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DEVELOPMENT OF A NEW ANALYTICAL METHOD AND VALIDATION OF TRIHEXYPHENIDYL HCL AND CHLORPROMAZINE HCL IN PURE AND PHARMACEUTICAL FORMULATION BY RP-HPLC

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ABSTRACT

A New Simple Precise, Rapid, Specific, Accurate, Robust And Rugged Reverse-Phase High-Performance Liquid Chromatographic Method For The Simultaneous Analysis of Trihexyphenidyl Hcl and Chlorpromazine HcL in pure and pharmaceutical dosage form has been developed and validated as per ICH Guidelines. Chromatography was performed on a Hypersil ODS C18 (4.6mm×250mm) 5µm Particle Size column with Acetonitrile: Phosphate Buffer (0.05M) (pH-3.6) (28:72% v/v) used as mobile phase at a flow rate of 1.0 ml/min. UV detection at 224nm; Trihexyphenidyl HCL and Chlorpromazine HCL were eluted with retention times of 2.133, 3.692min respectively. The method was validated in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable and reproducible. Calibration plots were linear over the concentration ranges 30–70µg/ml for Trihexyphenidyl HCL, 60–140µg/ml for Chlorpromazine HCL. Limits of detection were 1.3µg/ml and 3.9µg/ml and limits of quantification were 1.9µg/ml and 5.7µg/ml for Trihexyphenidyl HCL and Chlorpromazine HCL respectively. The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of the two drugs in tablets. Statistical analysis proves that the method is suitable for the analysis of Trihexyphenidyl HCL and Chlorpromazine HCL as a pure drug and in pharmaceutical formulation without any interference from the excipients.

KEYWORDS: Trihexyphenidyl HCL and Chlorpromazine HCL, RP-HPLC, Validation, ICH Guidelines, Accuracy.

INTRODUCTION

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the *mobile phase,* which carries it through a structure holding another material called the *stationary phase.* The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.[1]

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.[2]

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity or differences among their molecular weights.[1,2] Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster.[3]

Based on this approach three components form the basis of the chromatography technique.

- Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- Mobile phase: This phase is always composed of "liquid" or a "gaseous component."

- Separated molecules of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (ie. ion-exchange chromatography) are more effective in the separation of macromolecules as nucleic acids, and proteins. Paper chromatography is used in the separation of proteins, and in studies related to protein synthesis; gas-liquid chromatography is utilized in the separation of alcohol, esther, lipid, and amino groups, and observation of enzymatic interactions, while molecular-sieve chromatography is employed especially for the determination of molecular weights of proteins. Agarose-gel chromatography is used for the purification of RNA, DNA particles, and viruses.[4]

Stationary phase in chromatography, is a solid phase or a liquid phase coated on the surface of a solid phase. Mobile phase flowing over the stationary phase is a gaseous or liquid phase. If mobile phase is liquid it is termed as liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas chromatography is applied for gases, and mixtures of volatile liquids, and solid material. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.[5]

The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation, is to achive a satisfactory separation within a suitable timeinterval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.[6]

- Column chromatography
- Ion-exchange chromatography
- Gel-permeation (molecular sieve) chromatography
- Affinity chromatography
- Paper chromatography
- Thin-layer chromatography
- Gas chromatography
- Dye-ligand chromatography
- Hydrophobic interaction chromatography
- Pseudoaffinity chromatography
- High-pressure liquid chromatography (HPLC)

High-prssure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes throuıgh columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm//sec) flow rate. In this technique, use of small particles, and application of high presure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time.

Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled Essential components of a HPLC device are solvent depot, high- pressure pump, commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued.[25]

Types of HPLC

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. Normal Phase HPLC

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.

Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

2. Reverse Phase HPLC

The stationary phase is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

3. Size-exclusion HPLC

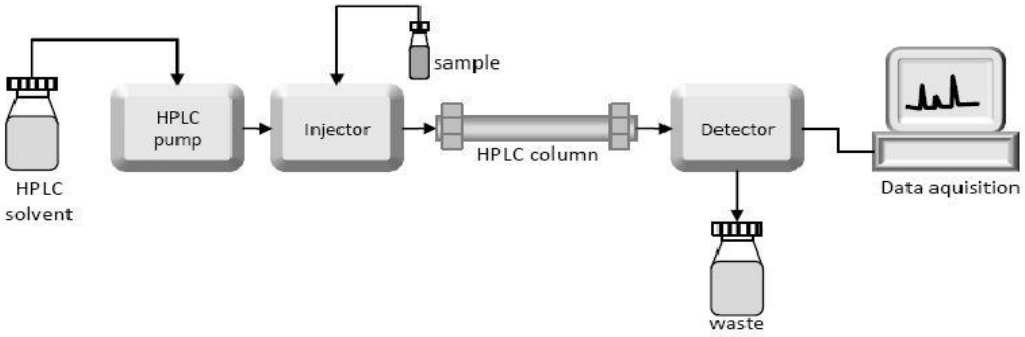
The column is filled with material having precisely controlled pore sizes, and the particles are separated according to its their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

4. Ion-Exchange HPLC

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples.

The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

Instrumentation of HPLC



As shown in the schematic diagram in Figure above, HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs.

1. Solvent Reservoir
Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

2. Pump
A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system's column and detecter. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.

3. Sample Injector
The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).

4. Columns
Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm.

Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

5. Detector
The HPLC detector, located at the end of the column detects the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.

6. Data Collection Devices
Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

Applications of HPLC
The information that can be obtained by HPLC includes resolution, identification and quantification of a compound. It also aids in chemical separation and purification. The other applications of HPLC include:

- **Pharmaceutical Applications**

1. To control drug stability.
2. Tablet dissolution study of pharmaceutical dosages form.
3. Pharmaceutical quality control.

- **Environmental Applications**

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

- **Applications in Forensics**

1. Quantification of drugs in biological samples.
2. Identification of steroids in blood, urine etc.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine etc.

- **Food and Flavour**

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.
3. Analysis of polycyclic compounds in vegetables.
4. Preservative analysis.

- **Applications in Clinical Tests**

1. Urine analysis, antibiotics analysis in blood.
2. Analysis of bilirubin, biliverdin in hepatic disorders.
3. Detection of endogenous Neuropeptides in extracellular fluid of brain etc.

METHOD DEVELOPMENT

Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters, mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytical method should be simple, used column, mobile phase and buffer should be common. It can be done easily step by step. Following are the common HPLC method development steps.

1. Selection of HPLC Analytical Method
2. Selection of Chromatographic Conditions
3. Parameter Optimization

1. Selection of HPLC Analytical Method: First of all consult the literature that is available on the product. It will help you to understand the nature of the product that will help to select the different parameters.

A. Sample Preparation: Select method to prepare the sample according to its solubility, filtration requirements, extraction requirements or other special requirements to make a clear solution of HPLC analysis.

B. Chromatography: Reverse phase chromatography is used for most of the samples but when acidic or basic molecules are present in the sample then reverse phase ion suppression (for weak acid or base) or reverse phase ion pairing (for strong acid or base) should be used. The stationary phase should be C18 bonded. Normal phase is used for low or medium polarity analyte especially when it is required to separate the product isomers. Choose cyano bonded phase for normal phase separations. Ion exchange chromatography is best to use for inorganic anion or cation analysis. If analyte has higher molecular weight than size exclusion chromatography is the best to use.

C. Gradient/Isotonic HPLC: Gradient HPLC is helpful in the analysis of complex samples having a number of components. It will help to get higher resolution than isotonic HPLC having constant peak width while in isotonic HPLC peak width increases with the retention time. Gradient HPLC has great sensitivity, especially for the products having longer retention time.

D. Column Size: 100-150 mm columns are used for most of the samples. It reduces the method development and analysis time for the sample. Bigger columns are used for complex samples those take more time in separation. Initially, a flow rate should be kept between 1 and 1.5 ml/min and column particle size should be between 3 and 5 µm.

E. HPLC Detectors: If the analyte has chromophores that enable the compound to be detected by UV than it is better to use UV detector. It is always better to use a UV detector than others. Fluorescence and electrochemical detectors should be used for trace analysis. Samples having high concentration should be analyzed using refractive index detectors.

F. Wavelength: λmax of the sample has the greatest sensitivity to the UV light. It detects the sample components that have chromophores. A wavelength above 200 nm gives greater sensitivity than the lower wavelengths. Wavelengths lower than 200 nm gives more noise, therefore, it should be avoided. Related: Analytical Method Development Protocol.

2. Selection of Chromatographic Conditions: After selection of analytical method, different chromatographic conditions are selected. The flow of the analytes through the column depends upon the concentration of the solvent in the mobile phase. The concentration of solvent is generally used to control the retention time. Mobile phase pH and ion pairing reagents also affect the retention time of the sample. Samples having a large number of components are analyzed using the gradient to avoid the large retention time while the samples containing one or two components are analyzed on an isotonic system.

3. Parameter Optimization: After taking the same sample runs some parameters including column dimensions, particle size, run time and flow rate are optimized. It is done to get the best resolution and minimum run time. After proper optimization of the analysis method, it is validated to ensure the consistency of the analytical method. Analytical method validation is now done mandatory by all regulatory authorities.

Performance calculations

Computing the accompanying qualities (which VAL be incorporated in a custom report) used to get to general framework execution.

1. Relative retention
2. Theoretical plates
3. Capacity factor
4. Resolution
5. Peak asymmetry
6. Plates per meter

Relative retention (Selectivity)

$$= (t_2 - ta) / (t_1 - ta)$$

Theoretical plates

$$n = 16 (t / W)_2$$

Capacity factor

$$K' = (t_2 / ta) - 1$$

Resolution

$$R = 2 (t_2 - t_1) / (W_2 + W_1)$$

Peak asymmetry

$$T = W0.05 / 2f$$

Plates per meter

$$N = n / L$$

HETP: L/n

Where,

t_2 = **Retention time** of the second peak measured from point of injection.

t_1 = Retention time of the first peak measured from point of injection.

ta = Retention time of an inert peak not retained by the column, measured from point of injection.
n = Theoretical plates.
t = Retention time of the component.
W = Width of the base of the component peak using tangent method.
K' = Capacity factor.
R = Resolution between a peak of interest (peak 2) and the peak preceding it
W_2 = Width of the base of component peak 2.
W_1 = Width of the base of component peak 1.
T = Peak asymmetry, or tailing factor.
W0.05 = Distance from the leading edge to the tailing edge of the peak, measured at a point 5 % of the peak height from the baseline.
f = Distance from the peak maximum to the leading edge of the peak.
N = Plates per meter.
L = Column length, in meters.

METHOD VALIDATION

Validation is a fundamental piece of value affirmation; it includes the deliberate study of frameworks, offices and procedures went for figuring out if they perform their planned capacities sufficiently and eliably as determined.[1,2] An accepted procedure is one which has been shown to give a high level of affirmation that uniform bunches will be created that meet the needed particulars and has in this manner been formally affirmed. Validation in itself does not enhance forms but rather affirms that the procedures have been legitimately created what's more, are under control.
Since a wide assortment of methodology, procedures, and exercises need to be approved, the field of validation is isolated into many subsections[3]
Equipment validation

Analytical Method validation
Cleaning validation
Process validation
Facilities validation
HVAC system validation etc.

