTLC meets all quality requirements of today's analytical labs even in a fully regulated environment.

- **Advantages of HPTLC:**

- The Chromatogram is visible to the eye.
- Contamination risk- free analysis
- Quite simple to use.
- Development time is very faster.
- The TLC plate is re-usable.
- Extremely flexible detection

➢ Characteristics of HPTLC:

- HPTLC applies physico-chemical phenomena more complex than HPLC.
- HPTLC corresponds to a three-phase system between which equilibrium is established solid (stationary), liquid(mobile) and vapour phases.
- The stationary phase is only partially equilibrated with the liquid phase before the migration of the compounds.
- Depending upon the manner in which the separation is obtained, the mobile phase may or may not be in equilibrium with the vapour phase.
- The adsorption phenomenon of the stationary phase is substantially reduced Once a large part of the adsorption sites is occupied.
- As a result, the Rf (retardation factor) of a compound in the pure state is slightly different form the Rf of the same compound present in a mixture.

❖ General steps and instrumentation of HPTLC

❖ Introduction:

- Method development in thin layer chromatography is one of the most crucial steps for a qualitative and quantitative analysis. Method development is divided into selection of the development mode, of the stationary phase of the vapour phase, of suitable solvents, optimization of the mobile phase, transfer of the optimized mobile phase to an appropriate forced flow planner chromatography method (FFPC)method, and selection of other operating parameters.

❖ Selection of stationary phase:

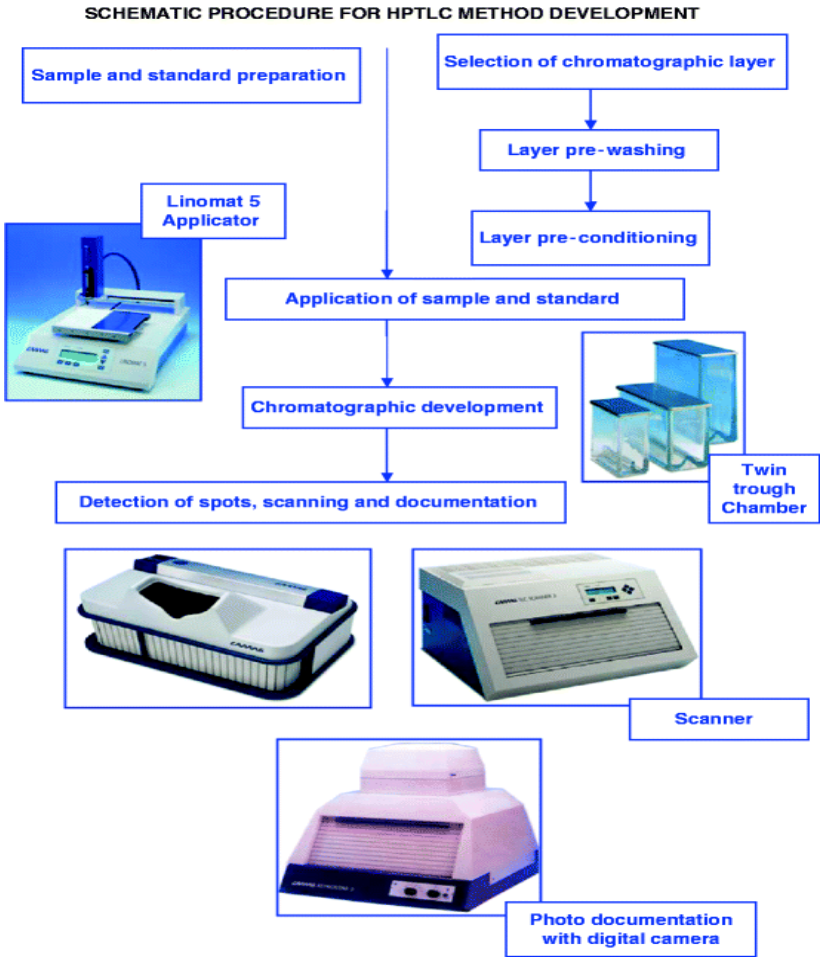
- During method development, stationary phase selection should be based on the type of compound to be separated. If the compounds are ionic adsorption chromatography can be used. IEC in which ion-exchangers
- Such as modified cellulose are used as layers, are applicable to the separation of ionic compound for instance, inorganic ions, purines, and pyrimidines.

➢ Selection and optimization mobile phase:

- Mobile phase components selection and optimization in many cases is done on a trial-and-error basis or the analyst's experience or following hints form a literature search. One's own experience and literature non-polar compounds eluted first because of lower affinity with stationary phase. Mainly selected 27 commonly used solvents in planer chromatography, from which he indicated nine solvents that can be used individually for running a solution of a compound mixture on a silica TLC.The selected solvent diethyl ether, ethanol, tetra hydrofuran, acetic acid, dioxane, chloroform.

❖ Sample application:

- Devices for application of sample solution on the chromatographic layers are available from different manufacturers. Example of such devices are the nanomate 4, the linomat 5, and the automatic TLC sampler Nanomate 4 is manually operated spot wise sample application device where dosing is done with a disposable capillary pipette guided by a universal capillary holder, Types of chromatography bases on run direction ascending, descending, two dimensional, multiple and step wise development, radical, anti-radical and flow planer chromatography. Major types of chambers available for HPTLC are the normal (N) type and sandwich(S) type.



❖ Detection:

- Detection of separated compounds on the sorbent layers in HPTLC analysis is facilitated with the impregnation of fluorescence indicators with 254 to 366 nm excitation wavelength, making an irradiated plate to fluorescence with green or blue colour, respectively. Fluorescence quenching by the UV-absorbing compounds enables detection. Electronic devices such as densitometers are available to translate the intensity of the quenching by detected compounds into chromatograms whose peak.

❖ Quantitation:

- Methods for the quantitative determination of separated compounds on the HPTLC plates include those in which the compound are scraped from the stationary phase before being examined and those in which separated compounds are examined directly on the stationary layer. In the former, the scraped compound is extracted from the layer using a suitable solvent, to obtain a solution that can be analyzed by conventional quantitative methods such as UV, HPLC, GC and Liquid scintillation counting.

❖ Application:

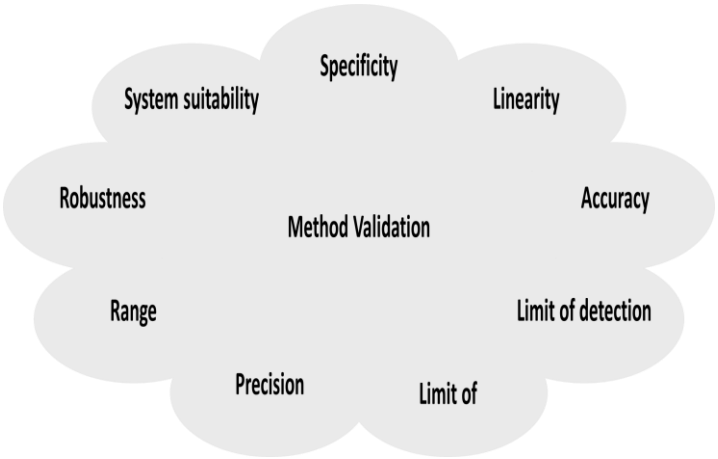
- HPTLC finds application in many fields of analytical sciences. Apart from being used to active ingredients in pharmaceutical formulation it is used for testing their stability. potential usefulness and contribution of HPTLC in food analysis as a quantitative method.

➢ Analytical method validation:

❖ Definition:

- Validation is to establish documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes.
- Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability, and consistency of analytical results. It is an integral part of any good analytical practice.

The USP has published specific guidelines for method validation for compound evaluation.



❖ Accuracy

- The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value.
- The accuracy of an analytical method should be established across its range. In the case of the assay of a drug in a formulated product, accuracy may be determined by application.
- analytical method to synthetic mixture of the drug product component to which known Amount of analyte have been added within the range of the method.

❖ Precision:

- The precision of an analytical procedure expresses the closeness of agreement between a Series of measurement obtained from multiple sampling of the same homogeneous sample Under the prescribed condition.
- Precision should be investigated using homogeneous, authentic samples. In the precision results of all samples should not have RSD >2%

❖ Reproducibility:

- Reproducibility expresses the precision between laboratories studies.
- Reproducibility can be assessed by means of an inter-laboratory trial.
- Reproducibility Should be considered in case of the standardization of an analytical procedure.

❖ Specificity:

- Specificity is the ability to assess unequivocally the analyte in the presence of Component which may be expected to be present. Typically, these might include Impurities, degradants, matrix etc.
- ICH documents state that when chromatographic procedure used representative Chromatogram should be used to demonstrate specificity and individual components should be appropriately detected.

❖ Limit of detection:

- The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

❖ Determination of limit of detection:

- For instrumental and non-instrumental methods detection limit is generally determined by the analysis of samples of known concentration of analyte and by establishing the minimum level at which the analyte can be reliability detected.

The limit of detection may be expressed:

LOD =3.3 ɑ/s

Where ɑ = the std deviation of curve

s= the slope of the calibration curve

Slope S may be estimated from the calibration curve of the analyte.

❖ Limit of quantitation:

- The limit of quantitation of an individual analytical procedure is the lowest amount of Analyte in a sample which can be quantitatively determined with suitable precision and Accuracy.
- The limit of quantitation is a parameter of qualitative assays for low level of compounds in sample matrices and is used particularly for the determination of Impurities or degradation products.

❖ Determination of limit of quantitation:

- For instrumental methods quantitation limit is generally determined by the analysis of samples of known concentration of analyte and by establishing the minimum level at which the analyte can be qualified with acceptable accuracy and precision.

- **The limit of quantitation may be expressed as:**

LOQ =10α/s

- Where, α=the std deviation of the response.

s=the slope of the calibration curve.

❖ Linearity and Range:

- The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample.
- The range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentration) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

❖ Determination of Linearity and Range:

- For the determination of linearity, a minimum of 5 concentration is recommended. Linearity can be determined by a series of samples whose concentration span 80-120% of the expected concentration range. Linearity is evaluated by graphically.

❖ Ruggedness:

- Degree of reproducibility of test results obtained by the same sample under different conditions such as, different analyst, different laboratories condition, different instruments etc. normally expressed as the lack of influence on the test result of operational & environmental variables of analytical method.
- Ruggedness is a measure of reproducibility of test results under the variation in the condition normally expected from the laboratory to laboratory and from analyst to analyst.

❖ Determination of Ruggedness:

- By analysis of aliquot from homogeneous lots in different laboratory, by different instruments and using operational and environmental conditions that may differ but still with the specified parameter of the assay. Degree of reproducibility of test results is then determined as a function of assay variable.
- Different operators in same laboratory, Different equipment in same laboratory.
- Different source of segment and solution, Different source of column.

❖ Robustness:

- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in parameter and provides an indication of its reliability during normal usage.

❖ Determination of robustness:

- 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 variation in method parameters.
- Examples of typical variation are:
- Stability of analytical solutions.
- Extraction time.

❖ **In the case of liquid chromatography, examples of typical variation are:**

- Influence of variation of pH in a mobile phase.
- Influence of variation in mobile phase composition.
- Different column (different lots/or suppliers).
- Temperature and flow rate.

❖ **Application and advantages:**

- An ideal method for separation of various compounds in plant extracts which resemble in structure and thus demand specific and extremely sensitive method.
- A premier separation technique capable of multi-component analysis of real-life samples and complex mixtures.
- This method is used for ascertaining of various pharmaceuticals. The analysis of the various degradation products can be done and thus stability indicating HPLC systems and method has developed.
- Highly automated, using sophisticated auto-samplers and data system for unattended analysis and report generation. Few techniques can match its versatility and precision of ±0.5%RSD.

➢ **Introduction of Analytical Method Development** [6]

❖ **Steps involve in method development**

1. Understand the physicochemical properties of drug molecules.
2. Set up HPTLC conditions.
3. Preparation of sample solution for method development.
4. Method optimization
5. Validation of method.

❖ The goal of the HPTLC-method is to Try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.

- Rapid, Specific, Accurate and Precise

HPTLC Allows qualitative and quantitative information of drug Used to provide information on the composition of drug related to Sample.

➢ **Forced degradation Method** [7]

- ICH harmonized Tripartite Guideline described Stability Testing of New Drug Substances and Products [ICH Q1A(R2)]
- The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity, and light.
- According to **US-FDA** stability guideline of 1998, Stability indicating assay method defined as "Validated quantitative analytical methods that can detect the changes with time in the chemical, physical, or microbiological properties of the drug substance and drug product, that are specific so that the contents of active ingredient, degradation products, and other components of interest can be accurately measured without interference."