A composed arrangement depicting the procedure to be approved, including production equipment and how validation will be conducted.[4] Such an arrangement would address target test parameters, item and procedure attributes, foreordained details, and elements, which will focus worthy results.[5-7]

Validation Parameters
The parameters, as defined by the ICH and by other organizations and authors, are summarized below and are described in brief in the following[8,9]

- **Specificity**
- Selectivity

- **Precision**
- Repeatability
- Intermediate precision
- Reproducibility
- Accuracy

- **Linearity**
- Range

- **Limit of detection**
- Limit of quantization

- **Robustness**
- Ruggedness

Specificity/Selectivity
Specificity, which is the capacity of the system to precisely gauge the analyte reaction in the vicinity of all potential specimen segments.[9-12] The reaction of the analyte in test blends containing the analyte and all potential example parts (placebo definition, combination intermediates, excipients, debasement items and procedure debasements) is contrasted and the reaction of an answer containing just the analyte.[13,14] Other potential example segments are created by presenting the analyte to push conditions adequate to debase it to 80–90% purity.[15-19]

Precision
Accuracy is the measure of how close the information qualities are to one another for various estimations under the same scientific conditions.[20] Accuracy is typically examined at three levels: repeatability, transitional exactness (intermediate precision), and reproducibility.[21-23]

Repeatability
Repeatability is a measure of the exactness under the same working conditions more than a short interim of time, that is, under ordinary working states of the scientific technique with the same hardware.[6] It is some of the time alluded to as intra - test accuracy.[24,25] The ICH prescribes that repeatability be surveyed utilizing at least nine determinations covering the predetermined extent for the technique (e.g., three focuses/ three recreates as in the exactness test) or utilizing at least six determinations at 100% of the test fixation.[26]

Intermediate Precision
Transitional exactness is characterized as the variety inside of the same lab. The degree to which middle of the road exactness needs to be built up relies on upon the circumstances under which the method is planned to be utilized.[27-29] Commonplace parameters that are researched incorporate day - to - day variety, examiner variety, and hardware variety. Contingent upon the degree of the study, the utilization of exploratory configuration is empowered.[30] Test outline will minimize the quantity of investigations that need to be performed.[2] It is essential to note that ICH permits exception from doing halfway accuracy when reproducibility is demonstrated. It is normal that the

transitional exactness ought to show variability that is in the same reach or not as much as repeatability variety.[15,19,31] ICH prescribes the reporting of standard deviation, relative standard deviation (coefficient of variety), and confi-dence interim of the information.[32,33]

Reproducibility

Reproducibility measures the accuracy between labs. This parameter is considered in the institutionalization of a diagnostic methodology (e.g., incorporation of methods in pharmacopeias and system exchange between distinctive labs).[34,35] To accept this trademark, comparable studies need to be performed at distinctive research centers utilizing the same homogeneous example part and the same exploratory configuration. On account of technique exchange between two labs, diverse methodologies may be taken to accomplish the fruitful exchange of the method.[36-38] The most widely recognized methodology is the direct - strategy exchange from the beginning lab to the getting research facility. The beginning research facility is characterized as the lab that has created and accepted the scientific technique or a lab that has beforehand been confirmed to perform the method and will take an interest in the system exchange studies.[39,40] The getting research center is characterized as the lab to which the diagnostic methodology will be exchanged and that will partake in the strategy exchange studies.[41]

Every quantitative result ought to be of high accuracy - there ought to be close to a ±2% variety in the examine framework.[42] A helpful paradigm is the relative standard deviation (RSD) or coefficient of variety (CV), which is an evidence of the imprecision of the framework.

The square of standard deviation is called change (S2). Relative standard deviation is the standard deviation imparted as a little measure of the mean, i.e., S/x.[43-45] It is a couple times expanded by 100 and imparted as a percent relative standard deviation. It transforms into a more strong verbalization of precision.[46]

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean Value}} \times 100$$

Accuracy and Recovery

A system is said to be precise in the event that it gives the right numerical response for the analyte.[47] The technique ought to have the capacity to figure out if the material being referred to complies with its detail for instance, it ought to have the capacity to supply the accurate measure of substance present. Then again, the careful sum present is obscure.[16,25,48] For medication substance, precision may be characterized by the use of the expository method to an analyte of known virtue (e.g., a reference standard).[49-52] For the medication item, precision will be controlled by use of the explanatory method to engineered blends of the medication item parts to which known measures of analyte have been included inside of the scope of the technique.[53]

Exactness is surveyed utilizing at least 9 determinations more than at least 3 focus levels covering the predefined extent (e.g. 3 focuses/3 imitates each of the aggregate scientific method).[54] Exactness is accounted for as percent recuperation by the examine of known included measure of analyte in the example or as the distinction between the mean and the acknowledged genuine esteem together with the certainty interims.[19,55-58]

$$\text{Absolute recovery} = \frac{\text{response of analyte spike into matrix(processed)}}{\text{response of analyte of pure standard (unprocessed)}} \times 100$$

Linearity

A straight relationship ought to be assessed over the scope of the logical method. It is exhibited specifically on the medication substance (by weakening of a standard stock arrangement) and/or separate weighings of engineered blends of the medication item parts, utilizing the proposed technique.[59,60] Linearity ought to be assessed by visual examination of a plot of signs as an element of analyte fixation or substance.[61] In the event that there is a straight relationship, test outcomes ought to be assessed by suitable measurable strategies.

At times, to acquire linearity in the middle of tests and test fixations, the test information may need to be subjected to a scientific change preceding the relapse examination.[62] For the establishment of linearity, a minimum of 5 concentrations are used as shown in **Figure 1**.

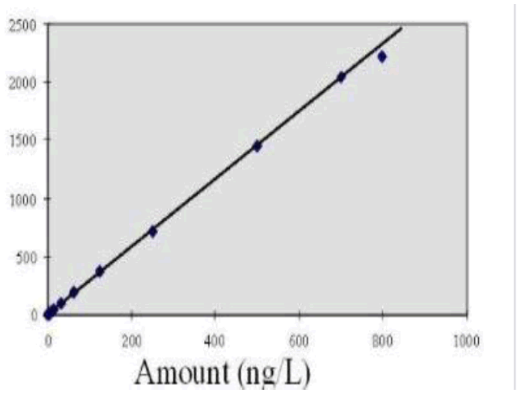


Figure 1: Linearity Graphy (Concentration Vs Peak Area)

Figure 1: Linearity Graphy (Concentration Vs Peak Area).

Limit of Detection

These cutoff points are regularly connected to related substances in the medication substance or medication item.[63-65] Details on these points of confinement are submitted with the administrative debasements system identifying with discharge and steadiness of both medication substance and medication item.[66]

Breaking point of discovery is the least centralization of analyte in a specimen that can be distinguished, yet not so much quantitated, under the expressed test conditions.[67,68]

The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\ \sigma}{S}$$

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S is estimated from the calibration curve of the analyte.

Limit of Quantification
Cutoff of quantitation is the most minimal amassing of analyte in a specimen that can be resolved with satisfactory accuracy and precision under the expressed trial conditions.[69,70]
The quantitation limit (QL) may be expressed as:

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

Where,
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Robustness
ICH characterizes power as a measure of the system's ability to stay unaffected by little, however ponder varieties in strategy parameters.[71] Vigor is incompletely guaranteed by great framework suitability determinations. The assessment of vigor ought to be considered amid the advancement stage and relies on upon the kind of technique under study. It demonstrates the dependability of an examination concerning conscious varieties in technique parameters.[72] In the event that estimations are helpless to varieties in systematic conditions, the explanatory conditions are suitably controlled or a safety oriented proclamation is incorporated in the technique.[73] One result of the assessment of strength ought to be that a progression of framework suitability parameters (e.g., determination test) is built up to guarantee that the legitimacy of the diagnostic technique is kept up at whatever point utilized.
Examples of typical variations are:

- Stability of analytical solutions
- Extraction time

System Suitability
As indicated by the USP, framework suitability tests are a fundamental piece of chromatographic routines. These tests are utilized to confirm that the determination and reproducibility of the framework are sufficient for the examination to be performed. Framework suitability tests are taking into account the idea that the hardware, gadgets, investigative operations, and tests constitute a vital framework that can be assessed all in all. The reason for the framework suitability test is to guarantee that the complete testing framework (counting instrument, reagents, segments, experts) is suitable for the planned application.[74]

Like the scientific technique advancement, the framework suitability test method ought to be updated as the examiners grow more involvement with the measure. All in all, consistency of framework execution (e.g., imitate infusions of the standard) and chromatographic suitability (e.g. tailing component, segment effectiveness and determination of the discriminating pair) are the principle segments of framework suitability.[45]

Amid the early phase of the system improvement transform a portion of the more advanced framework suitability tests may not be pragmatic because of the absence of involvement with the technique. In this stage, for the most part a more "non specific" methodology is utilized. For instance, assessment of the tailing component to check chromatographic suitability, and repeat infusions of the framework suitability answer for check infusion exactness may be adequate for a HPLC polluting influences examine.[52] As the system develops more experience is obtained for this strategy, a more advanced framework suitability tests are fundamental.

Framework suitability is the checking of a framework to guarantee framework execution before or amid the investigation of questions. Parameters, for example, plate tally, tailing components, determination and reproducibility (%RSD maintenance time and region for six redundancies) are resolved and thought about against the determinations set for the system.[16,75] These parameters are measured amid the examination of a framework suitability "test" that is a blend of fundamental parts and expected by-items [19]. **Table 1** rundowns the terms to be measured and their prescribed cutoff points acquired from the examination of the framework suitability test according to current FDA rules on "Validation of Chromatographic Methods" (**Table 1**).

Table 1: System Suitability Parameters and Recommendations.

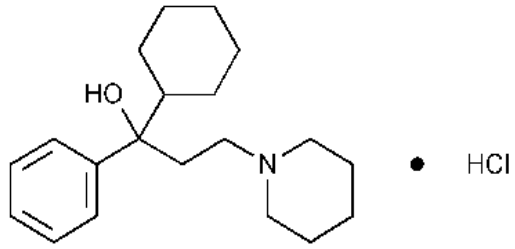
Parameter	RECOMMENDATION
Capacity Factor (k′)	The peak should be well-resolved from other peaks and the void volume, generally k′>2.0
Repeatability	RSD ≤ 1% for N ≥ 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T of ≤ 2
Theoretical Plates (N)	N > 2000

DRUG PROFILE[25-27]

Drug Name: Trihexyphenidyl Hydrochloride

Description: One of the centrally acting muscarinic antagonists used for treatment of parkinsonian disorders and drug-induced extrapyramidal movement disorders and as an antispasmodic.

Structure :



Chemical name : 1-cyclohexyl-1-phenyl-3-(piperidin-1-yl) propan-1-ol hydrochloride

Molecular formulae: $C_{20}H_{32}ClNO$

Molecular Weight : 337.927g/mol

Category : Trihexyphenidyl is used to treat symptoms of Parkinson's disease.

Dose : 5mg tablets.

PHYSICOCHEMICAL PROPERTIES

Description (Physical State): Solid

Solubility: Trihexyphenidyl hydrochloride is soluble in water. It dissolves in methanol at 50 mg/ml to yield a clear to hazy, colorless solution. It is very slightly soluble in ether and benzene.

Storage conditions: Store at controlled room temperature (between 68 and 77 degrees F).

Indications : Indicated for the treatment of parkinson's disease and extrapyramidal reactions caused by drugs.