- **Objectives of Forced degradation study**

To reveal the degradation mechanisms

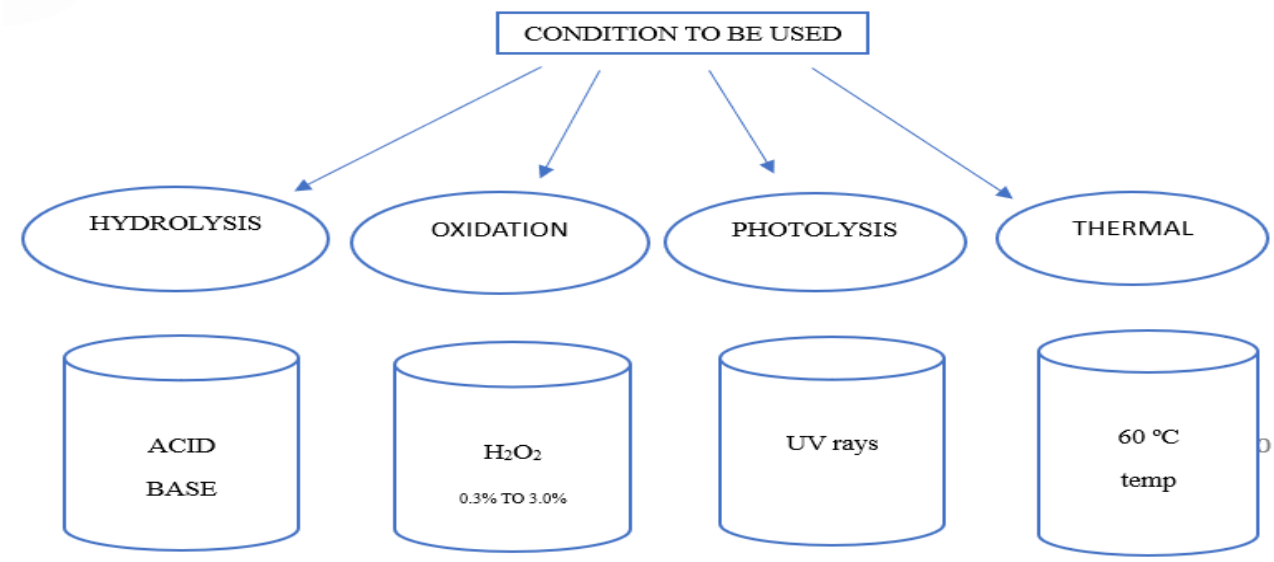
To understand the chemical properties of drug

To elucidate the structure of degradation

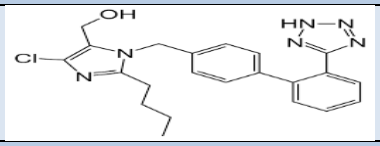
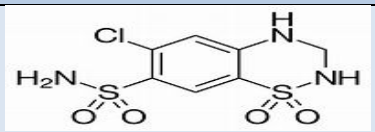
To determine the intrinsic stability of a drug substance

To established stability indicating nature of a developed method

➢ **Forced degradation parameters:**



DRUG PROFILE:

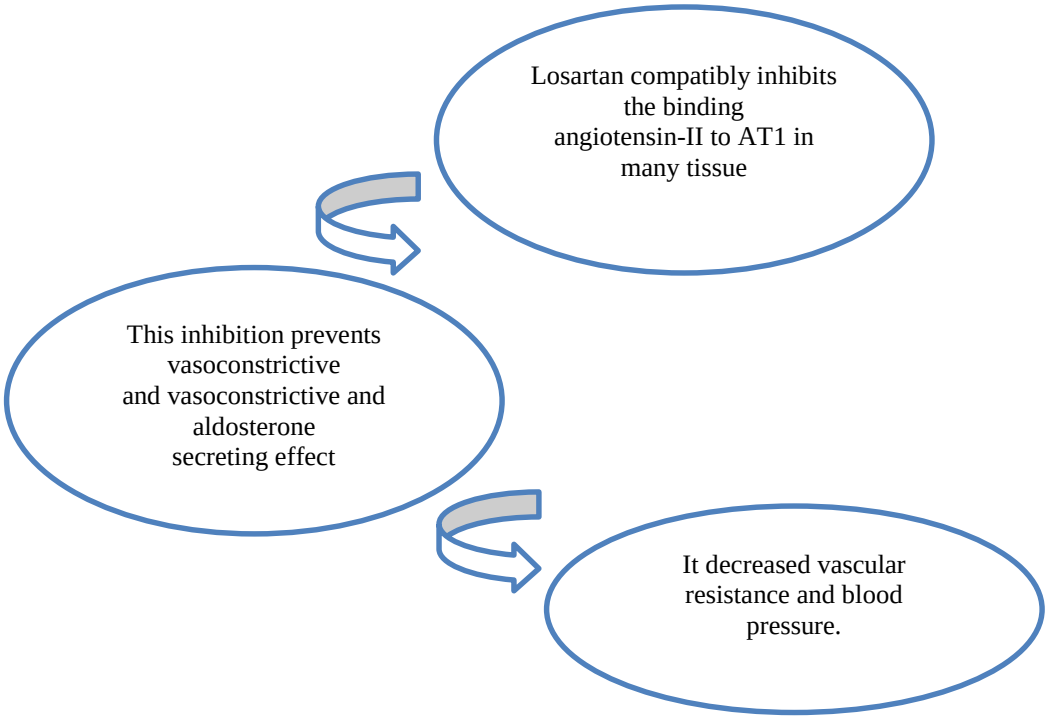
PROPERTIES	LOSARTAN POTASSIUM	HYDROCHOLORO-THIAZIDE
Category	Anti-hypertensive	Diuretic
Structure		
Chemical Formula	$C_{22}H_{23}C_1N_6O$	$C_7H_8C_lN_3O_4S_2$
IUPAC Name	[2-butyl-5-chloro-3-[4-[2-(2H-tetrazolyl) phenyl] phenyl] methyl] imidazol-4-yl] methanol	6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ6,2,4-benzothiadiazine-7-sulfonamide
Molecular Weight	422 g /mol	297.9 g/mol

PROPERTIES	LOSARTAN POTASSIUM	LOSARTAN POTASSIUM
Solubility	Ethanol, Dimethyl formamide	NaOH, Methanol Dimethyl formamide
State	solid	solid
Melting point	178-184 ºC	273-275 ºC
pKa	178-184 ºC	7.9
Log P	1.19	-0.07

LOSARTAN POTASSIUM

- Losartan is a selective, competitive angiotensin II receptor type 1 (AT1) antagonist, reducing the end organ responses to angiotensin II.
- Losartan administration results in a decrease in total peripheral resistance (afterload) and cardiac venous return (preload).
- All of the physiological effects of angiotensin II, including release of aldosterone, are antagonized in the presence of losartan.
- Reduction in blood pressure occurs independently of the status of the renin–angiotensin system.
- As a result of losartan dosing, plasma renin activity increases due to removal of the angiotensin II feedback. Renin is released from the kidneys when there is reduced renal arterial pressure, sympathetic activation, or increased sodium delivery to the distal renal tubule.
- Renin then acts by converting angiotensinogen to angiotensin I; angiotensin converting enzyme (ACE) converts angiotensin I to angiotensin II; angiotensin II causes vasoconstriction and aldosterone release.
- Aldosterone serves to retain sodium from the distal renal tubule. Sodium retention ultimately results in increased blood pressure.
- Therefore, the use of angiotensin II receptor antagonists like losartan result in blocking the downstream effect of renin, angiotensin II, and ultimately decreasing blood pressure.

MECHANISM OF ACTION:



HYDROCHLOROTHIAZIDE:

- The mechanism of the antihypertensive effect of thiazides is unknown. Hydrochlorothiazide does not usually affect normal blood pressure.
- Hydrochlorothiazide affects the distal renal tubular mechanism of electrolyte reabsorption. At maximal therapeutic dosage, all thiazides are approximately equal in their diuretic efficacy.
- Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. Natriuresis may be accompanied by some loss of potassium and bicarbonate.
- After oral use diuresis begins within 2 hours, peaks in about 4 hours and lasts about 6 to 12 hours.

LITERATURE REVIEW:

BOOKS

PATENTS

THESIS

JOURNAL

INTERNET

PHARMACOPOEIA

➢ REVIEW OF LITERATURE

Official Methods for Losartan Potassium:

Sr. no	Drug	Method	Analytical Description	Ref. no.
1.	Losartan Potassium	LC	**mobile phase: Ortho** phosphoric acid: water (75:25) v/v **column:** octaylsalin (250*4) mm **wavelength :**220nm **flow rate :**1.0 ml/min	11
2.	Losartan Tablet	LC	**mobile phase: Buffer**: Acetonitrile (5:25) v/v **column: Lichrospe**-re RP8e (250*4.0) mm **wavelength :**235nm **flow rate :**1.5 ml/min	11
3.	Losartan Potassium and Amlodipine	LC	**Mobile phase**: Buffer: Orthophosphoric acid (70:30) v/v **Column**: octaylsalin (250*4) mm **Wavelength :**254nm **Flow rate:** 1.5 ml/min	11
4.	Losartan Potassium and hydrochlorothiazide	LC	**Mobile phase: Acetonitrile**: Buffer (75:25) /v **Column**: Liposphere 8e (150*4.0) mm **Wavelength** :235nm **Flow rate** :1.0 ml/min	11

Official Methods of LC And TLC For Hydrochlorothiazide:

Sr. no	Drug	Method	Analytical Description	Ref. no
1.	Hydrochlorothiazide	LC	**Mobile phase:** Methanol: Phosphate Buffer: Acetonitrile (9.4:0.6:0.1) v/v **Column**: OctadecylsyliteSilica gel (250*4.6) mm **Wavelength** :273nm **Flow rate** :1.0 ml/min	11
2.	Hydrochlorothiazide	TLC	**Mobile phase: Ethyl** acetate **Column**: Silica gel 60F254 **Wavelength** :254nm	11
3.	Hydrochlorothiazide	TLC	**Mobile phase: Coating** Substance and ethyl acetate **Column: Silica** gel G F_{254} **Wavelength** :254nm	12
4.	Hydrochlorothiazide	LC	**Mobile phase:** Methanol: Acetonitrile: Phosphate buffer (7:2:1) v/v **Column: PhenosphereODS** (100*4.6) mm **Wavelength** :254nm **Flow rate** :1 ml/min	12

Official Method of UV For Hydrochlorothiazide:

Sr. no	Drug	Method	Analytical Description	Ref. no
1.	Hydrochlorothiazide	UV	**Solvent**: NaOH and water **Absorbance Ratio** :5.4 to5.7 **Absorbance Max** :273nmTo 323nm **Spectrial Range** :250-350 nm	11

➢ **REVIEW OF LITERATURE: Reported Methods of RP-HPLC For Losartan Potassium:**

Reported Methods of RP-HPLC For Losartan Potassium:

Sr no.	Drug	Method	Analytical Description	Ref. No.
1.	losartan potassium and amlodipine	RP-HPLC	**Mobile phase** :o -phosphoric acid **Column** -c18 (100-5,250*4.6 nm) **Wavelength** -226 nm **Flow rate** -1.0 ml/min	12
2.	losartan potassium and hydrochlorothiazide	RP-HPLC	**Mobile phase** -methanol and acetone (70:30) v/v **Column** -gold c18 (150*4.6 nm) **Wavelength** -271 nm **Flow rate** -1.5 ml/min	13
3.	Losartan Potassium	RP-HPLC	**Mobile phase**: Ammonium phosphate buffer: acetonitrile (65:35) v/v **Column**: SpherisorhC18 (250*4.6) mm **Wavelength** :254 nm **Flow rate** :1.5 ml/min	14
4.	Losartan Potassium	RP-HPLC	**Mobile phase**: Orthophospheric acid: acetonitrile (55:45) v/v **Column**: Zorlax C18(150*4.6) mm **Wavelength** :254 nm **Flow rate** :1.0 ml/min	15
5.	Losartan Potassium	RP-HPLC	**Mobile phase:** Orthophosphericacid: methanol (40:60) v/v **Column**: C18(150*4.6) mm **Wavelength**:230nm **Flow rate:** 1.0 ml/min	16
6.	Losartan Potassium and Hydrochlorothiazide	RP-HPLC	**Mobile phase**: Acetonitrile: water (60:40) v/v **Column**: ACE 3 C- 18(250*4.6) mm **Wavelength**:226nm **Flow rate:**1.0 ml/min	17
7.	Losartan potassium and Amlodipine	RP-HPLC	**Mobile phase: Triethylamine**: Acetonitrile (70:30) v/v **Column**:C-18 (250*4.6) mm **Wavelength** :246 nm **Flow rate** :1.0 ml/min	18
8.	Losartan potassium and Atenolol	HPLC	**Mobile phase: Potassium** hydroxide: Phosphate buffer (45:55) v/v **Column**: SuperlecosilODS (250*4.6) mm **Wavelength** :227nm **Flow rate** :1.2 ml/min	19
9.	Losartan potassium and Ramiprile	RP-HPLC	**Mobile phase: Acetonitrile**: methanol: Butyl: ammonium (30:30:40) v/v **Column:** Hypersil ODS (250*4.6) mm **Wavelength** :210nm **Flow rate** :1 .0ml/min	20
10.	Losartan Potassium and chlorthalidone	RP-HPLC	**Mobile phase:** Acetonitrile: water (80:20) v/v **Column**: PhenomenexC18 (150*4.6) mm **Wavelength** :284nm **Flow rate** :1.0 ml/mi	21
11.	Losartan potassium and Peridoprile	RP-HPLC	**Mobile phase** Methanol: phosphate buffer (85:15) v/v **Column**: LUNA C-18(250*4.6) mm **Wavelength** :227nm **Flow rate** :0.8 ml/min	22
12.	Losartan potassium, amlodipine, and hydrochlorothiazide	HPLC	**Mobile phase:** Phosphate buffer: CAN (57:43) v/v **Column**: Kromasil C8 (250*4.6) mm	23

			Wavelength :232nm **Flow rate** :1to 6.3ml/min	
13.	Losartan potassium, Amlodipine, and hydrochlorothiazide	HPLC	**Mobile phase:** Acetonitrile: phosphate Buffer (70:30) v/v **Column**: C8 (150*6.4) mm **Wavelength** :254nm **Flow rate** :1ml/min	24
14.	Losartan potassium and amlodipine hydrochlorothiazide	HPLC	**Mobile phase** Methanol: water (95:5) v/v **Column**: Hypersil Gold (250*4.6) mm **Wavelength:** 230nm **Flow rate:** 0.8ml/min	25
15.	Losartan potassium	HPTLC	**Mobile phase:** Methanol: acetic acid: acetonitrile (3.5:2.6:3.9) v/v **Column:** Silica gel $60F_{254}$ **Wavelength:** 270nm **Flow rate:** 1 ml/min	26
16.	Losartan potassium and chlorothilidone	HPTLC	**Mobile phase: Methanol**: acetic acid: acetonitrile (3.5:2.6:3.9) v/v **Column:** Silica gel $60F_{254}$ **Wavelength:**270nm **Flow rate:** 1 ml/min	27
17.	Losartan potassium and amlodipine	Stability Indicating HPTLC	**Mobile phase** Chloroform: methanol: toluene: acetonitrile (5.5:2.5:2:0.5) v/v **Column:** Silica gel $60F_{254}$ **Wavelength**:237nm **Flow rate:**1.5ml/min	28
18.	Losartan Potassium, Hydrochlorothiazide And amlodipine	HPTLC with UV	**Mobile phase** Chloroform: acetone: Methanol: formic acid (7.5:0.5:1.3:0.03) v/v **Column**: Silica gel plate **Wavelength** :226nm **Flow rate** :1.0ml/min	29
19.	Losartan potassium, Hydrochlorothiazide and amlodipine	HPTLC	**Mobile phase** Chloroform: methanol: ACN: formic acid (7.5:1.3:0.5:0.03) v/v **Column:** Silica gel plate **Wavelength** :254nm **Flow rate:**1.0ml/min	30
20.	Amlodipine, losartan Potassium and hydrochlorothiazide	UPLC	**Mobile phase**: ACN: ammonium acetate (98:2) v/v **Column**: C18(2.1*50) mm **Wavelength:** 254nm **Flow rate** :0.4ml/min	31