Mechanism of action: Trihexyphenidyl is a selective M1 muscarinic acetylcholine receptor antagonist. It is able to discriminate between the M1 (cortical or neuronal) and the peripheral muscarinic subtypes (cardiac and glandular). Trihexyphenidyl partially blocks cholinergic activity in the CNS, which is responsible for the symptoms of Parkinson's disease. It is also thought to increase the availability of dopamine, a brain chemical that is critical in the initiation and smooth control of voluntary muscle movement.

Official : Indian *Pharmacopoeia*, British *Pharmacopoeia*

Melting point : 223-226 °C

pKa (Strongest basic): 9.32

log p **:** 4.23

Adverse effects : Drowsiness, dizziness, constipation, flushing, nausea, nervousness, blurred vision, or dry mouth may occur. These effects usually lessen as your body gets used to the medicine. If any of these effects persist or worsen, contact your doctor or pharmacist promptly.

Pharmacodynamics: Trihexyphenidyl is an anticholinergic used in the symptomatic treatment of all etiologic groups of Parkinsonism and drug induced extrapyramidal reactions (except tardive dyskinesia). Trihexyphenidyl possesses both anticholinergic and antihistaminic effects, although only the former has been established as therapeutically significant in the management of Parkinsonism.

Absorption: Trihexyphenidyl is rapidly absorbed from the gastrointestinal tract.

Half life: 3.3-4.1 hours

INTERACTIONS

7-Nitroindazole: 7-Nitroindazole may increase the central nervous system depressant (CNS depressant) activities of Trihexyphenidyl.

Abediterol: The risk or severity of Tachycardia can be increased when Trihexyphenidyl is combined with Abediterol.

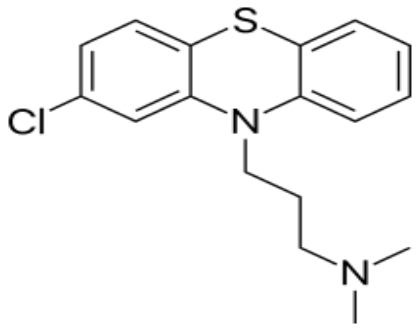
Acepromazine: Acepromazine may increase the central nervous system depressant (CNS depressant) activities of Trihexyphenidyl.

Aceprometazine: Aceprometazine may increase the central nervous system depressant (CNS depressant) activities of Trihexyphenidyl.
Acetaminophen: The risk or severity of adverse effects can be increased when Trihexyphenidyl is combined with Acetaminophen.
Contraindications: ARTANE (Trihexyphenidyl) is contraindicated in patients with hypersensitivity to trihexyphenidyl HCl or to any of the tablet or elixir ingredients. Artane (trihexyphenidyl) is also contraindicated in patients with narrow angle glaucoma. Blindness after long-term use due to narrow angle glaucoma has been reported.
Medical Uses: Trihexyphenidyl oral tablet is used to treat all forms of Parkinsonism, including Parkinson's disease. It's also used to treat severe movement side effects caused by antipsychotic drugs.

DRUG FORMULATION

S.No	Drug name	Label Claim	Brand name	Company
1	Trihexyphenidyl	2mg	Artane	INTAS

DRUG PROFILE[28-30]
Drug : Chlorpromazine
Synonym **:** Chlorpromazine
Drug category : Anticholinergic Agents
Structure :



Chemical name/ Nomenclature / IUPAC Name **:** 3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-propan-1-amine
Molecular Formula : $C_{17}H_{19}ClN_2S$
Molecular Weight : 318.86 gm/mole.
Official Pharmacopoeia **:** USP, EP

PHYSICOCHEMICAL PROPERTIES
Description (Physical State): Liquid
Solubility: water solubility **2.55 mg/L**
Dosage: Tablet
Melting point: 177-178 °C
pKa (strongest Basic): 9.2
Log P: 5.41

PHARMACOKINETIC PROPERTIES
Bioavailability : 10–80 %
Half-life : 30 hrs
Absorption : Readily absorbed from the GI tract. Bioavailability varies due to first-pass metabolism by the liver.
Volume of Distribution: 20 L/kg
Protein binding : 90–99 %
Metabolism **:** Extensively metabolized in the liver and kidneys. It is extensively metabolized by cytochrome P450 isozymes CYP2D6 (major pathway), CYP1A2 and CYP3A4. Approximately 10 to 12 major metabolite have been identified. Hydroxylation at positions 3 and 7 of the phenothiazine nucleus and the N-dimethylaminopropyl side chain undergoes demethylation and is also metabolized to an N-oxide. In urine, 20% of chlopromazine and its metabolites are excreted unconjugated in the urine as unchanged drug, demonomethylchlorpromazine, dedimethylchlorpromazine, their sulfoxide metabolites, and chlorpromazine-N-oxide. The remaining 80% consists of conjugated metabolites, principally O-glucuronides and small amounts of ethereal sulfates of the mono- and dihydroxy-derivatives of chlorpromazine and their sulfoxide metabolites. The major metabolites are the monoglucuronide of N-dedimethylchlorpromazine and 7-hydroxychlorpromazine. Approximately 37% of the administered dose of chlorpromazine is excreted in urine.

Excretion : Kidneys, ~ 37% excreted in urine
Adverse effects/Side effects **:** Drowsiness, Dizziness, Lightheadedness,

PHARMACODYNAMICS: Chlorpromazine is a psychotropic agent indicated for the treatment of schizophrenia. It also exerts sedative and antiemetic activity. Chlorpromazine has actions at all levels of the central nervous system-primarily at subcortical levels-as well as on multiple organ systems. Chlorpromazine has strong antiadrenergic and weaker peripheral anticholinergic activity; ganglionic blocking action is relatively slight. It also possesses slight antihistaminic and antiserotonin activity.

Mechanism of action: Chlorpromazine acts as an antagonist (blocking agent) on different postsysnaptic receptors -on dopaminergic-receptors (subtypes D1, D2, D3 and D4 - different antipsychotic properties on productive and unproductive symptoms), on serotonergic-receptors (5-HT1 and 5-HT2, with anxiolytic, antidepressive and antiaggressive properties as well as an attenuation of extrapypramidal side-effects, but also leading to weight gain, fall in blood pressure, sedation and ejaculation difficulties), on histaminergic-receptors (H1-receptors, sedation, antiemesis, vertigo, fall in blood pressure and weight gain), alpha1/alpha2-receptors (antisympathomimetic properties, lowering of blood pressure, reflex tachycardia, vertigo, sedation, hypersalivation and incontinence as well as sexual

dysfunction, but may also attenuate pseudoparkinsonism - controversial) and finally on muscarinic (cholinergic) M1/M2-receptors (causing anticholinergic symptoms like dry mouth, blurred vision, obstipation, difficulty/inability to urinate, sinus tachycardia, ECG-changes and loss of memory, but the anticholinergic action may attenuate extrapyramidal side-effects). Additionally, Chlorpromazine is a weak presynaptic inhibitor of Dopamine reuptake, which may lead to (mild) antidepressive and antiparkinsonian effects. This action could also account for psychomotor agitation and amplification of psychosis (very rarely noted in clinical use).

Therapeutic efficacy/ Indications: For the treatment of schizophrenia; to control nausea and vomiting; for relief of restlessness and apprehension before surgery; for acute intermittent porphyria; as an adjunct in the treatment of tetanus; to control the manifestations of the manic type of manic-depressive illness; for relief of intractable hiccups; for the treatment of severe behavioral problems in children (1 to 12 years of age) marked by combativeness and/or explosive hyperexcitable behavior (out of proportion to immediate provocations), and in the short-term treatment of hyperactive children who show excessive motor activity with accompanying conduct disorders consisting of some or all of the following symptoms: impulsivity, difficulty sustaining attention, aggressivity, mood lability, and poor frustration tolerance.

Contraindications: Asthma, benzyl alcohol hypersensitivity, phenothiazine hypersensitivity, sulfite hypersensitivity

INTERACTIONS

Drug interactions

7-Nitroindazole: The risk or severity of adverse effects can be increased when Chlorpromazine is combined with 7-Nitroindazole.

7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline: The risk or severity of serotonin syndrome can be increased when Chlorpromazine is combined with 7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline.

7,9-Dimethylguanine: The serum concentration of 7,9-Dimethylguanine can be increased when it is combined with Chlorpromazine.

8-azaguanine: The serum concentration of 8-azaguanine can be increased when it is combined with Chlorpromazine.

Food interactions

Avoid alcohol.

Take with food to reduce irritation.

DRUG FORMULATION

S.No	Drug name	Label Claim	Brand name	Company
1	Chlorpromazine	25mg	Thorazine	SANDOZ

Combined Drug Formulation

S.No.	Drug name	Label Claim	Brand name	Company
1	Chlorpromazine HCL/ Trihexyphenidyl	100mg/2mg	Talentil Plus Tab	Talent India

AIM AND OBJECTIVES

Literature review reveals that there is less analytical methods are reported for the analysis of Trihexyphenidyl HCL and Chlorpromazine HCL by simultaneous estimation by RP-HPLC. There is a need of new analytical method development for the simultaneous estimation of Trihexyphenidyl HCL and Chlorpromazine HCL in pharmaceutical dosage form.

Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Trihexyphenidyl HCL and Chlorpromazine HCL. The developed method will be validated according to ICH guidelines.

Objectives of the Work

- The analytical method for the simultaneous estimation of Trihexyphenidyl HCL and Chlorpromazine HCL will be developed by RP-HPLC method by optimizing the chromatographic conditions.
- The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1).

Plan of Work

In order to develop a simple, reliable and an accurate method development and validation of Trihexyphenidyl HCL and Chlorpromazine HCL in bulk and pharmaceutical dosage form by reverse phase HPLC and validate the method for its repeatability and reproducibility.

Plan of the proposed work includes the following steps

- Selection of drug and literature survey.
- Solubility studies and optimization of conditions.
- Analytical method(s) development using HPLC etc.
- Assay of the drugs(s) in marketed formulations using the proposed method(s).
- Procurement of raw materials.
- Establishment of system suitability parameters.

- Trails for the method development of Trihexyphenidyl HCL and Chlorpromazine HCL.
- Setting of the optimized method.
- Validation of the optimized method for Trihexyphenidyl HCL and Chlorpromazine HCL.
- Validation parameters include:
  - System suitability
  - Specificity
  - Method precision
  - Linearity
  - Accuracy
  - Range
  - Robustness

EXPERIMENTAL METHODS

INSTRUMENTS USED

Table: Instruments used.

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

CHEMICALS USED

Table: Chemicals used.

S.No	Chemical	Brand names
1	Trihexyphenidyl HCL (Pure)	Sura labs
2	Chlorpromazine HCL (Pure)	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Trihexyphenidyl HCL and Chlorpromazine HCL working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

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

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 75:25 v/v respectively.