Reported methods of RP-HPLC for Hydrochlorothiazide:

Sr. no	Drug	Method	Analytical Description	Ref. no
1.	Hydrochlorothiazide	RP-HPLC	**Mobile phase** Acetonitrile: water (50:50) v/v **Column:** ODS 3 (250*4.6) mm **Wavelength** :272nm **Flow rate :**1.0 ml/min	32
2.	Hydrochlorothiazide And Lisnoprile	RP-HPLC	**Mobile phase: Acetate** buffer: acetonitrile (75:25) v/v **Column: C** 18column (150*4.6) mm **Wavelength** :230nm **Flow rate** :1.0 ml/min	33
3.	Hydrochlorothiazide And Enaprile	RP-HPLC	**Mobile phase:** ACN: water: trimethylamine (14:85.5:6.4) v/v **Column:** Supelocil-Le8(150*4.6) mm **Wavelength:**254nm **Flow rate :**2.0ml/min	34

4.	Hydrochlorothiazide And Captopril	RP-HPLC	**Mobile phase** Acetonitrile: triflurosen: Water (70:15:15) v/v **Column:** C 18(150*4.0) mm **Wavelength :**263nm **Flow rate :**1.2 ml/min	35
5.	Hydrochlorothiazide And Metoprolol	RP-HPLC	**Mobile phase:** Sodium hydrogen: methanol: acetonitrile (525:225:250) v/v **Column:** C-18(150*4.6) mm **Wavelength:** 222nm **Flow rate :**1.0 ml/min	36
6.	Hydrochlorothiazide And amloride	RP-HPLC	**Mobile phase** KH_2PO_4: acetonitrile: Triethylamine (90:10:0.3) v/v **Column:** ODS C-18(250*4.6) mm **Wavelength**: 260nm **Flow rate :**1.5 ml/min	37
7.	Hydrochlorothiazide And Trimterene	RP-HPLC	**Mobile phase:** Acetonitrile: phosphate Buffer (75:25) v/v **Column**: Lunn C18(250*4.6) mm **Wavelength:** 272nm **Flow rate :**1.0 ml/min	38
8.	Hydrochlorothiazide , Atenolol and Amloride	RP-HPLC	**Mobile phase:** Aerohydrous: acetonitrile: triethylamine (95:5:0.1) v/v **Column:** C 18(250*4.6) mm **Wavelength:** 280nm **Flow rate :**0.9 ml/min	39
9.	Hydrochlorothiazide and Timolol	HPLC	**Mobile phase**: Methanol: water: tea (85:15:0.25) v/v **Column:** C-18 column (150*4.6) mm **Wavelength**: 225nm **Flow rate :**1.0 ml/min	40
10.	Hydrochlorothiazide and propranolol	HPLC	**Mobile phase: Water**: acetonitrile (45:55) v/v **Column:** Phenomenex C18(150*4.6) mm **Wavelength** :270nm **Flow rate :**1.0 ml/min	41

Reported methods of RP-HPLC for Hydrochlorothiazide:

Sr. no	Drug	Method	Analytical Description	Ref. no
1.	Hydrochlorothiazide and Spironolactone	HPTLC	**Mobile phase: Ethyl** ether: chloroform: Formic acid: methanol (7:3:0.1:0.1) v/v **Column:** Silica gel $60F_{254}$ **Wavelength:** 230nm **Flow rate** :2.0 ml/min	42
2.	Hydrochlorothiazide and Quinapril	HPTLC	**Mobile phase: Ethyl** acetate: acetone: acetic acid (6.5:3:0.5) v/v **Column**: Silica gel $60F_{254}$ **Wavelength:**208nm **Flow rate:**1.3 ml/min	43
3.	Hydrochlorothiazide and Bisoprolol	HPTLC	**Mobile phase: Ethyl** acetate: methanol: ammonium (10:0.5:0.5) v/v **Column:** Silica gel $60F_{254}$ **Wavelength:** 225nm **Flow rate** :1.0 ml/min	44
4.	Hydrochlorothiazide	HPTLC	**Mobile phase: Triethyl** amine: toluene: dioxane (2:3:5) v/v **Column:** Silica gel $60F_{254}$ **Wavelength:** 281nm **Flow rate :**1.0 ml/min	45
5.	Hydrochlorothiazide and Irbesartan	HPTLC	**Mobile phase** Acetonitrile: chloroform (4:6) v/v **Column:** Silica gel $60F_{254}$ **Wavelength:** 270nm **Flow rate :**1.0 ml/min	46
6.	Hydrochlorothiazide,	HPTLC	**Mobile phase** Toluene: methanol: ethyl acetate: acetone	47

	Olmesartan Medoxomil		(2.5:1:0.5:2) v/v **Column:** Silica gel 60F $_{254}$ **Wavelength:**254nm **Flow rate** :1.0 ml/min	
7.	Telmisartan and hydrochlorothiazide	HPTLC	**Mobile phase** methanol: chloroform: acetone (30:30:40) v/v **Column:** silica gel F254 **Wavelength** 272nm **Flow rate:**1.0 ml/min	48

Reported Methods of HPTLC For Hydrochlorothiazide:

Sr. no	Drug	Method	Analytical Description	Ref. no
1.	Hydrochlorothiazide and Spironolactone	HPTLC	**Mobile phase: Ethyl** ether: chloroform: Formic acid: methanol (7:3:0.1:0.1) v/v **Column:** Silica gel 60F$_{254}$ **Wavelength:** 230nm **Flow rate** :2.0 ml/min	42
2.	Hydrochlorothiazide and Quinapril	HPTLC	**Mobile phase: Ethyl** acetate: acetone: acetic acid (6.5:3:0.5) v/v **Column**: Silica gel 60F$_{254}$ **Wavelength:**208nm **Flow rate:**1.3 ml/min	43
3.	Hydrochlorothiazide and Bisoprolol	HPTLC	**Mobile phase: Ethyl** acetate: methanol: ammonium (10:0.5:0.5) v/v **Column:** Silica gel 60F$_{254}$ **Wavelength:** 225nm **Flow rate** :1.0 ml/min	44
4.	Hydrochlorothiazide	HPTLC	**Mobile phase: Triethyl** amine: toluene: dioxane (2:3:5) v/v **Column:** Silica gel 60F$_{254}$ **Wavelength:** 281nm **Flow rate :**1.0 ml/min	45
5.	Hydrochlorothiazide and Irbesartan	HPTLC	**Mobile phase** Acetonitrile: chloroform (4:6) v/v **Column:** Silica gel 60F$_{254}$ **Wavelength:** 270nm **Flow rate :**1.0 ml/min	46
6.	Hydrochlorothiazide, Olmesartan Medoxomil	HPTLC	**Mobile phase** Toluene: methanol: ethyl acetate: acetone (2.5:1:0.5:2) v/v **Column:** Silica gel 60F $_{254}$ **Wavelength:**254nm **Flow rate** :1.0 ml/min	47
7.	Telmisartan and hydrochlorothiazide	HPTLC	**Mobile phase** methanol: chloroform: acetone (30:30:40) v/v **Column:** silica gel F254 **Wavelength** 272nm **Flow rate:**1.0 ml/min	48

Other Reported Methods:

Sr. no	Drug	Method	Analytical Description	Ref. no
1.	Hydrochlorothiazide and valsartan	Simultaneous Absorption method	**Solvent**: 0.1 NaOH RSD >2% **Wavelength:** 249to276nm **Flow rate:** 1.0 ml/min	49
2.	Hydrochlorothiazide and Enaprile	Simultaneous Determination LC	**Mobile phase:** Octanol: propanol: trimethylamine (1:9:0.3) v/v **Column:**C-18 60F$_{254}$ **Wavelength:** 210nm **Flow rate :**1.0 ml/min	50
3.	Hydrochlorothiazide and Benazepril	Stability indicating LC	**Mobile phase:** Water: methanol (55:45) v/v	51

			Column: C-18 60F_{254} **Wavelength :**233nm **Flow rate :**1.0 ml/min	
4.	Hydrochlorothiazide and Spironolactone	TLC	**Mobile phase**: Water: acetonitrile (97:3) v/v **Column:** C-18 60F_{254} **Wavelength :**230nm **Flow rate :**2.0 ml/min	52

SUMMARY OF REVIEWS:

DRUGS	METHODS					
	RP-HPLC	HPLC	HPTLC	UPLC	LC	TLC
LOSARTAN	8	4	5	-	3	1
HYDROCHLORO - THIAZIDE	7	3	4	-	4	3
COMBINED	1	2	-	1	1	-

SUMMARY OF PSAR:

Sr no.	Patent application no.	Title of patent	Ref no.
1.	US 7,915,425 B2	PROCESS FOR PREPRATION OF LOSARTAN	53
2.	US 2011/189281 A1	TELMISARTAN AND HYDROCHLOROTHIAZIDE THERAPY	54
3.	US 2017/0157094 A1	PHRMACEUTICAL FORMULATION COMPARISING LOSARTAN AND CHLOROTHALIDON	55
4.	WO 2013/098578A1	IMMIDIATE RELEASE VALSARTAN AND HYDROCHLOROTHIAZIDE	56
5.	EP 203791A1	AMLODIPIN AND LOSARTAN	57

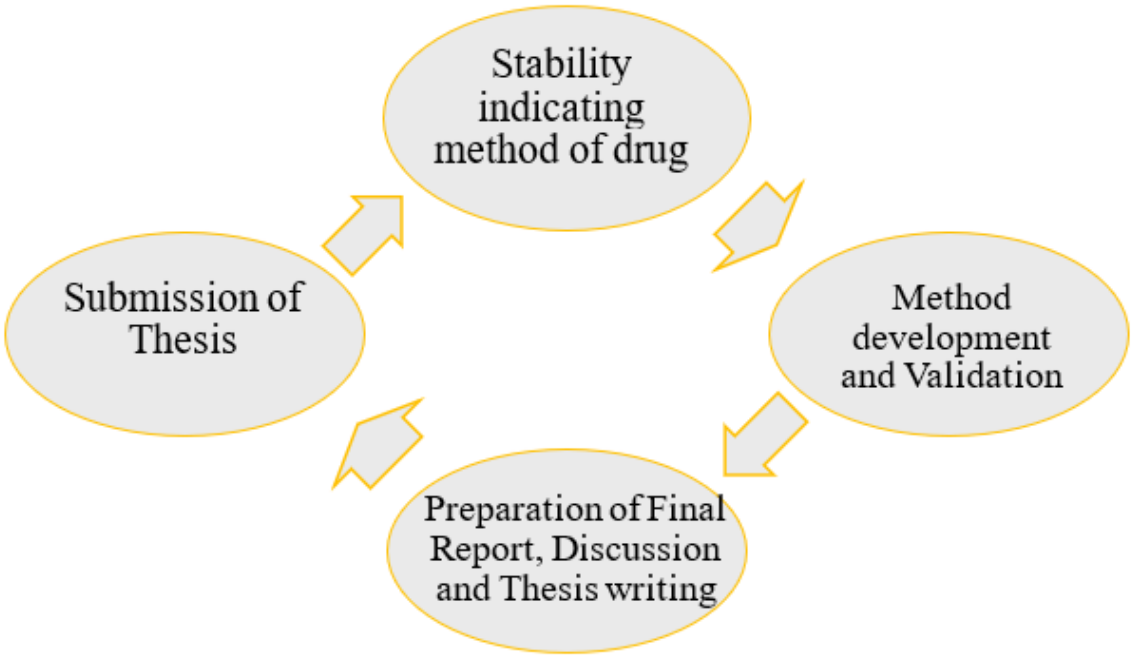
- **AIM OF WORK:**

ANALYTICAL METHOD DEVELOPMENT

STABILITY INDICATING STUDY

ANALYTICAL METHOD VALIDATION

➢ **PLAN OF WORK:**



➢ **RATIONALE OF SELECTION OF DRUG:**

- Losartan potassium is used as an anti-hypertensive drug in the market. It is widely used in treat high blood pressure and heart failure.
- Hydrochlorothiazide is used to treat edema caused by various medical problems including liver, heart, and kidney disease.
- Various methods are reported for the analysis of individual drug and in combination with other drugs but no Stability indicating HPTLC method reported for these drugs in combined dosage form.
- Therefore, it was thought worthwhile to develop stability indicating HPTLC Method for the losartan and hydrochlorothiazide in their Combined Pharmaceutical dosage form

- **EXPERIMENTAL WORK: Standards and Reagents**

- **Standards**

Standard	Source
Losartan Potassium	Alembic Pharmaceutical
Hydrochlorothiazide	Alembic Pharmaceutical

- **Sample**

Sample	Source
ZAART H Tablet	Cipla Pharmaceutical

- **Chemical and reagents:**

Sr No	Name	Grade	Manufacturers
1.	Methanol	AR	Merck Life Science Pvt. Ltd
2.	Ethyl Acetate	AR	Merck Life Science Pvt. Ltd
3.	Acetone	AR	Merck Life Science Pvt. Ltd
4.	Formic acid	AR	Merck Life Science Pvt. Ltd
5.	Chloroform	AR	Merck Life Science Pvt. Ltd

➢ **Apparatus /Equipment:**

Sr No	Components	Volume	Type
1.	Volumetric Flask	10 ml,25ml ,50 ml and 100 ml	Borosilicate Glass type I
2.	Measuring Cylinder	10 ml and 100 ml	Borosilicate Glass type I
3.	Pipettes	1 ml ,5 ml, and 10 ml	Borosilicate Glass type I
4.	Beaker	100 ml ,250 ml	Borosilicate Glass type I
5.	Whitman Filter Paper	-	Filter Paper No.42

➢ **Identification of Drugs:**

- **Determination of Solubility**

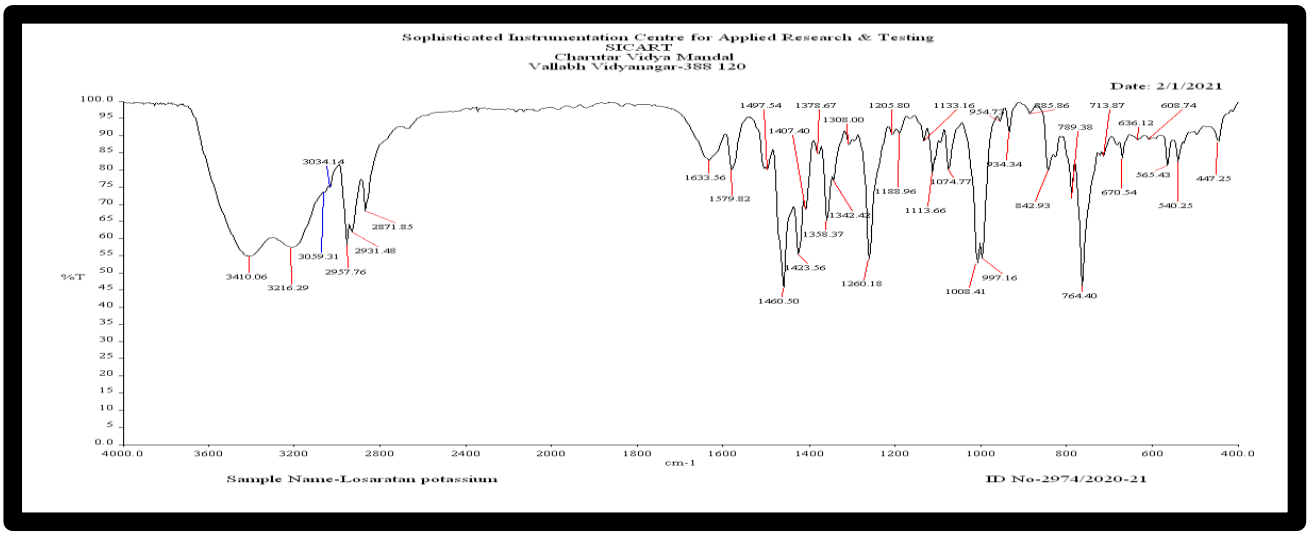
Drug	Solubility			
	Water	Methanol	Ethyl acetate	Diethyl amine
Losartan Potassium	Freely soluble	Freely soluble	Sparingly	Slightly
Hydrochlorothiazide	Slightly	Freely soluble	Sparingly	Slightly

- **Determination of Melting point**

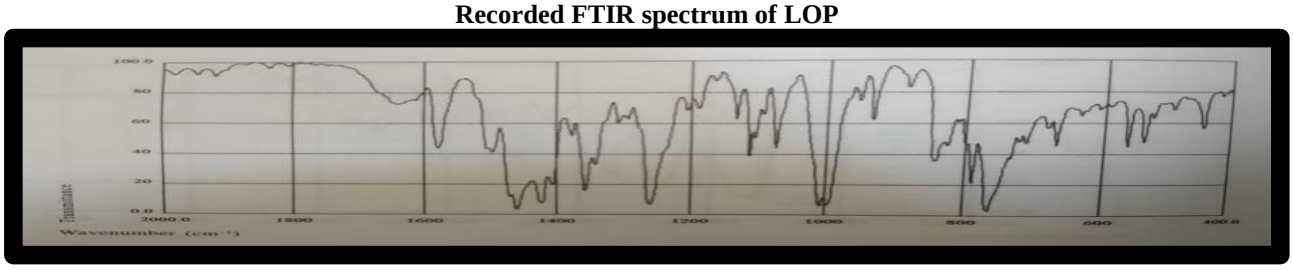
Drug Determination of Melting point	Melting point observed	Melting point standard
Losartan Potassium	179-182 ºC	178-184ºC
Hydrochlorothiazide	272-275 ºC	273-275ºC

➢ Identification by IR

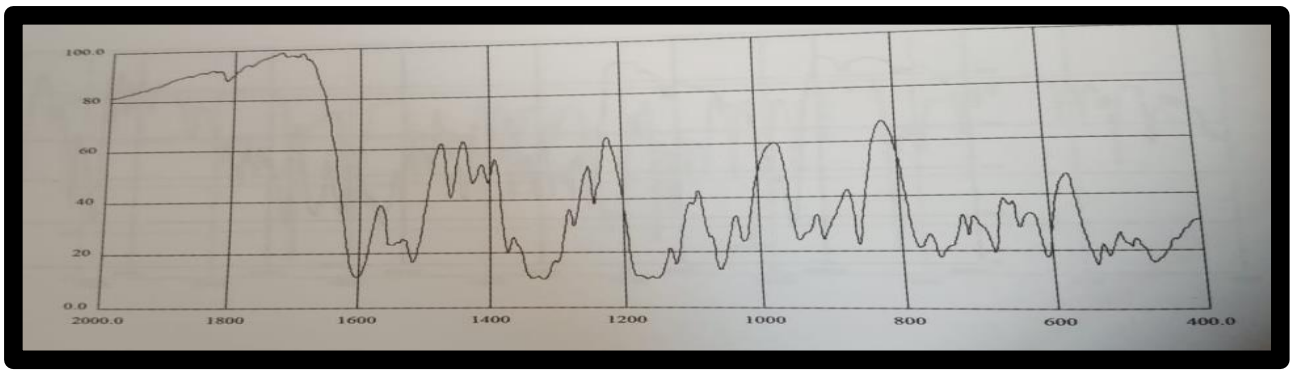
- The recorded IR spectra of LOP and HCTZ was compared with the reference spectra. The major Peaks in the fingerprint region were the comparable indicating identity of the sample. The recorded and reference IR spectra of LOP and HCTZ shown in figure.



Recorded FTIR spectrum of LOP



Reference FTIR spectrum of LOP



Reference FTIR spectrum of HCTZ

➢ Preparation of stock solution:

- **LOP standard stock solution :(400µg/ml)**

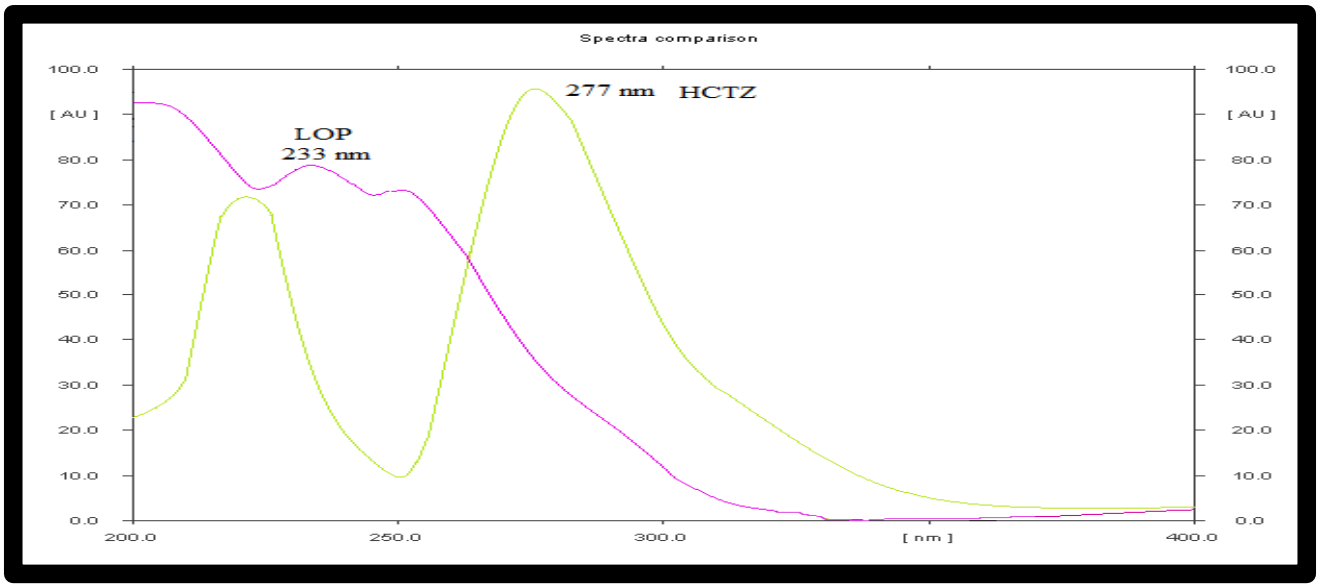
  A 10 mg of LOP was weighed and transferred to a 10 ml volumetric flask (1000 µg/ml) and volume make up by methanol take 4 ml from this solution in 10 ml volumetric flask and volume was make up to the mark with methanol.

- **HCTZ standard stock solution :(100µg/ml)**

  A 10 mg of HCTZ was weighed and transferred to a 10 ml volumetric flask (1000 µg/ml) and volume make up by methanol take 1 ml from this solution in 10 ml volumetric flask and volume was make up to the mark with methanol.

➢ Selection of detection wavelength

- Standard solution of losartan potassium and hydrochlorothiazide was applied on the plate in from of band. After chromatographic development plate was viewed in UV chamber. Bands were scanned over the range 200-400 nm for absorption spectrum at scan speed 20 nm/s and the spectra were recorded. Single wavelength showing maximum absorbance was selected as a detection wavelength for HPTLC method.



❖ **UV overlain spectrum of LOP (400ppm) and HCTZ (100ppm)**

Continue …

The mobile phase consisted of Methanol: Chloroform: Acetone: Formic Acid (1.3:7.5:0.5:0.2) v/v. The optimized chamber saturation time before chromatographic development was 20 min at room temperature (25°C±2). The length of chromatographic run was 8 cm which took average 15 min to develop.

Subsequent to the development TLC plates was dried in current of air with the help of air Dryer. Densitometry scanning was performed using camag TLC scanner IV with win CATS Software (V 1.4.6.2002) All measurements were made in the reflectance absorbance mode at 232nm, slit dimension (6.00*0.30 mm, micro), Scanning speed 20mm/s, data resolution 100µm/step, optical filter (second order), filter factor (Savisky golay7). The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190 to 400 nm.

➢ **Instrumentation:**

➢ **UV-Visible double beam spectrophotometer with matched quartz cell (1cm)**

- Model: UV 1601
- Make: Shimadzu, Kyoto, Japan
- Wavelength Range: -3.99=+3.99Abs
- Scan Speed: 40 nm/min
- Photometric Accuracy: ± 0.003

➢ **UV-Visible double beam spectrophotometer with matched quartz cell (1cm)**

- Model: UV 1800
- Serial no: A114548
- Make: Shimadzu, Kyoto, Japan
- Wavelength Range: -3.99=+3.99Abs
- Scan Speed: 3000 nm/min

➢ **FT-IR Spectrophotometer**

- Model: Spectrum GX FT-IR
- Make: Perkin Elmer, USA
- Scan Range: 15600-30 cm -1

➢ **Analytical Balance**

- Model: K-EA 210
- Serial No.: KE-129
- Make: K-Roy Instrument Pvt. Ltd.
- Maximum capacity: 200g
- List count: 0.001 g

➢ **Ultrasonic Bath Sonicator**

- Electric supply: 230 V AC 50 HC
- Model: 1-SLSOH

➢ **Instrumentation of HPTLC**

- 100 microliter syringes (Lino mat syringe 659.0014, Hamilton Bonaduz Schweiz, Camag Lino mat V sample applicator (Switzerland), Twin through chamber (Camag, Switzerland) Camag TLC scanner IV with Win CATS software (V 1.4.6.2002, Camag) the source of radiation was deuterium lamp.

➢ **Chromatographic Condition**

- The samples were spotted in the form of bands having band width 8mm with a microliter micro-Syringe (Lion mat syringe 659.0014, Hamilton Bonaduz Schweiz, Camag, Switzerland) on Precoated silica gel aluminum plate 60 F_{254} (20 cm *10cm)100µm thickness; (E. Merck, Darmstadt, Germany) using a Camag Lino mat V sample applicator (Switzerland). Linear ascending development was carried out in 20*10 cm twin through glass chamber (Camag, Switzerland).

➢ **Method Development**

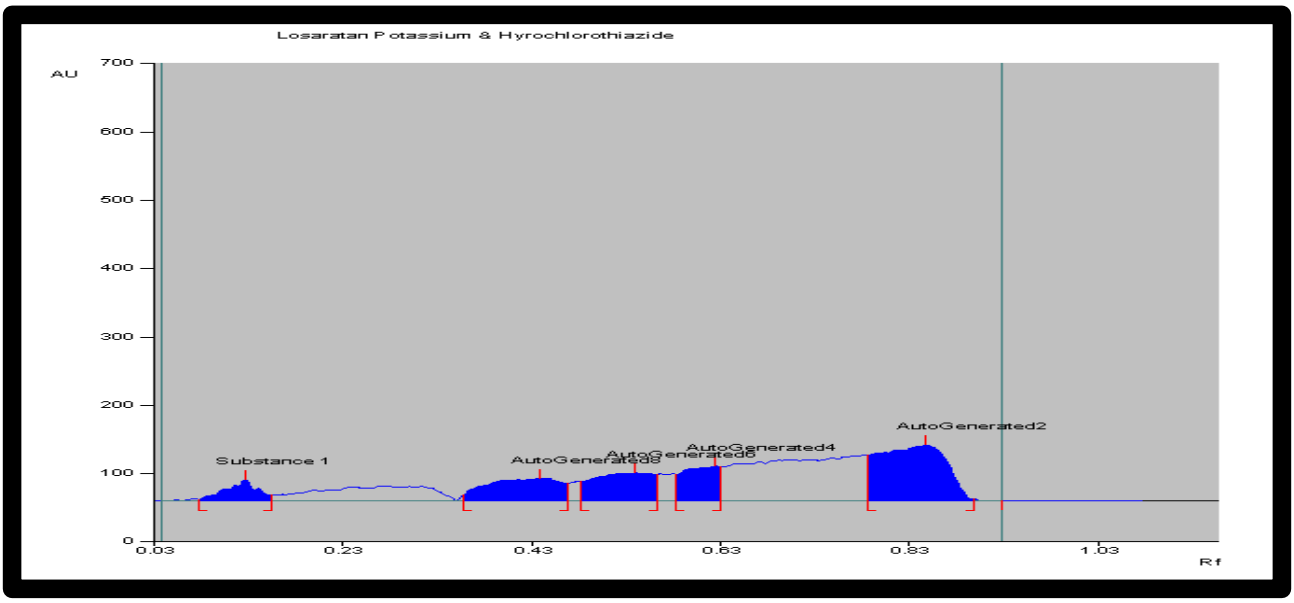
- **Optimization of mobile phase**

Various mobile phase trials were taken using number of solvents like methanol, toluene, ammonium acetate, ethyl acetate with different ratios. In case of ethyl acetate, methanol, Diethyl amine, acetone peak was not obtained. Then different ratio of toluene, methanol Ethyl acetate and diethyl amine were tried. Moreover, various trials were carried out to check that the Mobile phase was capable of resolving degradation product peak from peak of both drugs. Hence, the nearly optimized mobile phase was chloroform: methanol: formic acid: acetone in (7.5:1.3:0.2:0.5) v/v resulted in good resolution with R_f 0.68 for LOP and R_f 0.40 for HCTZ.