Optimization of Column

The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Column Temperature : 38ºC
Column : Hypersil ODS C18 (4.6mm×250mm) 5µm Particle Size
Mobile phase : Acetonitrile: Phosphate Buffer (0.05M) (pH-3.6) (28:72% v/v)
Flow rate : 1ml/min
Wavelength : 224nm
Injection volume : 10µl
Run time : 6minutes

METHOD VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of Mobile Phase

Accurately measured 280ml of Acetonitrile (28%) of and 720ml of Phosphate Buffer (72%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

VALIDATION PARAMETERS
System Suitability
Accurately weigh and transfer 10 mg of Trihexyphenidyl HCL and Chlorpromazine HCL working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG
Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Trihexyphenidyl HCL and Chlorpromazine HCL working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Trihexyphenidyl HCL and Chlorpromazine HCL sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.
Further pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity
Accurately weigh and transfer 10 mg of Trihexyphenidyl HCL and Chlorpromazine HCL working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (30ppm of Trihexyphenidyl HCL and 60ppm of Chlorpromazine HCL)
Pipette out 0.3ml of Trihexyphenidyl HCL and 0.6ml of Chlorpromazine HCL in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – II (40ppm of Trihexyphenidyl HCL and 80ppm of Chlorpromazine HCL)
Pipette out 0.4ml of Trihexyphenidyl HCL and 0.8ml of Chlorpromazine HCL in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – III (50ppm of Trihexyphenidyl HCL and 100ppm of Chlorpromazine HCL)
Pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – IV (60ppm of Trihexyphenidyl HCL and 120ppm of Chlorpromazine HCL)
Pipette out 0.6ml of Trihexyphenidyl HCL and 1.2ml of Chlorpromazine HCL in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – V (70ppm of Trihexyphenidyl HCL and 140ppm of Chlorpromazine HCL)
Pipette out 0.7ml of Trihexyphenidyl HCL and 1.4ml of Chlorpromazine HCL in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Procedure
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

Repeatability

Preparation of Trihexyphenidyl HCL and Chlorpromazine HCL Product Solution for Precision

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

Further pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

DAY 1

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy

For preparation of 50% Standard stock solution

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

Further pipette out 0.25ml of Trihexyphenidyl HCL and 0.5ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution

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

Further pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution

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

Further pipette out 0.75ml of Trihexyphenidyl HCL and 1.5ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Trihexyphenidyl HCL and Chlorpromazine HCL and calculate the individual recovery and mean recovery values.

ROBUSTNESS

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

For preparation of Standard solution

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

Further pipette out 0.5ml of Trihexyphenidyl HCL and 1ml of Chlorpromazine HCL from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer (0.05M) (pH-3.6) (28:72% v/v) and water was taken in the ratio and 33:77, 13:87 instead of 28:72 remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trails

Trail 1:

Column : Inertsil C18 (4.6mm×250mm) 5µm
Column temperature : 35ºc
Wavelength : 224nm
Mobile phase ratio : Acetonitrile: Water (70:30) V/V
Flow rate : 0.8ml/min
Injection volume : 20µl
Run time : 10minutes

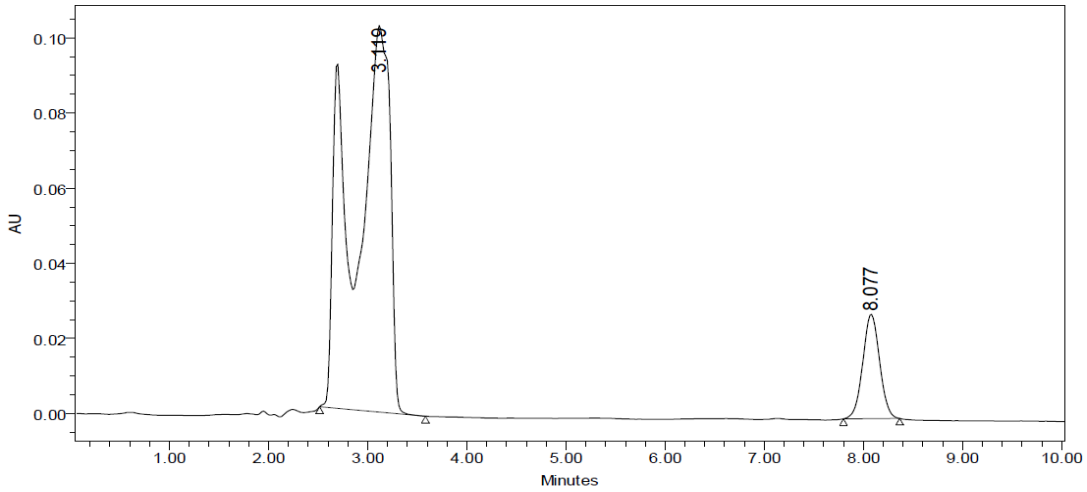


Figure: Chromatogram for Trail 1.

Table: Peak Results for Trail 1.

S.No.	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Trihexyphenidyl HCL	3.118	8569852	98698	0.96	985
2	Chlorpromazine HCL	8.077	58645	652	0.98	967

OBSERVATION

In this trial it shows less plate count, improper separation of two peaks and shows improper baseline, resolution in the chromatogram. So it's required more trials to obtain good peaks.

Trail 2

Column : Xterra C18 (4.6mm×250mm) 5µm
Column temperature : 34°C
Wavelength : 224nm
Mobile phase ratio : Methanol: Acetonitrile (20:80) V/V
Flow rate : 0.8ml/min
Injection volume : 10µl
Run time : 2.5minutes

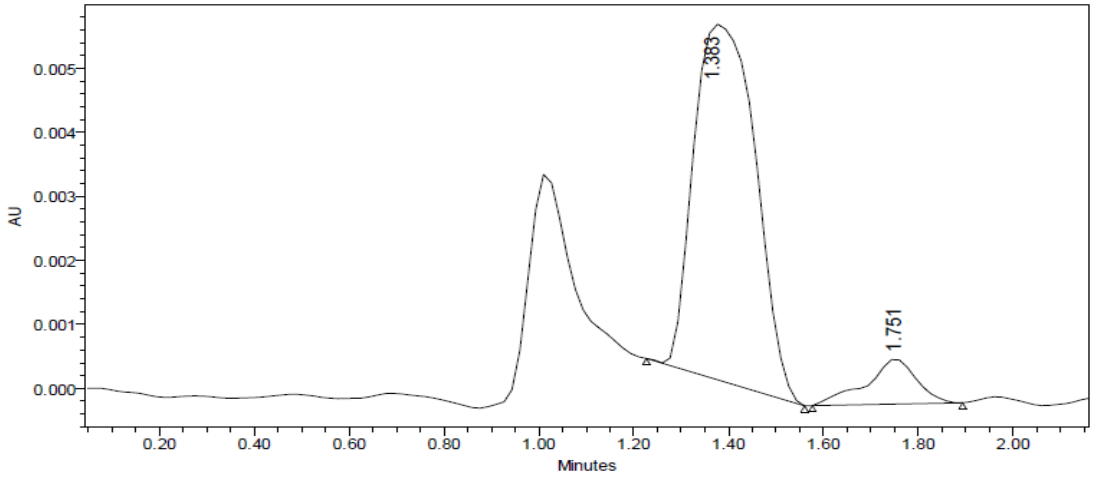


Figure: Chromatogram for Trail 2.

Table: Peak Results for Trail 2.

S. No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Trihexyphenidyl HCL	1.383	12698544	65984	0.99	2658
2	Chlorpromazine HCL	1.751	52652	4521	0.93	1235

Observation: In this above trail it shows improper separation of two peaks, less plate count and resolution in the chromatogram. More trails required for to obtained proper peaks.

Trail 3

Column : Develosil ODS C18 (4.6mm×250mm) 5µm
Column temperature : 35ºc
Wavelength : 224nm
Mobile phase ratio : Acetonitrile: Methanol (50:50) V/V
Flow rate : 0.8ml/min
Injection volume : 20µl
Run time : 10minutes

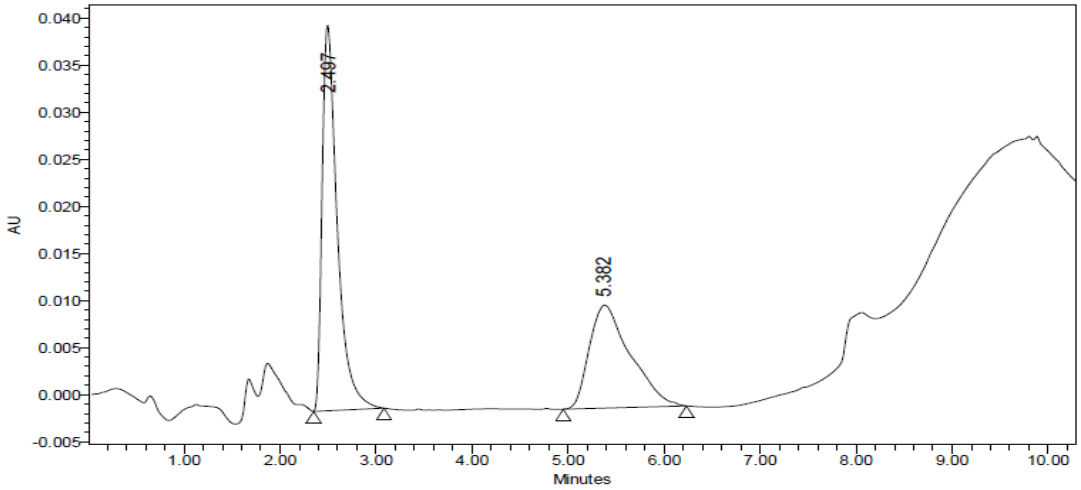


Figure-: Chromatogram for Trail 3.

Table: Peak Results for Trail 3.

S. No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Trihexyphenidyl HCL	2.497	854784	96587	1.06	1365
2	Chlorpromazine HCL	5.382	75847	4526	0.92	1748

Observation

In this above chromatogram shows improper separation of two peaks and baseline in the chromatogram. So it's required more trails to get good peaks.

Trail 4

Column : Zorbax ODS C18 (4.6mm×250mm) 5µm
Column temperature : 37ºc
Wavelength : 224nm
Mobile phase ratio : Methanol: Phosphate Buffer (30:70) V/V
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10minutes

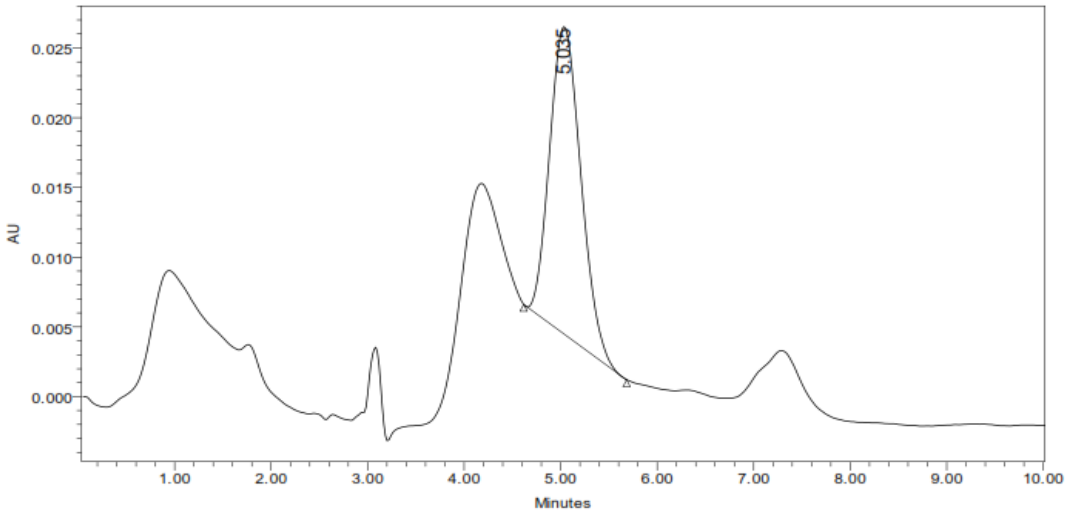


Figure: Chromatogram for Trail 4.