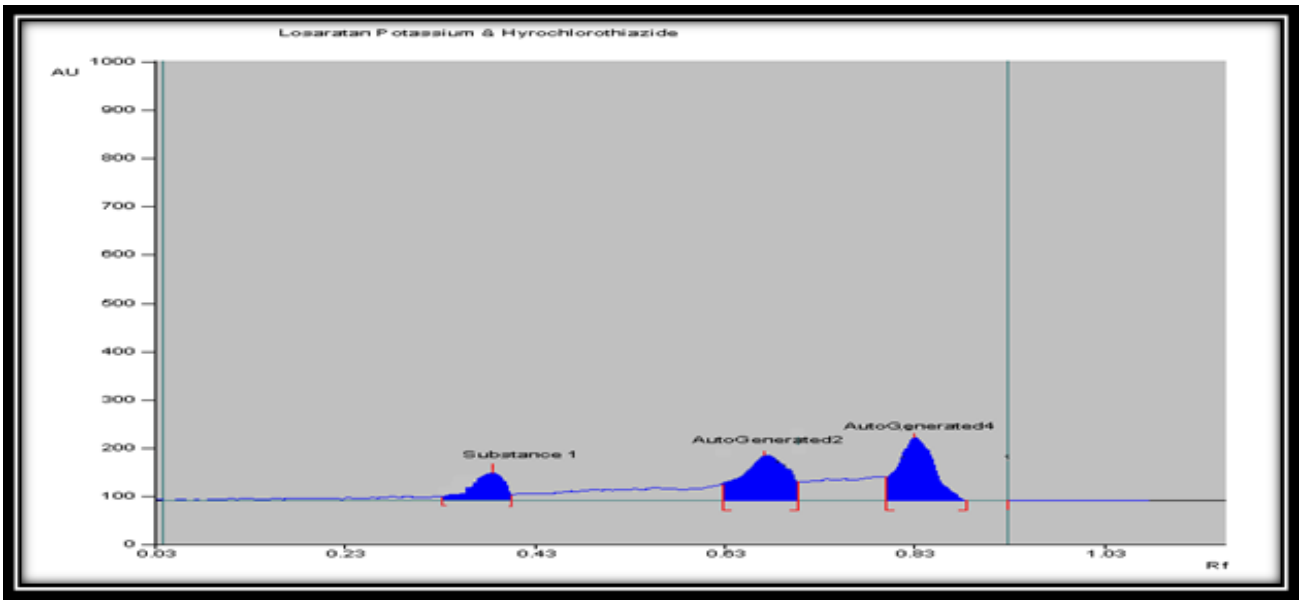
➢ **Development of optimum mobile phase**

Sr No.	Mobile phase composition	R_f of LOP	R_f of HCTZ	Remarks
1.	Methanol:acetonitrile:ethyl acetate (5:4:1) v/v	-	-	No peak observed
2.	Methanol:ethylacetate:diethyl amine: toluene (5:3:1:1) v/v	0.46	0.28	Low R_f value of both Substance
3.	Methanol:ethylacetate:acetone: diethyl amine (4:3:1:2) v/v	0.68	0.34	Low R_f value of HCTZ
4.	Methanol:ethylacetate:acetone: diethyl amine (5:2.5:0.5:2) v/v	0.65	0.40	LOP peak shape was not proper
5.	Chloroform:methanol:formic acid: acetone (7:2:0.5:0.5) v/v	0.79	0.43	Better resolution but One small peak appears with LOP
6.	Chloroform: methanol: formic acid: acetone (7.5:1.3:0.2:0.5) v/v	0.72	0.43	Both peaks are well Resolute and peak shapes are also better.

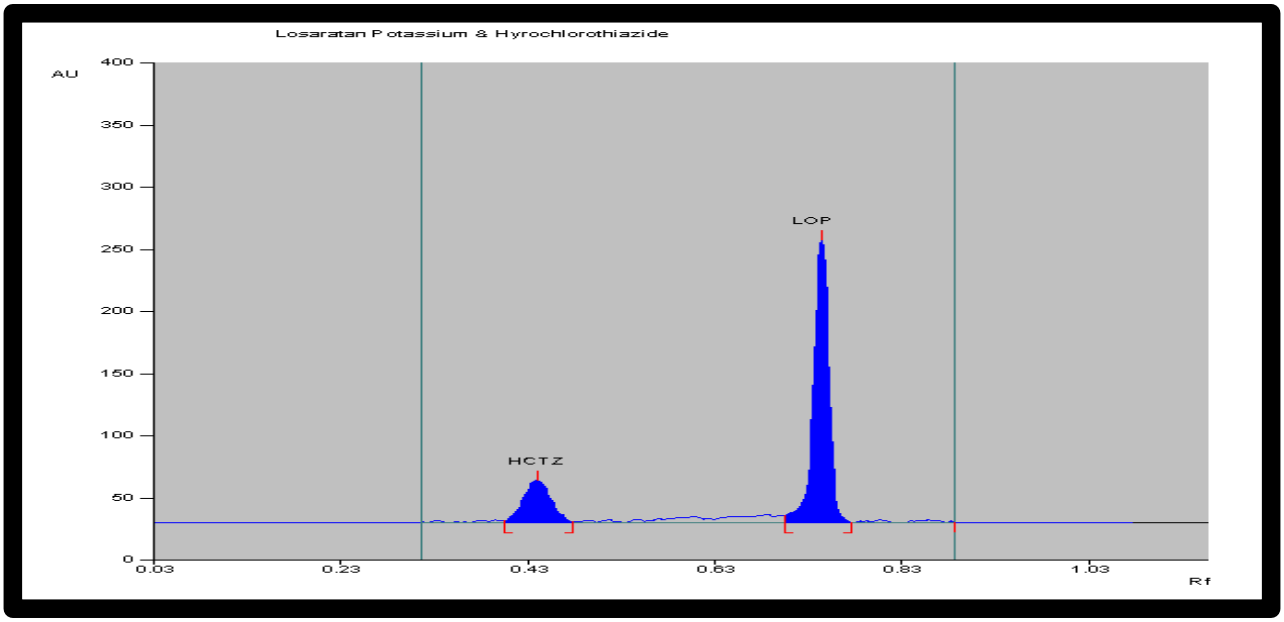
Trial no.1 [methanol: acetonitrile: ethyl acetate (5:4:1) v/v]



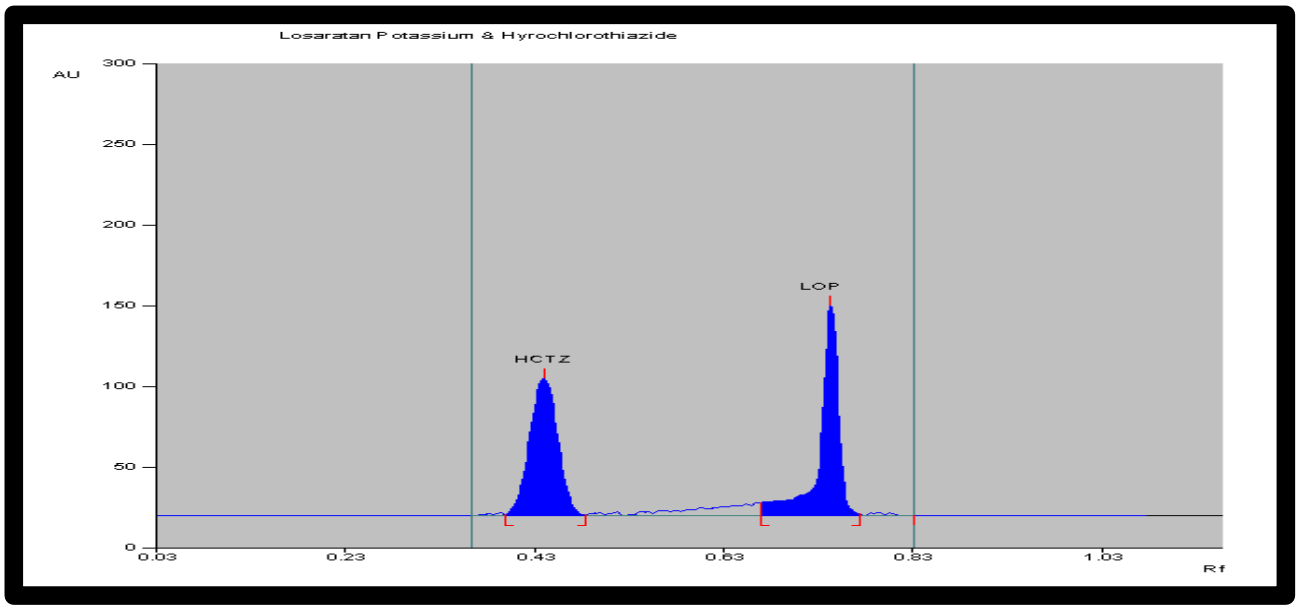
Trial no.2 [methanol: ethyl acetate: diethyl amine: toluene (5:3:1:1) v/v]



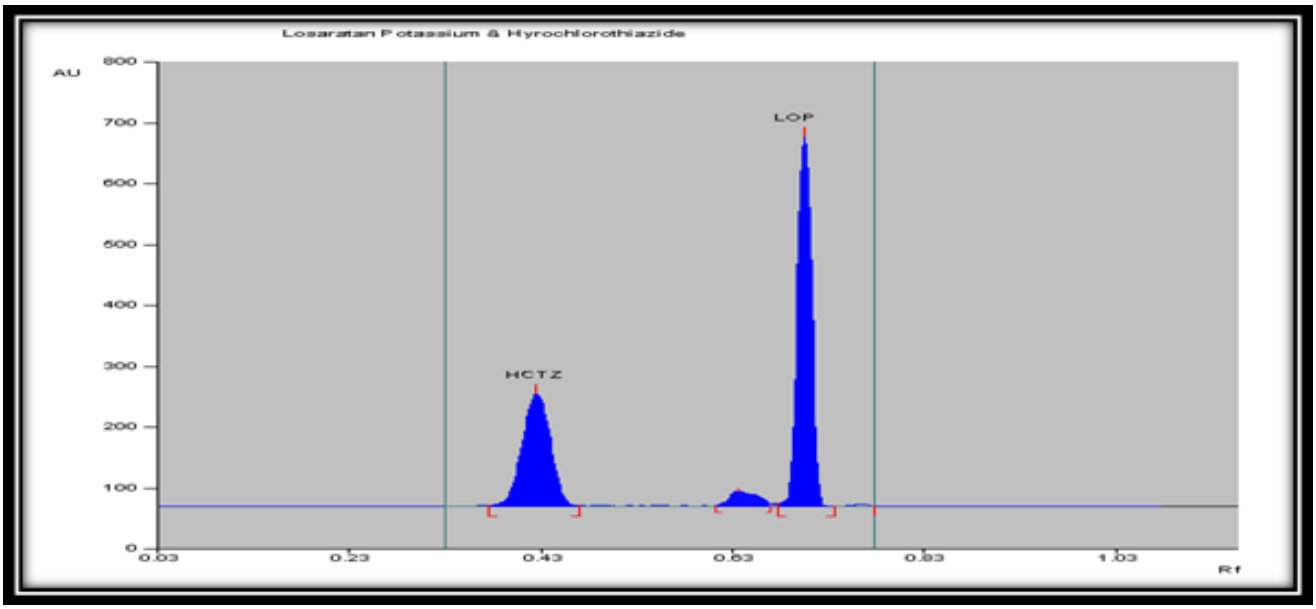
Trial no.3 [methanol: ethyl acetate: acetone: diethyl amine (4:3:1:2) v/v]



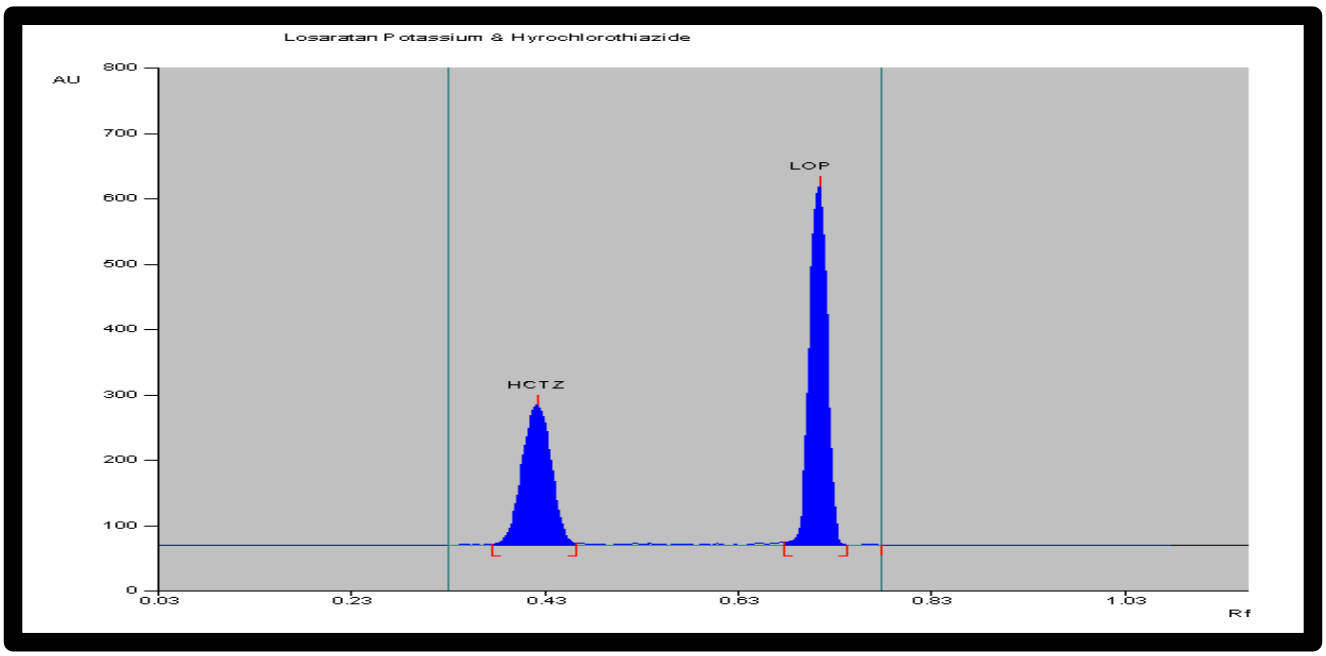
Trial no.4 [methanol: ethyl acetate: acetone: diethyl amine (5:2.5:0.5:0.2) v/v]



Trial no.5 [chloroform: methanol: formic acid: acetone (7:2:0.5:0.5) v/v]



Trial no.6 [chloroform: methanol: formic acid: acetone (7.5:1.3:0.2:0.5) v/v]

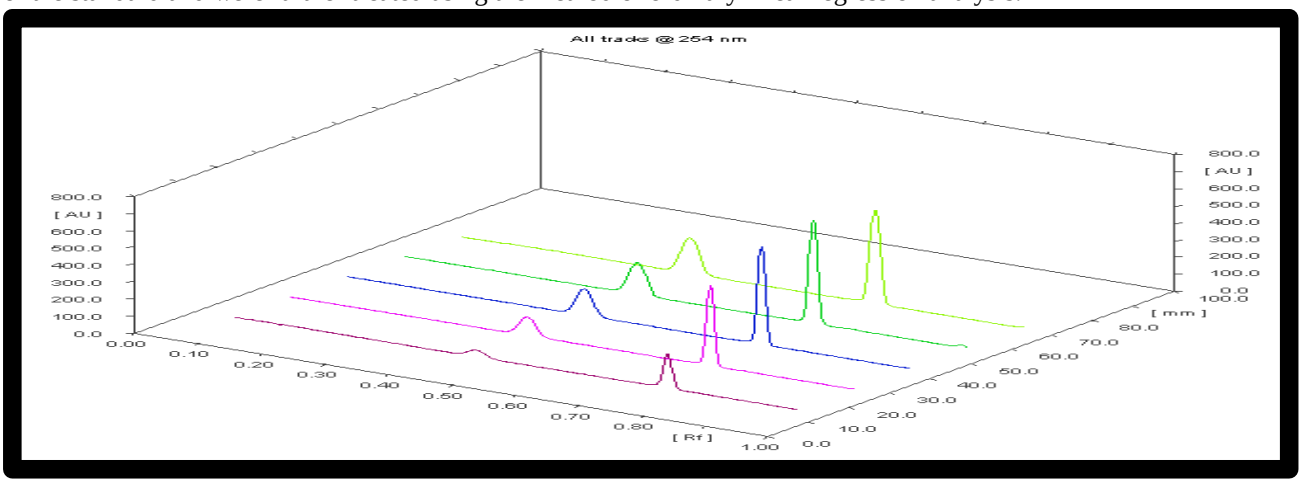


➢ Method validation

- The HPTLC method was validated for linearity, accuracy, precision, LOD, LOQ, repeatability in Accordance with ICH Q2 (R1) guidelines.

➢ Linearity:

- Linear relationship between peak area and concentration of standard was evaluated over the concentration range expressed in ng/band by making five replicative measurements in the concentration range of 800-4000 ng/band and 200-1000 ng/band, respectively for losartan potassium and hydrochlorothiazide, by applying different volume (2 to 10 µl) of working standard solution on the HPTLC plate. Calibration plots were constructed by plotting the area of the peak of band versus the concentration of the standard and were further treated using the method of ordinary linear regression analysis.



Linearity 3-D graph of LOP & HCTZ

➢ Table for Linearity

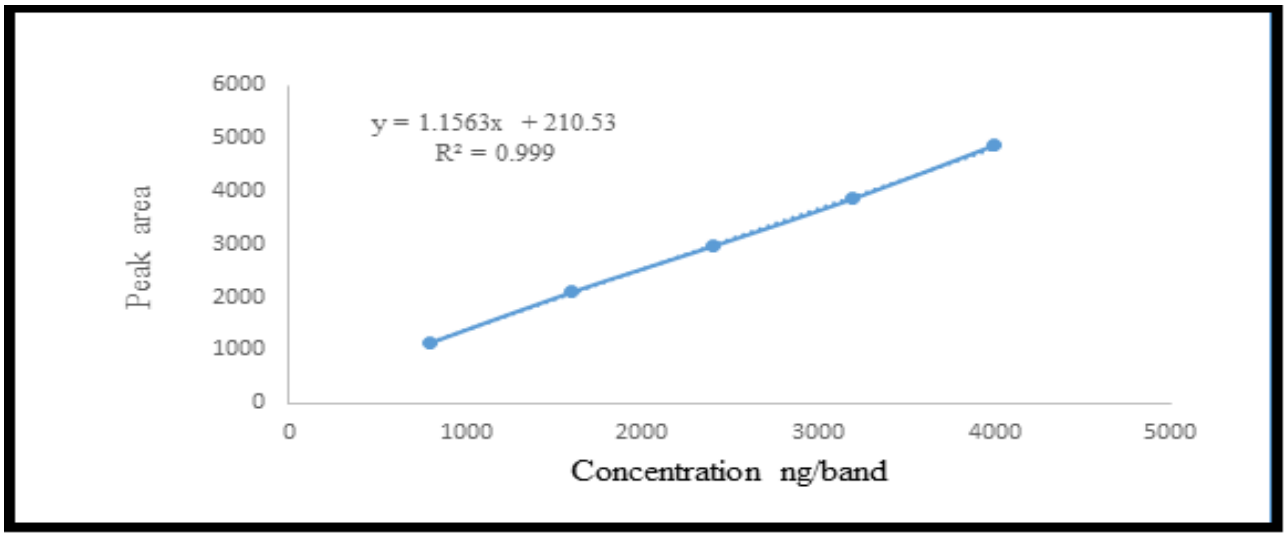
- **Losartan Potassium**

Concentration of LOP ng/band	Peak area					Average	SD	%RSD
	1	2	3	4	5			
800	1157.2	1145.1	1112.3	1142.2	1123.0	1135.1	18.037	1.59
1600	2078.1	2136.2	2054.1	2072.0	2132.3	2094.2	37.333	1.78
2400	2957.2	2962.4	2953.1	2936.3	2986.4	2959.4	18.135	0.61
3200	3868.4	3889.0	3854.4	3847.2	3864.3	3864.5	15.942	0.41
4000	4866.3	4842.5	4889.2	4892.4	4888.2	4875.2	21.260	0.44
LOD	90.70							
LOQ	274.86							

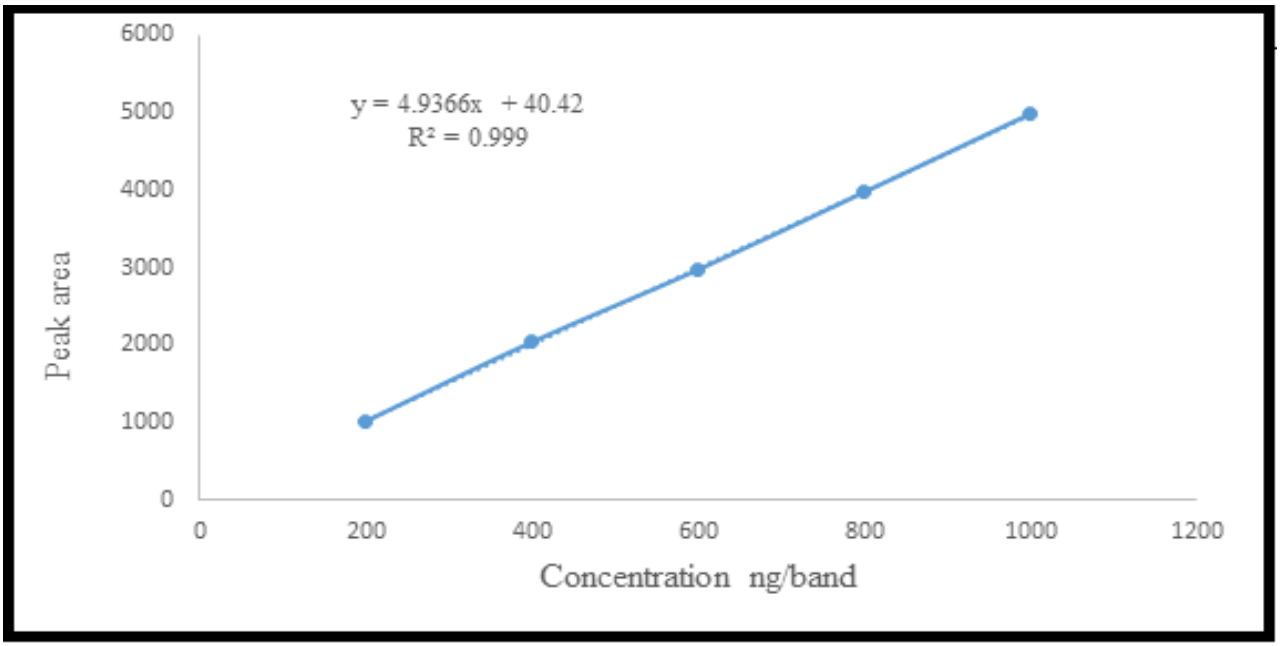
- **Hydrochlorothiazide**

Concentration of HCTZ ng/band	Peak area					Average	SD	%RSD
	1	2	3	4	5			
200	1025.2	1015.1	1030.0	1010.2	1022.0	1020.5	7.968	0.78
400	2036.1	2050.3	2047.1	2044.2		2045.1	5.612	0.27
600	2989.2	2965.3	2967.1	2965.1	2998.2	2976.9	15.610	0.52
800	3965.0	3987.4	3988.2		3982.0	3979.5	9.680	0.24
1000	4998.2	4996.3	4988.2	4985.0	4982.2	4989.9	7.006	0.14
LOD	4.24							
LOQ	12.87							

- **Concentration vs Peak area for LOP.**



- **Concentration vs Peak area for HCTZ**



➢ **Precision**

- The experiment was repeated three times in a day (intraday precision), and the average %RSD values of the results were calculated. Similarly, the experiment was repeated on three different days (inter day precision) and the average %RSD values for peak area of standard was calculated. Results of intraday precision expressed in terms of %RSD was found to be less than two demonstrating good repeatability and reproducibility of method. The low value of %RSD depicts the high precision of..

Losartan potassium						
Concentration ng/band	**Peak area**			**Average**	**SD**	**%RSD**
	1	**2**	**3**			
800	**1149.2**	**1152.3**	**1155.1**	**1152.2**	**2.9512**	**0.26**
1600	**2133.2**	**2137.2**	**2141.2**	**2137.2**	**4.2394**	**0.19**
2400	**2946.3**	**2949.3**	**2944.1**	**2946.5**	**2.6102**	**0.09**
3200	**3867.4**	**3862.1**	**3864.3**	**3864.6**	**2.6627**	**0.07**
4000	**4856.2**	**4852.1**	**4853.1**	**4853.8**	**2.1377**	**0.04**
Hydrochlorothiazide						
200	**1245.1**	**1249.2**	**1238.0**	**1244.1**	**5.6666**	**0.46**
400	**2035.2**	**2042.1**	**2032.1**	**2036.4**	**5.1189**	**0.25**
600	**2889.1**	**2884.2**	**2878.3**	**2883.8**	**5.4077**	**0.19**
800	**3862.0**	**3858.1**	**3854.2**	**3858.1**	**3.9143**	**0.10**
1000	**4986.3**	**4982.2**	**4990.1**	**4986.2**	**3.9509**	**0.08**

- **Intraday Precision:**

Losartan Potassium						
Concentration ng/band	Peak area			Average	SD	%RSD
	1	2	3			
800	1149.2	1167.1	1178.0	1164.7	14.5410	1.25
1600	2133.2	2176.1	2162.3	2157.2	21.9342	1.02
2400	2946.3	2987.0	2972.1	2968.4	20.5918	0.69
3200	3867.4	3846.3	3854.2	3855.9	10.6603	0.28
4000	4856.2	4822.0	4843.1	4840.4	17.2552	0.36
Hydrochlorothiazide						
200	1245.1	1258.0	1289.0	1264.0	22.560	1.79
400	2035.2	2063.1	2048.1	2048.8	13.963	0.68
600	2889.1	2895.3	2967.0	2917.1	43.296	1.48
800	3862.0	3876.1	3852.1	3863.4	12.061	0.31
1000	4986.3	4974.2	4994.1	4984.8	10.027	0.20

➢ **Repeatability of measurement**

- The precision of instrument was checked by scanning same spot (working standard solution, 15 µL) seven times without changing plate position. These results indicate that the method is precise for measurement of LOP & HCTZ. Data for repeatability of measurement is depicted in Table.

Drugs	Concentration (ng/band)	Peak area (Mean ± SD) (n=7)	%RSD
LOP	2400	2968.4	0.12
HCTZ	600	2905.0	0.14

- **Repeatability of the sample application:** Repeatability of sample application (15 µL) seven spots is placed on one plate for LOP &HCTZ. These results indicate that the method is precise for measurement of LOP &HCTZ. Data for repeatability of the sample application is depicted this Table.