Table: Peak Results for Trail 4.

S.No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Trihexyphenidyl HCL	5.035	568955	4547	2.06	1526

Observation

In this above trail it shows more tailing and less plate count in the chromatogram. More trails required to obtained proper peaks.

Trail 5

Column : Hypersil ODS C18 (4.6mm×250mm) 5µm Particle Size
Column temperature : 40ºc
Wavelength : 224nm
Mobile phase ratio : Acetonitrile: Phosphate Buffer (0.03M) (pH-4.8) (40:60% v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

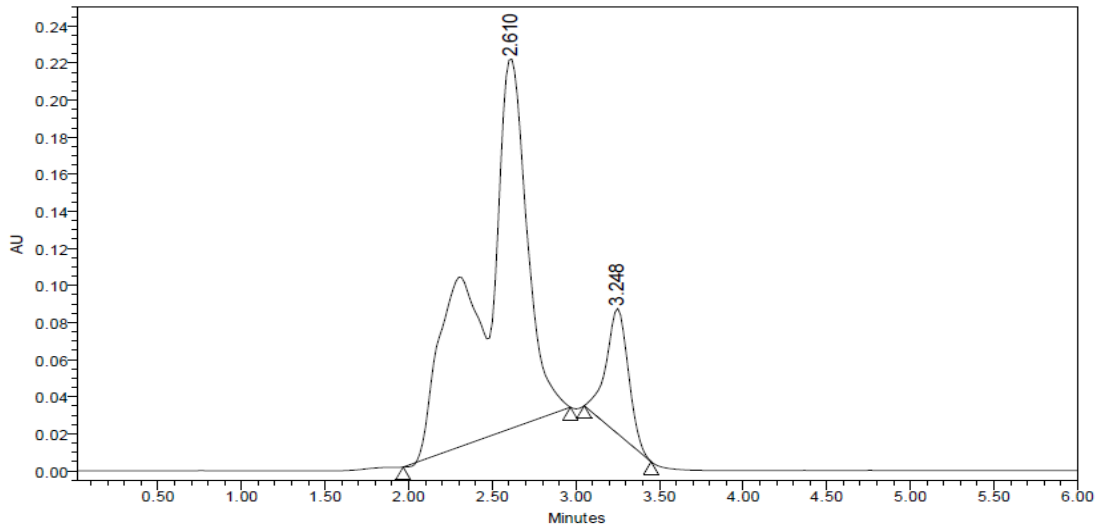


Figure: Chromatogram for Trail 5.

Table-: Peak Results for Trail 5

S.No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Trihexyphenidyl HCL	2..610	185986	45265	2.01	3879
2	Chlorpromazine HCL	3.248	46528	3658	1.14	2659

Observation

In this above trail it shows more tailing and less plate count in the chromatogram. More trails required to obtained proper peaks.

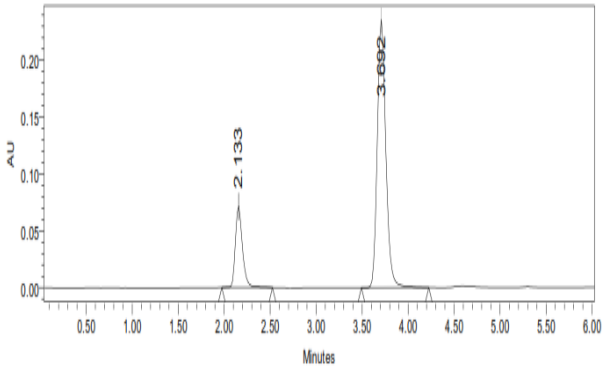


Figure: Optimized Chromatogram (Standard).

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Phosphate Buffer (0.05M) (pH-3.6) (28:72% v/v)
Column : Hypersil ODS C18 (4.6mm×250mm) 5µm Particle Size
Column temperature : 38ºC
Wavelength : 224nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 6minutes

Table: Optimized Chromatogram (Standard).

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Trihexyphenidyl HCL	2.133	58985	8654	1.36	6859	7.54
2	Chlorpromazine HCL	3.692	102569	96578	1.42	8475	

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

Optimized Chromatogram (Sample)

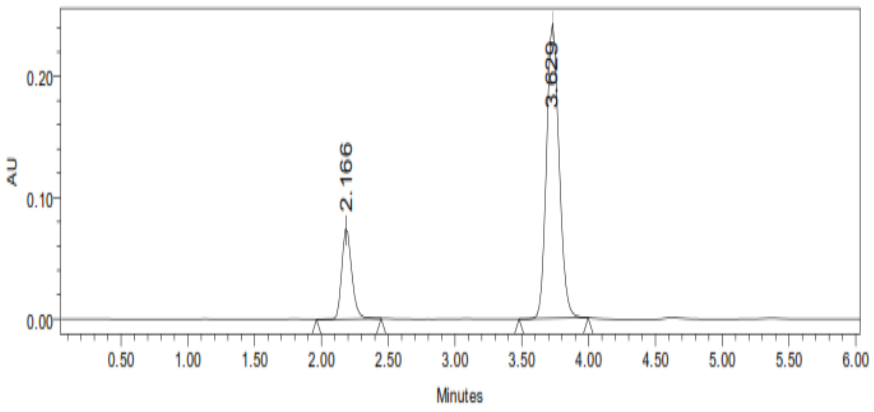


Figure: Optimized Chromatogram (Sample).

Table: Optimized Chromatogram (Sample).

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Trihexyphenidyl HCL	2.166	59865	8759	1.38	6985	8.52
2	Chlorpromazine HCL	3.629	104568	97856	1.43	8598	

Acceptance Criteria

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank

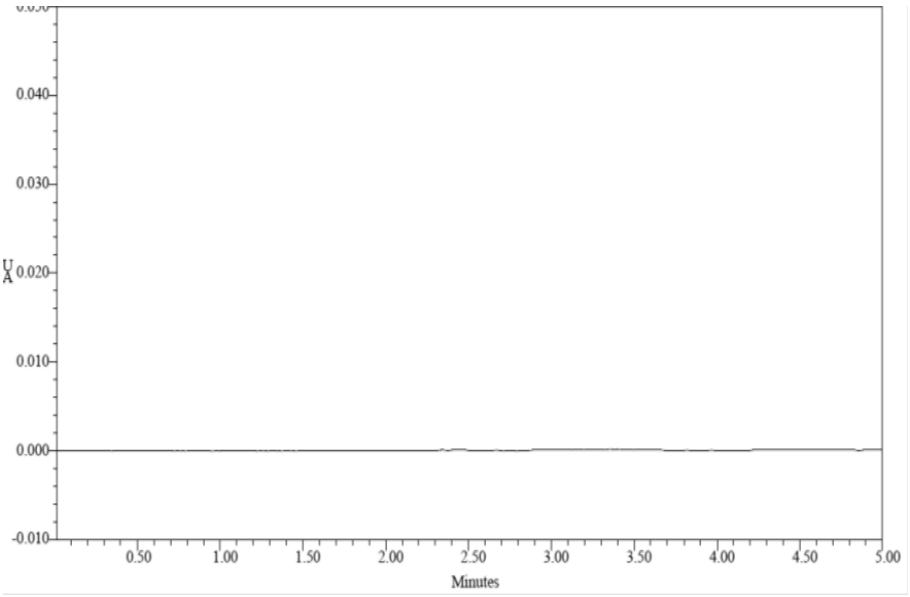


Fig: Chromatogram showing blank (mobile phase preparation).

System Suitability

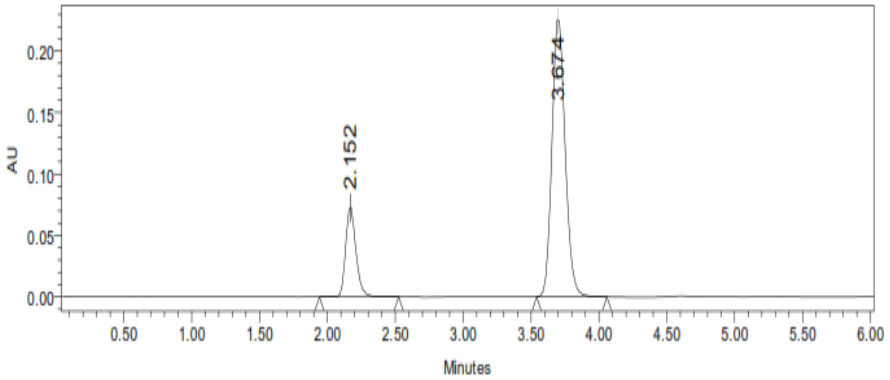


Fig: Chromatogram showing injection -1.

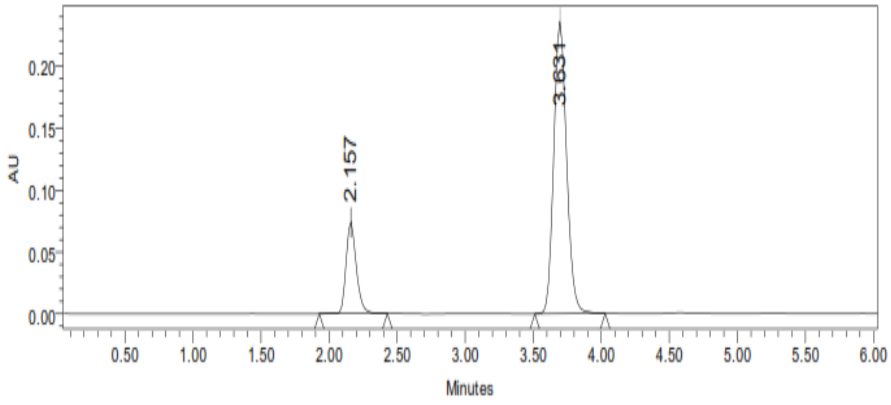


Fig: Chromatogram showing injection -2.

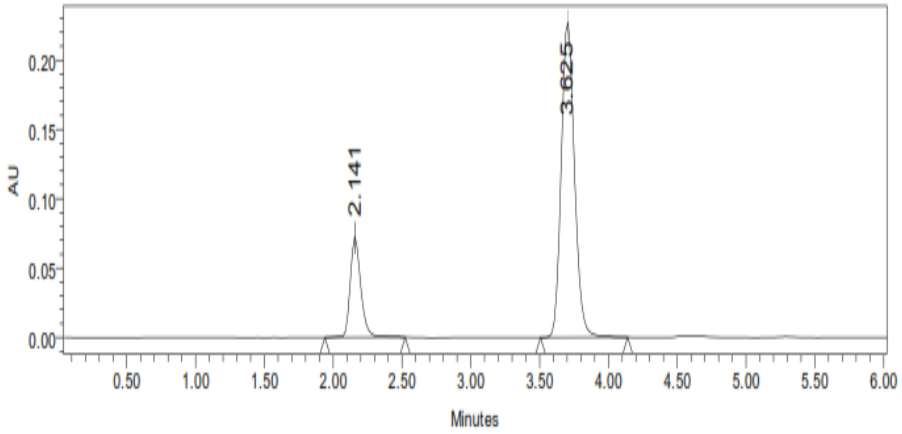


Fig: Chromatogram showing injection-3.