Drugs	Concentration (ng/band)	Peak area (Mean ± SD) (n=7)	%RSD
LOP	2400	2966.9	0.20
HCTZ	600	2912.0	0.41

Accuracy: The proposed method when used for evaluation of recovery at three concentration levels, 80%, 100%, and 120%. The % recovery of Losartan Potassium was found to be in the range of 98.62-99 %, and for Hydrochlorothiazide the %recovery was found to be in range of 98.9- 99.65 %. These results indicate that the method is accurate in the measurement of LOP and HCTZ. The data for the accuracy of the method for LOP and HCTZ is depicted in table.

- **Losartan potassium:**

Spiked Level %	Amount of LOP from analyzed tablet powder (mg)	Spiked LOP amount (mg)	Average peak area (n=3)	Amount found (mg)	Recovery (%)
80	10	8	2056.2	7.89	98.62
100	10	10	2276.5	9.96	99.68
120	10	12	2478.3	11.86	98.83

- **Hydrochlorothiazide:**

Spiked Level %	Amount of HCTZ from analyzed tablet powder (mg)	Spiked HCTZ amount (mg)	Average peak area (n=3)	Amount found (mg)	Recovery (%)
80	10	8	2684.1	7.93	99.12
100	10	10	2976.2	9.88	98.8
120	10	12	3284.3	11.98	99.83

Robustness:

The %RSD for different wavelengths, scanning speed and different saturation time of LOP &HCTZ was found to be in the range of and % respectively. These results indicate that the method is robust for measurement of LOP &HCTZ. The data for different wavelength, scanning speed and different chamber saturation time for LOP & HCTZ is depicted in Table.

- **LOP Data for Robustness (Changing in Wavelength)**

Concentration (ng/band)	Wavelength			Average	SD	%RSD
	231	233	235			
800	1126.1	1135.1	1147.2	1136.1	10.58	0.93
1600	2068.3	2094.2	2110.3	2090.6	21.18	1.01
2400	2936.1	2959.4	2969.2	2954.9	17.00	0.58
3200	3828.2	3864.5	3878.1	3856.8	25.79	0.67
4000	4839.3	4875.2	4889.2	4867.9	25.73	0.53

- **HCTZ Data for Robustness (Changing in Wavelength):**

Concentration (ng/band)	Wavelength			Average	SD	%RSD
	275	277	279			
200	1015.1	1020.5	1035.2	1035.6	15.30	1.48
400	2026.1	2045.1	2066.3	2045.8	20.11	0.98
600	2943.1	2676.9	2982.1	2967.3	21.17	0.71
800	3958.2	3979.5	3985.1	3974.2	14.19	0.36
1000	4948.1	4989.9	4993.2	4977.0	25.90	0.50

- **LOP Data for Robustness (Saturation time)**

Concentration (ng/band)	Saturation time			Average	SD	%RSD
	15	30	45			
800	1126.1	1110.1	1154.2	1130.1	22.36	1.88
1600	2068.3	2046.2	2189.1	2067.8	21.45	1.04
2400	2936.1	2922.3	2974.0	2944.1	26.78	0.91
3200	3828.2	3816.1	3884.3	3842.8	36.38	0.95
4000	4839.3	4828.2	4868.0	4845.1	20.33	0.45

- **HCTZ Data for Robustness (Saturation time):**

Concentration (ng/band)	Saturation time			Average	SD	%RSD
	15	30	45			
200	1015.1	1012.2	1033.1	1020.2	11.35	1.11
400	2026.1	2018.3	2048.2	2030.8	15.50	0.76
600	2943.1	2938.1	2959.3	2947.7	11.13	0.38
800	3958.2	3942.1	3964.1	3954.8	11.48	0.31
1000	4948.1	4936.2	4972.2	4952.0	18.24	0.37

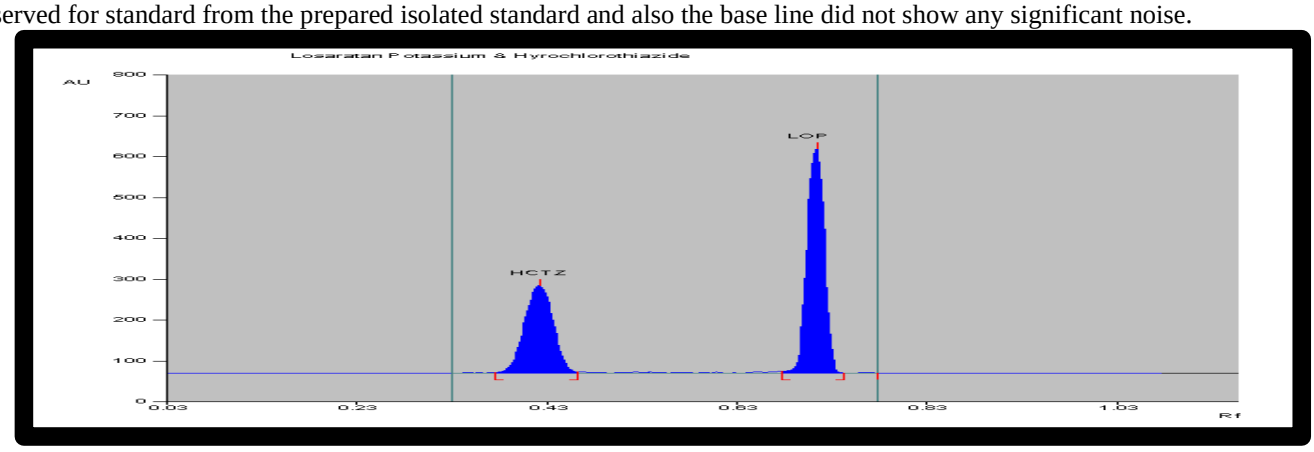
- **LOP Data for Robustness (Scanning speed):**

Concentration (ng/band)	Scanning speed			Average	SD	%RSD
	10	20	40			
800	1126.1	1134.1	1158.2	1139.4	16.70	1.47
1600	2068.3	2074.2	2078.3	2073.5	4.92	0.24
2400	2936.1	2956.1	2952.2	2948.1	10.52	0.36
3200	3828.2	3842.2	3848.1	3839.4	10.71	0.27
4000	4839.3	4852.1	4845.2	4845.5	6.35	0.15

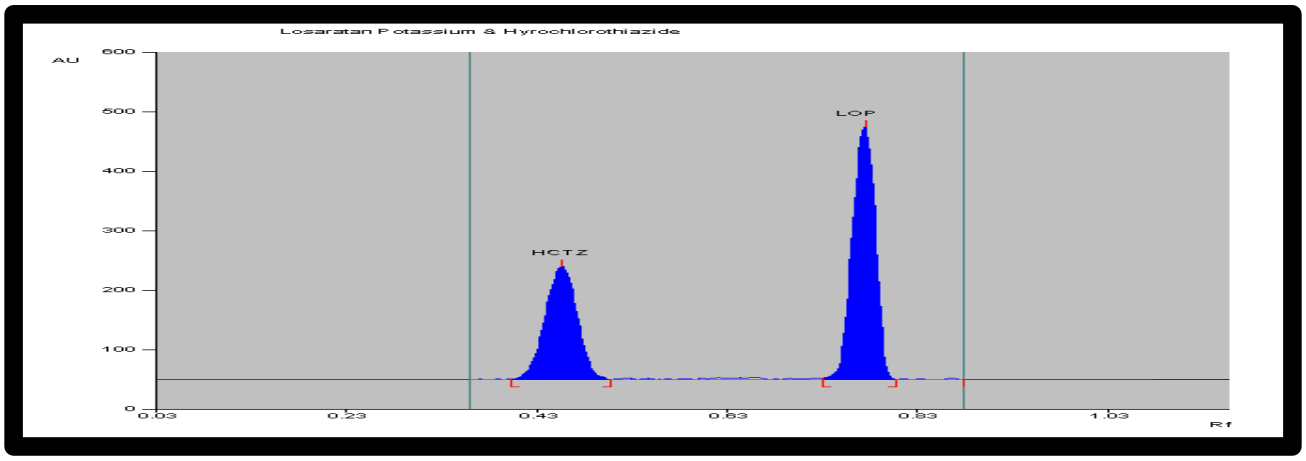
- **HCTZ Data for Robustness (Scanning speed):**

Concentration (ng/band)	Scanning speed			Average	SD	%RSD
	10	20	40			
200	1015.1	1033.2	1048.2	1032.1	16.52	1.60
400	2026.1	2064.1	2058.2	2049.4	20.41	1.08
600	2943.1	2666.1	2974.3	2961.3	16.09	0.54
800	3958.2	3974.1	3969.1	3967.0	8.07	0.24
1000	4948.1	4956.2	4972.3	4958.8	12.36	0.29

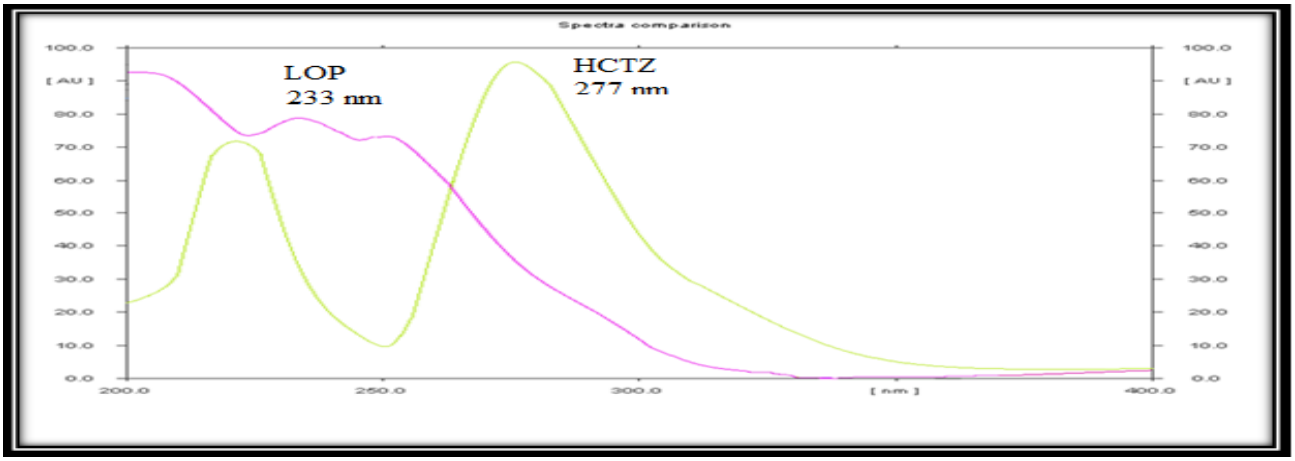
Specificity: Specificity of the method ascertained by comparing retardation factor and peak purity of standard and sample in chromatogram and UV. The retardation factor and UV spectra of the standard and sample for both LOP & HCTZ was found to be same, so the method was found to be specific. Moreover, there was no interference from other active ingredients at the peaks observed for standard from the prepared isolated standard and also the base line did not show any significant noise.



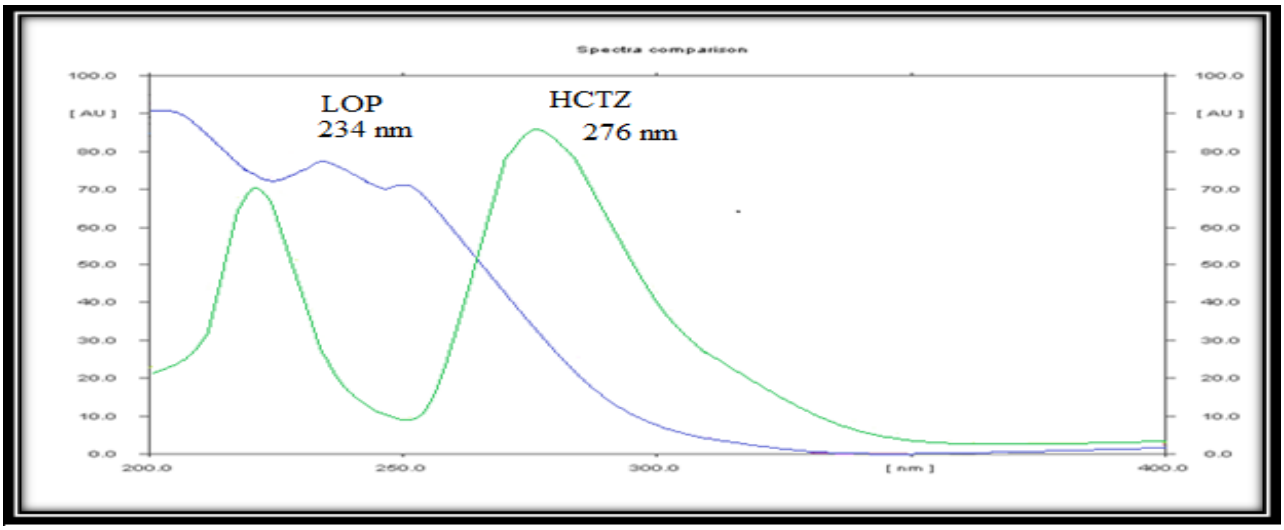
Densitogram of Standard LOP & HCTZ



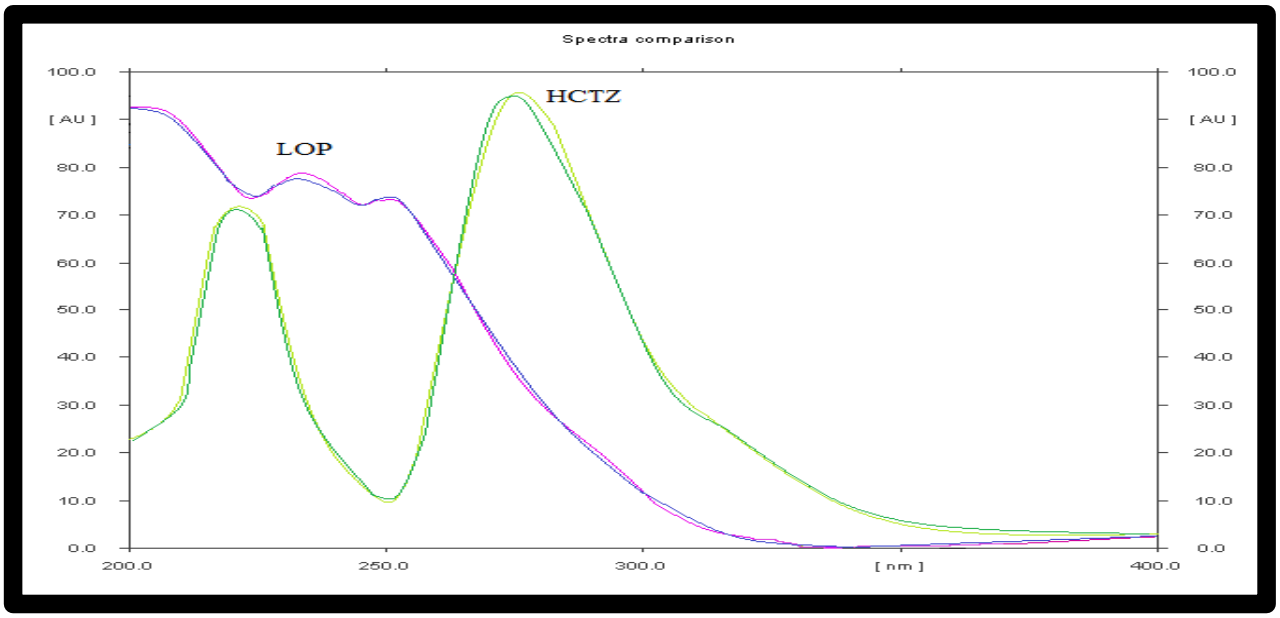
Densitogram of Dosage form of LOP & HCTZ



Peak purity spectra of Standard LOP & HCTZ



Peak purity spectra of Dosage form of LOP &HCTZ



Overlain spectra of LOP & HCTZ standard and pharmaceutical dosage form.