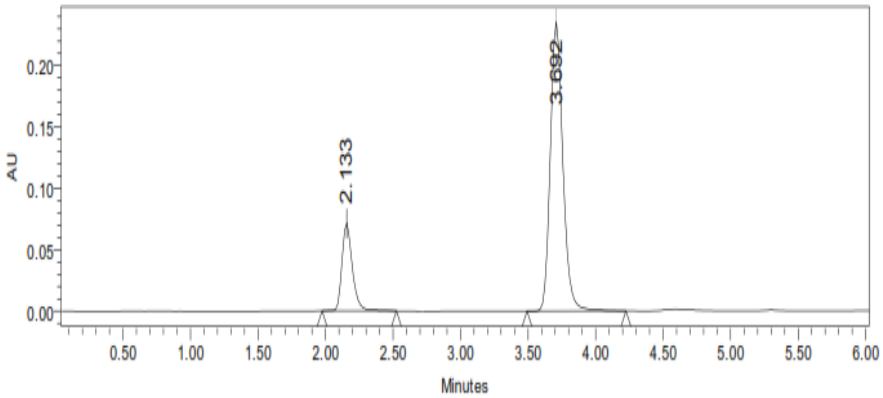


Fig: Chromatogram showing injection -4.

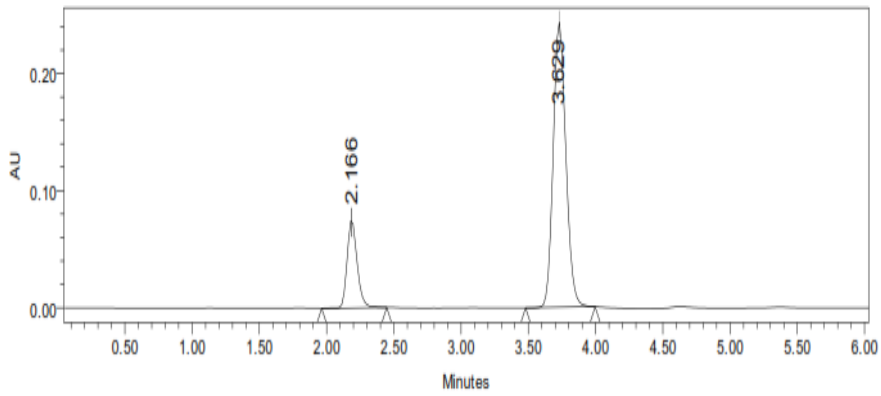


Fig: Chromatogram showing injection-5.

Table: Results of system suitability for Trihexyphenidyl HCL.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Trihexyphenidyl HCL	2.152	58986	8659	6859	1.36
2	Trihexyphenidyl HCL	2.157	58798	8645	6829	1.37
3	Trihexyphenidyl HCL	2.141	58965	8624	6878	1.36
4	Trihexyphenidyl HCL	2.133	58469	8649	6895	1.37
5	Trihexyphenidyl HCL	2.166	58957	8697	6849	1.36
Mean			58835			
Std. Dev.			217.8933			
% RSD			0.370346			

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Chlorpromazine HCL.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Chlorpromazine HCL	3.674	102365	96895	8459	1.42	7.61
2	Chlorpromazine HCL	3.631	102659	96598	8475	1.43	7.62
3	Chlorpromazine HCL	3.625	102658	96874	8496	1.42	7.61
4	Chlorpromazine HCL	3.692	102698	96258	8465	1.42	7.62
5	Chlorpromazine HCL	3.629	102879	96982	8495	1.43	7.61
Mean			102651.8				
Std. Dev.			184.4579				
% RSD			0.179693				

Acceptance criteria

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Trihexyphenidyl HCL and Chlorpromazine HCL in drug product.

Assay (Standard)

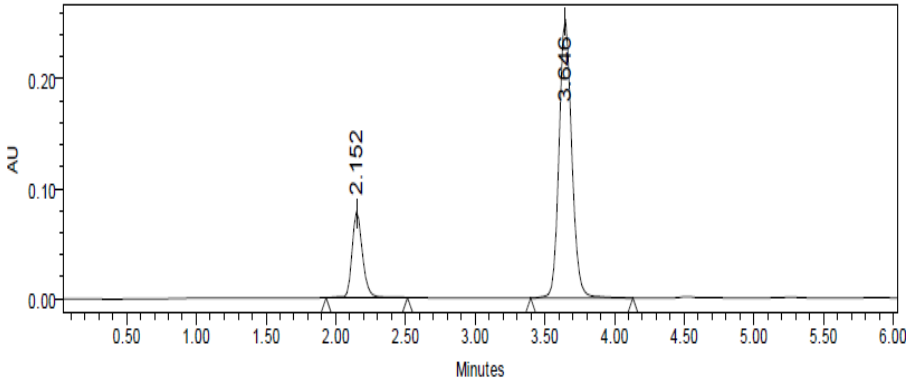


Fig: Chromatogram showing assay of standard injection -1.

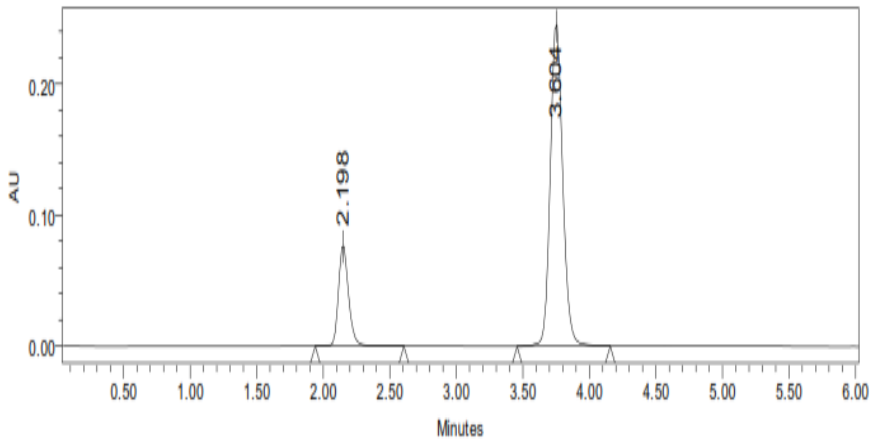


Fig: Chromatogram showing assay of standard injection-2.

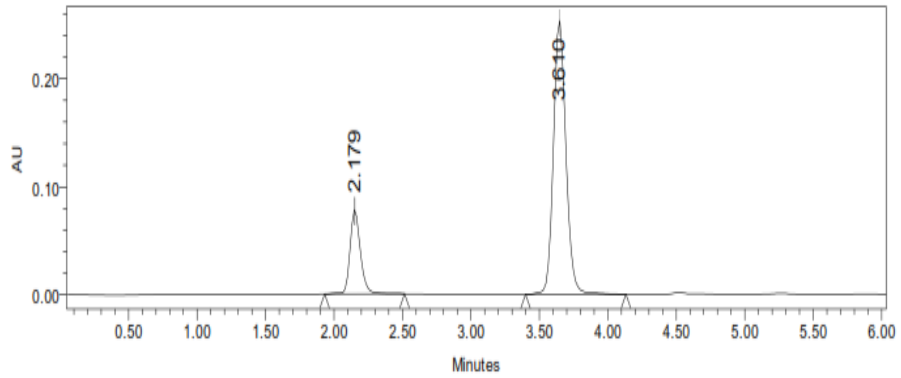


Fig: Chromatogram showing assay of standard injection-3.

Table: Peak results for assay standard of Trihexyphenidyl HCL.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trihexyphenidyl HCL	2.152	58986	8695	1.36	6895	1
2	Trihexyphenidyl HCL	2.198	58978	8689	1.37	6829	2
3	Trihexyphenidyl HCL	2.179	58798	8654	1.36	6872	3

Table: Peak Results for Assay Standard of Chlorpromazine HCL.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Chlorpromazine HCL	3.646	102658	96859	1.42	8469	1
2	Chlorpromazine HCL	3.604	102598	96256	1.43	8449	2
3	Chlorpromazine HCL	3.610	102854	96865	1.42	8426	3

Assay (Sample)

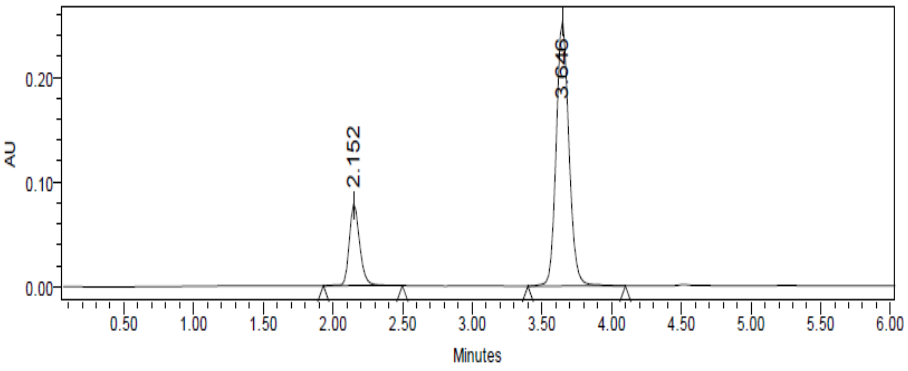


Fig: Chromatogram Showing Assay of Sample Injection-1.

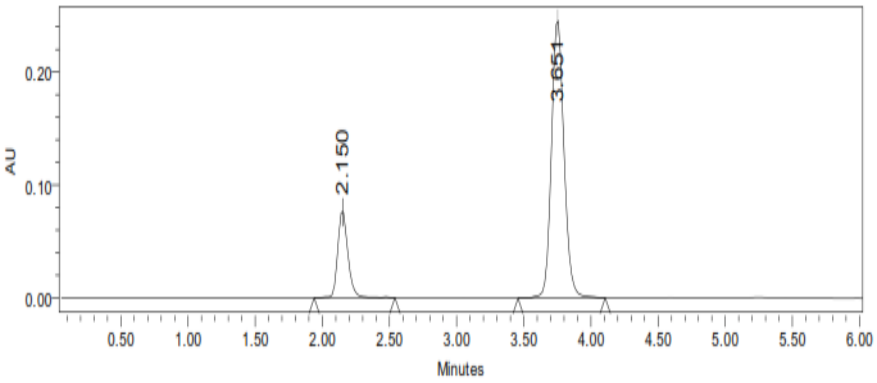


Fig: Chromatogram Showing Assay of Sample Injection-2.

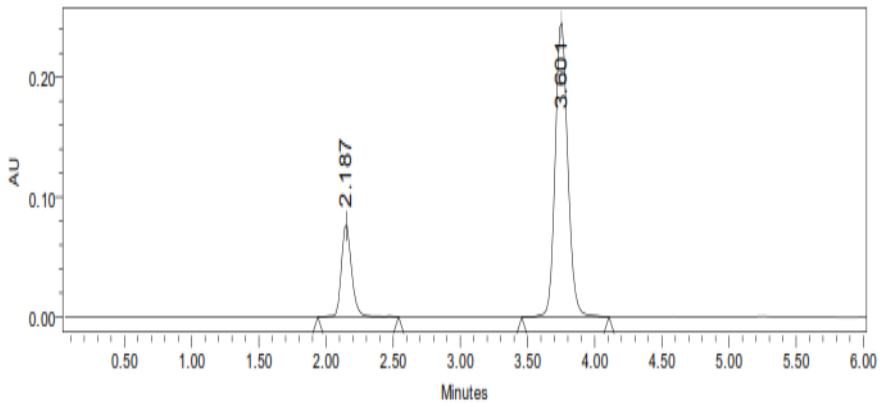


Fig: Chromatogram showing assay of sample injection-3.

Table: Peak results for Assay sample of Trihexyphenidyl HCL.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trihexyphenidyl HCL	2.152	59869	8798	1.37	6985	1
2	Trihexyphenidyl HCL	2.150	59865	8726	1.38	6925	2
3	Trihexyphenidyl HCL	2.187	59885	8795	1.37	6938	3

Table: Peak results for Assay sample of Chlorpromazine HCL.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Chlorpromazine HCL	3.646	103659	97895	1.43	8596	1
2	Chlorpromazine HCL	3.651	103852	97864	1.44	8527	2
3	Chlorpromazine HCL	3.601	103698	97268	1.43	8569	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Trihexyphenidyl HCL and Chlorpromazine HCL in pharmaceutical dosage form was found to be 99.75%

LINEARITY

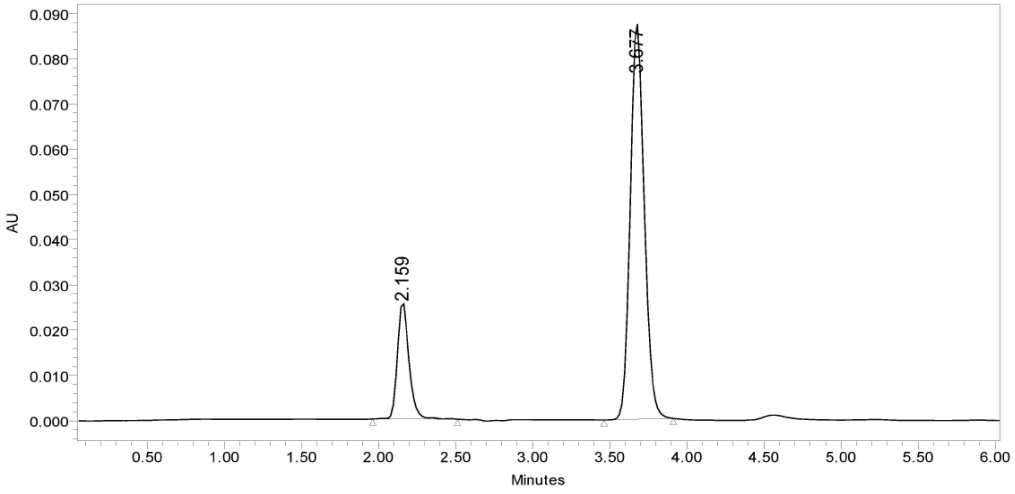


Fig-: Chromatogram showing linearity level-1

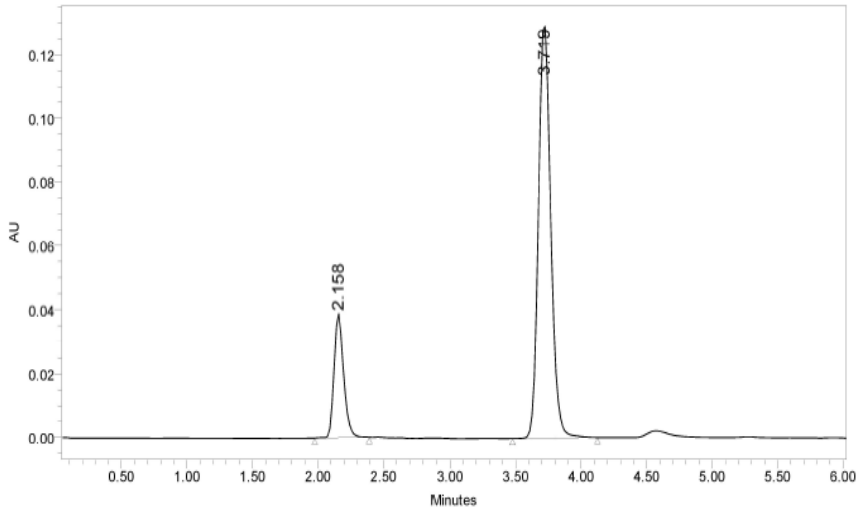


Fig: Chromatogram showing linearity level-2.

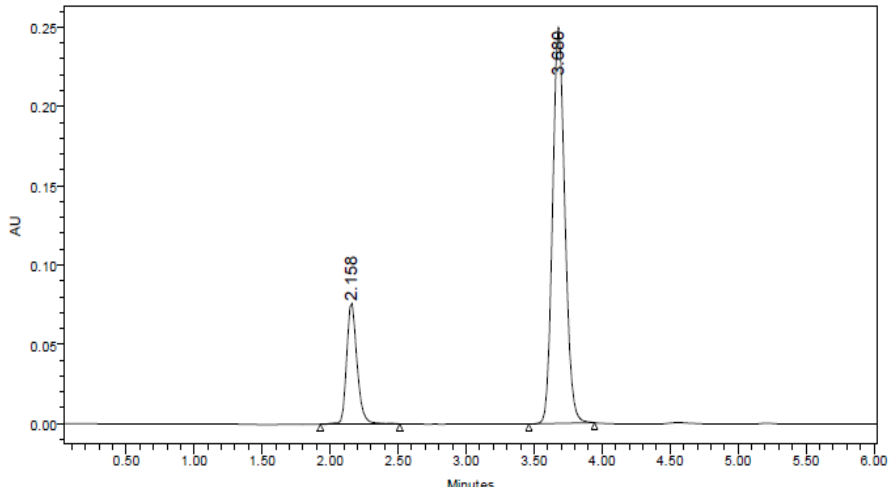


Fig: Chromatogram showing linearity level-3.

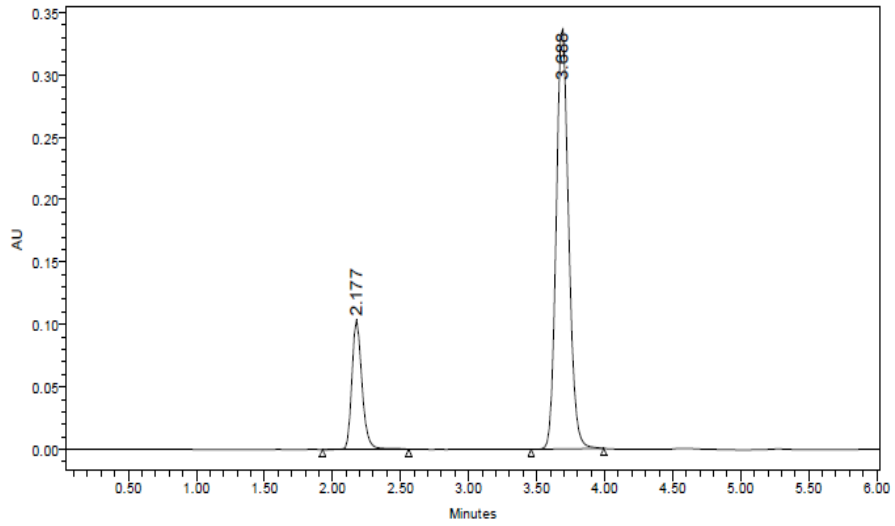


Fig: Chromatogram Showing Linearity Level-4.

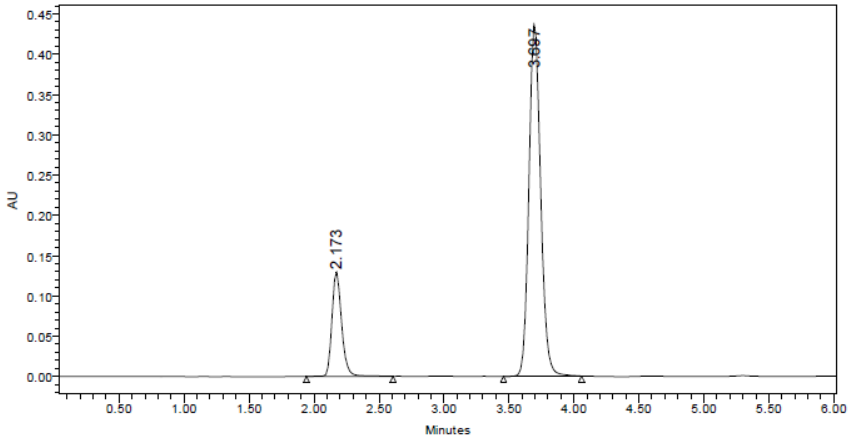
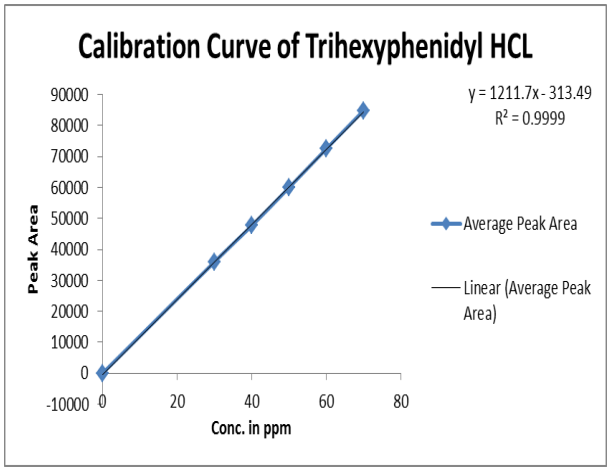


Fig: Chromatogram showing linearity level-5.

Chromatographic Data for Linearity Study of Trihexyphenidyl Hcl.

Concentration µg/ml	Average Peak Area
30	35965
40	47659
50	59986
60	72524
70	84899



LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Trihexyphenidyl HCL is a straight line.

Y = mx + c

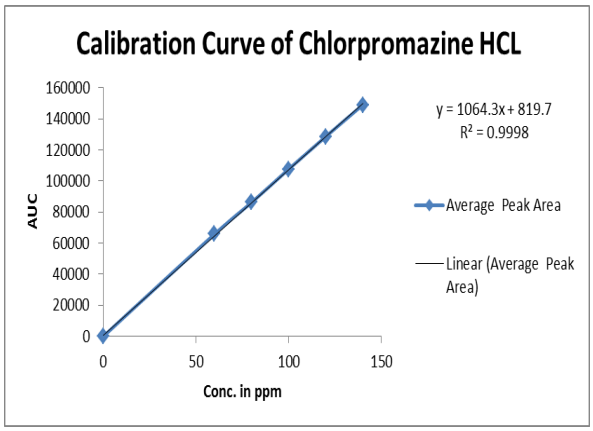
Slope (m) = 1211
Intercept (c) = 313.4
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 313.4. These values meet the validation criteria.

Chromatographic Data For Linearity Study Of Chlorpromazine Hcl.

Concentration µg/ml	Average Peak Area
60	65784
80	86185
100	107569
120	128544
140	148985



LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Chlorpromazine HCL is a straight line.