➢ **Analysis of Pharmaceutical dosage form:** Applicability of the proposed method was tested by analyzing pharmaceutical dosage form. The percentage of LOP & HCTZ in the pharmaceutical dosage form was calculated. The assay value for the pharmaceutical dosage form of LOP & HCTZ was found to be 99.3%, 99.7%. This result is within the range of precise limits. Assay result of the pharmaceutical dosage form of LOP & HCTZ is depicted in table

Drugs	Label claim amount (mg)	Amount of drug found (mg)	%Label claim	%RSD
LOP	50	49.69	99.39	0.246
HCTZ	12.5	12.47	99.74	0.116

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LOP	50	49.69	99.39	0.246
HCTZ	12.5	12.47	99.74	0.116

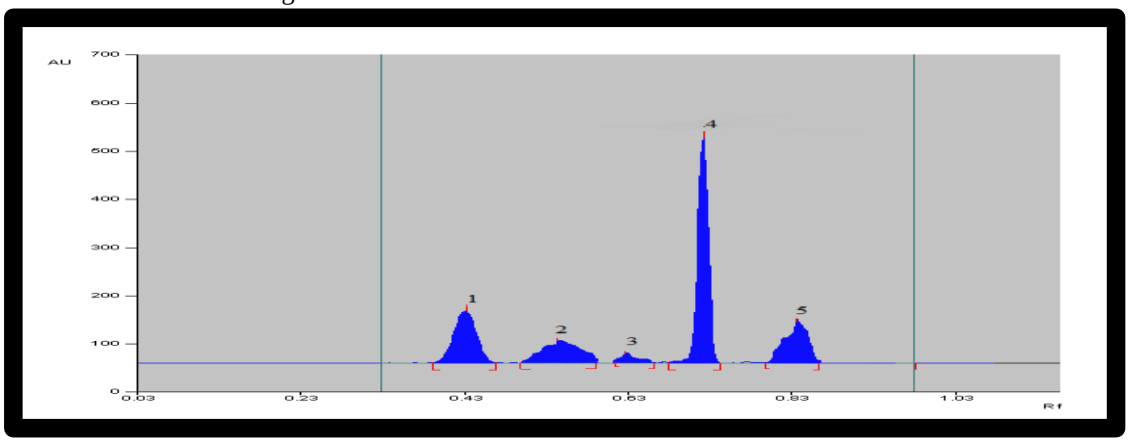
➢ **Summary of Validation Parameter:**

Sr. No.	Parameters	Results	
		LOP	HCTZ
1	Linearity Range	800-4000 ng/band	200-1000 ng/band
2	Regression equation	y = 1.563x + 210.53	y=4.9366+40.42
3	Regression coefficient(R2)	0.999	0.999
4	Precision (%RSD)		
	Repeatability of measurement(n=7))	0.12	0.14
	Repeatability of sample application(n=7)	0.20	0.41

	Intermediate precision		
	Intra-day precision(n=3)	**0.04-0.26**	**0.08-0.46**
	Inter-day precision(n=3)	**0.28-1.25**	**0.20-1.79**
5	**Limit of detection (LOD)**	**90.70 ng/band**	**4.24 ng/band**
6	**Limit of quantification (LOQ)**	**274.86 ng/band**	**12.87 ng/band**
7	**Accuracy(n=3)**	**99.62-100.50%**	**99.12-99.90%**
8	**Robustness**	**Robust**	**Robust**

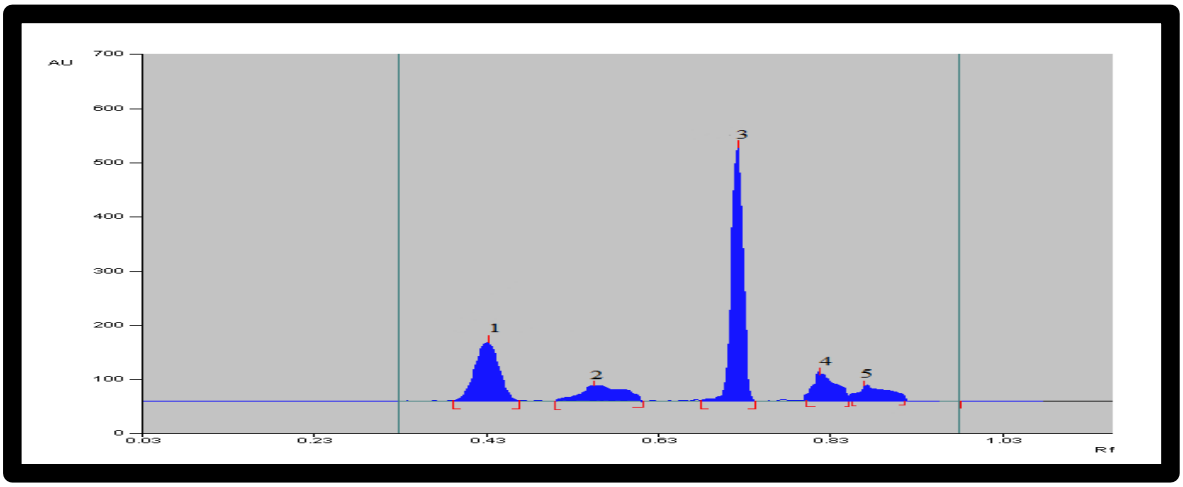
➢ **Stability Studies:**

1. **Acid degradation:** Degradation of LOP & HCTZ was observed in acidic condition. Peak area for LOP & HCTZ has decreased and an additional peak in degradation product was found in the chromatogram. LOP & HCTZ was found to be degraded about 18.26 % &12.24% after heating with 0.5 N HCl at 25 º± 2°C for 4 hours.



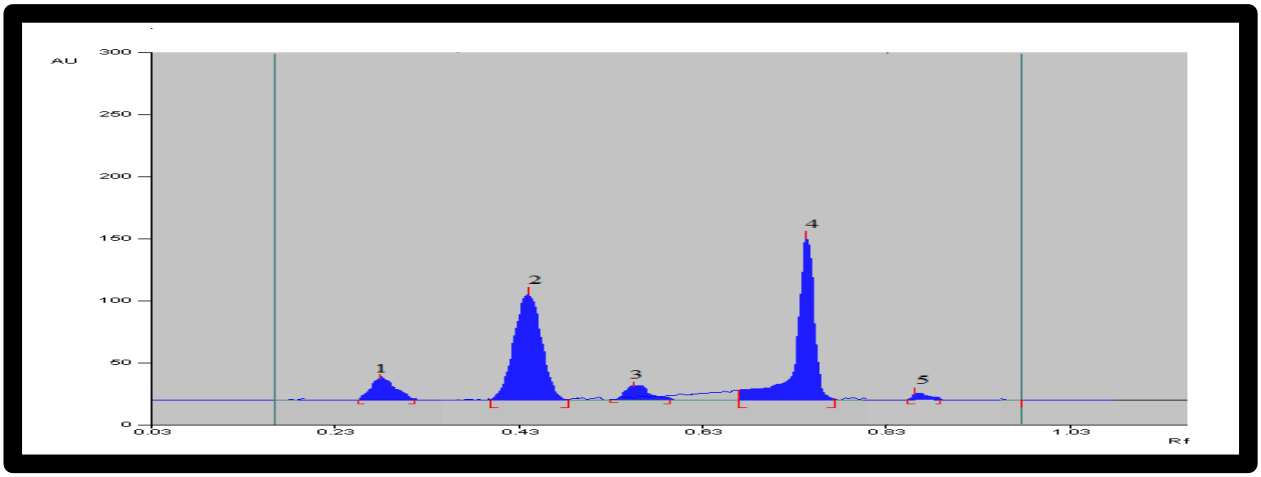
2. **Base degradation**

- Degradation of LOP & HCTZ was observed in basic condition. Peak area for LOP & HCTZ has decreased and an additional peak of degradation product was found in the chromatogram. LOP & HCTZ was found to be degraded about 17.86 % & 10.29 % with 0.5 N NaOH at 25± 2°C for 4 hours.



3. Neutral Degradation

- Degradation of LOP & HCTZ was observed in neutral conditions. Peak area for LOP & HCTZ has decreased and an additional peak of degradation product was not found in the chromatogram. LOP & HCTZ was found to be degraded about 13.50% & 9.86 % after heating with water at 25^0 ± 2°C for 4 hours.

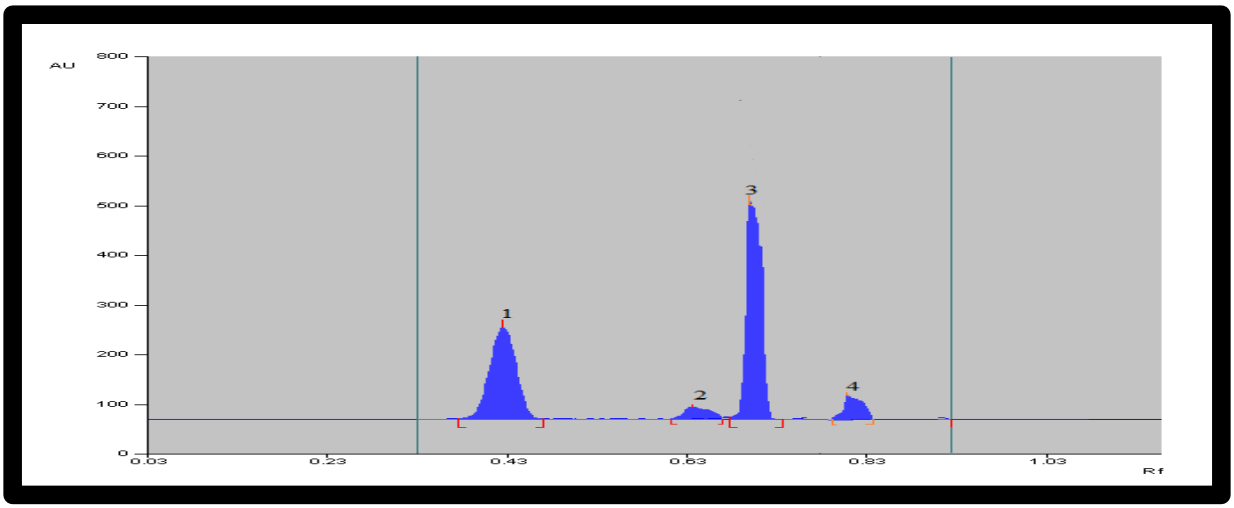


4. Oxidative degradation

- Degradation of LOP & HCTZ was observed in oxidative condition. Peak area for LOP & HCTZ has decreased and an additional peak of degradation product was found in the chromatogram. LOP & HCTZ was found to be degraded about 12.10 % & 10.74 % after heating with 6% H_2O_2 at 25º± 2°C for 6 hours.

5. Photolytic degradation

- Degradation of LOP & HCTZ was observed in Photolytic condition. Peak area for LOP & HCTZ has decreased and an additional peak of degradation product was not found in the chromatogram. LOP & HCTZ was found to be degraded about 8.54 % & 11.59 in photo-stability chamber at 25^0 ± 2°C for 6 hours.



➢ **Summary of forced degradation study of LOP & HCTZ:**

No.	Stress type	Stress conditions	%Degradation LOP	%Degradation HCTZ
1	Acid hydrolysis	0.5N HCl at 25°C for 4 hours	18.26	12.24
2	Alkaline hydrolysis	0.5N NaOH at 25°C for 4 hours	17.86	10.29
3	Oxidative degradation	6% H2O2 at 25°C for 6 hours	12.10	10.74
4	Neutral hydrolysis	25°C for 4 hours	13.50	9.86
5	Photolytic degradation	Stability chamber for 1 week 25±2°C	8.54	11.59

CONCLUSION

A simple, rapid, and reliable stability-indicating HPTLC method was successfully developed and validated for the simultaneous estimation of losartan potassium and hydrochlorothiazide in tablet dosage form. The method demonstrated acceptable accuracy, precision, sensitivity, and specificity in accordance with ICH guidelines. The optimized chromatographic conditions resulted in well-resolved peaks with reproducible Rf values. The method was found to be suitable for routine quality control analysis due to its simplicity, cost-effectiveness, and ability to analyze multiple samples simultaneously. Furthermore, forced degradation studies confirmed that the method is stability-indicating, as degradation products were effectively separated from the active pharmaceutical ingredients. Hence, the developed HPTLC method can be successfully applied for routine analysis and stability testing of losartan potassium and hydrochlorothiazide in combined tablet formulations.

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