Y = mx + c

Slope (m) = 1064
Intercept (c) = 819.7
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 819.7. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling

of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

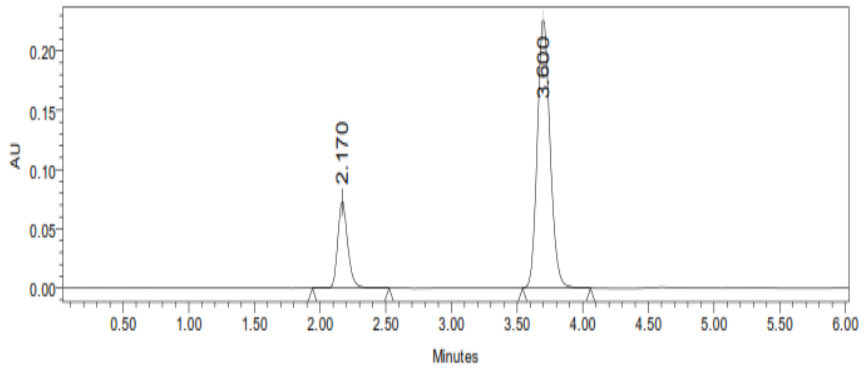


Fig: Chromatogram Showing Precision Injection-1.

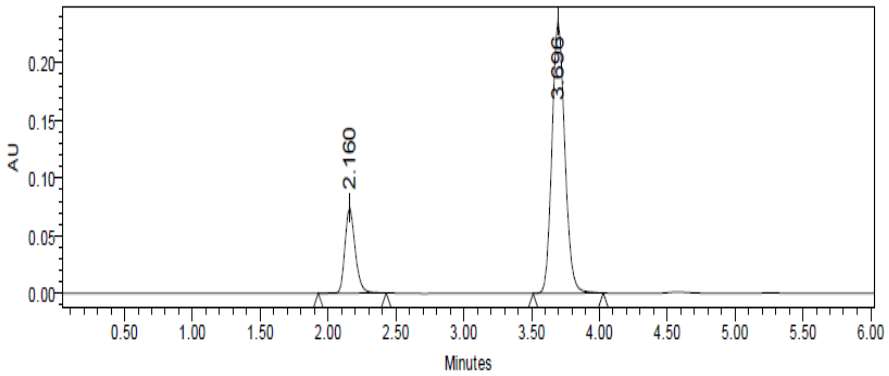


Fig: Chromatogram showing precision injection -2.

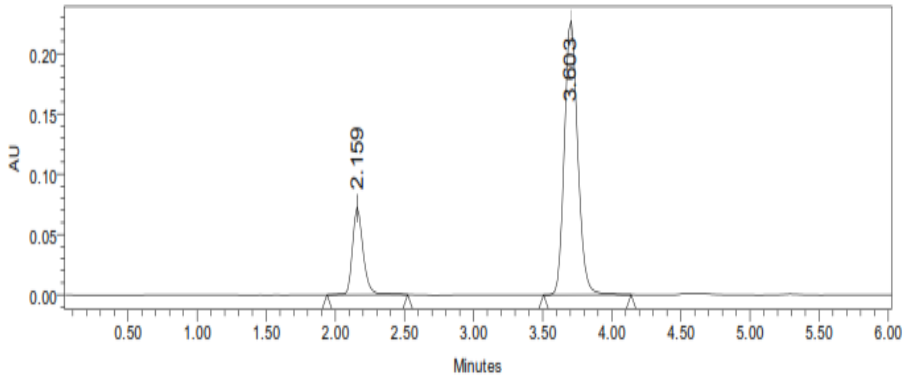


Fig: Chromatogram showing precision injection-3.

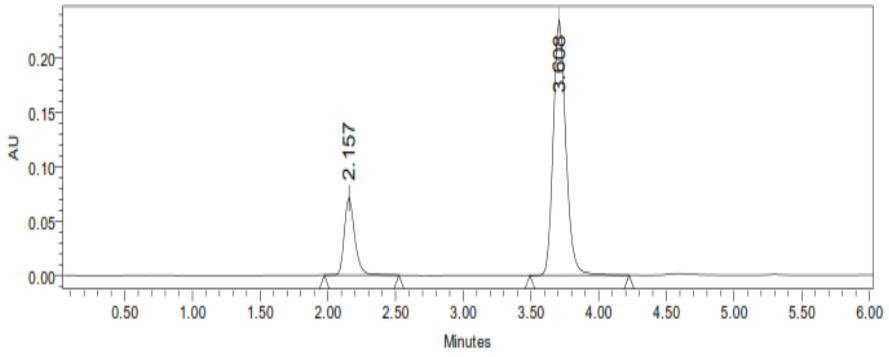


Fig: Chromatogram showing precision injection-4.

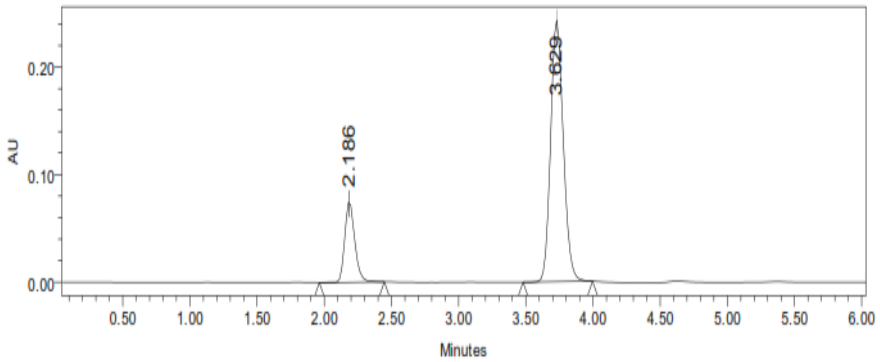


Fig: Chromatogram showing precision injection-5.

Table: Results of Repeatability for Trihexyphenidyl HCL.

S. No.	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Trihexyphenidyl HCL	2.157	58987	8659	6895	1.36
2	Trihexyphenidyl HCL	2.159	58963	8625	6829	1.35
3	Trihexyphenidyl HCL	2.186	58694	8629	6587	1.36
4	Trihexyphenidyl HCL	2.160	58489	8675	6582	1.36
5	Trihexyphenidyl HCL	2.170	58796	8629	6592	1.35
Mean			58785.8			
Std.dev			205.2455			
%RSD			0.349141			

Acceptance Criteria

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of Repeatability for Chlorpromazine HCL.

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Chlorpromazine HCL	3.603	102365	96895	8459	1.42
2	Chlorpromazine HCL	3.608	102659	96854	8463	1.43
3	Chlorpromazine HCL	3.600	102856	96254	8475	1.42
4	Chlorpromazine HCL	3.696	102845	96892	8492	1.43
5	Chlorpromazine HCL	3.629	102698	96847	8426	1.42
Mean			102684.6			
Std.dev			198.7996			
%RSD			0.193602			

Intermediate precision
Day 1

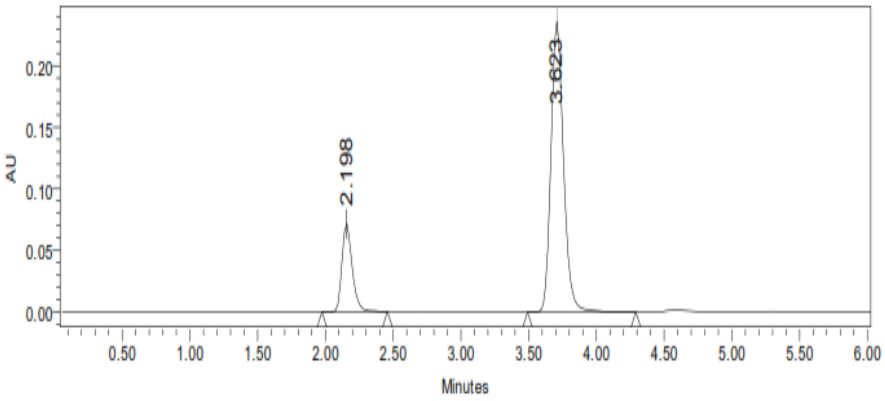


Fig: Chromatogram showing Day1 injection -1.

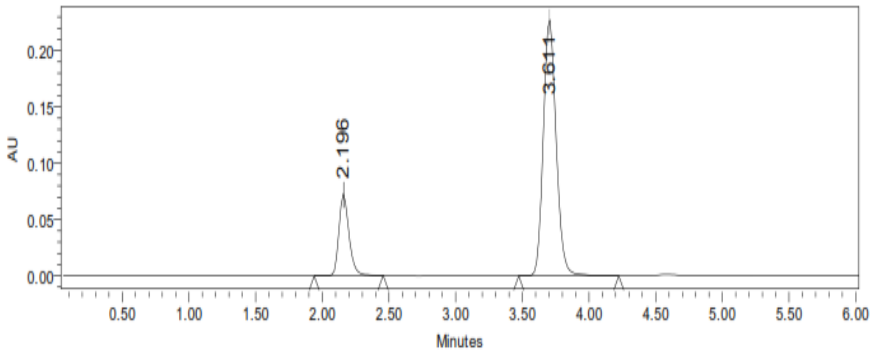


Fig: Chromatogram Showing Day1 Injection-2.

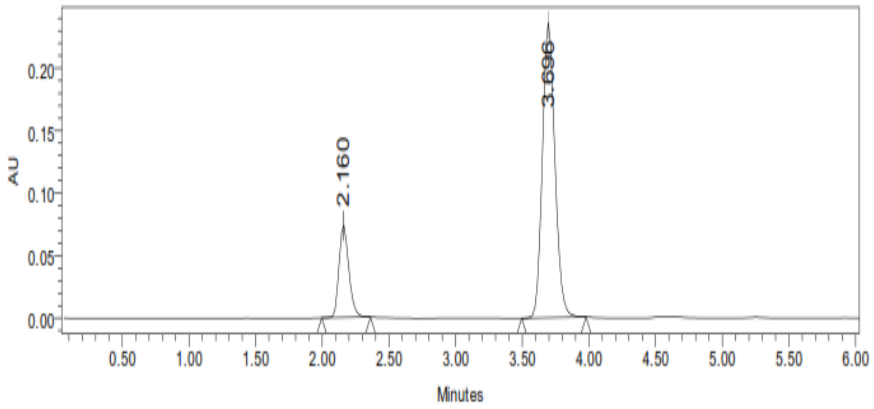


Fig-: Chromatogram showing Day1 injection -3.

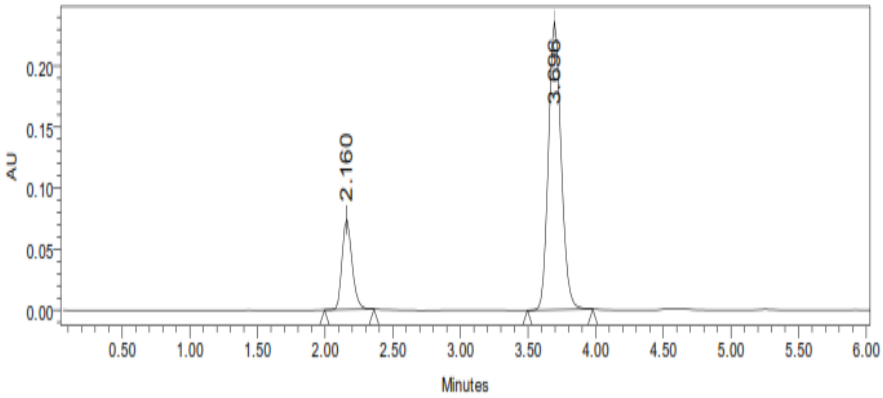


Fig: Chromatogram Showing Day1 injection-4.

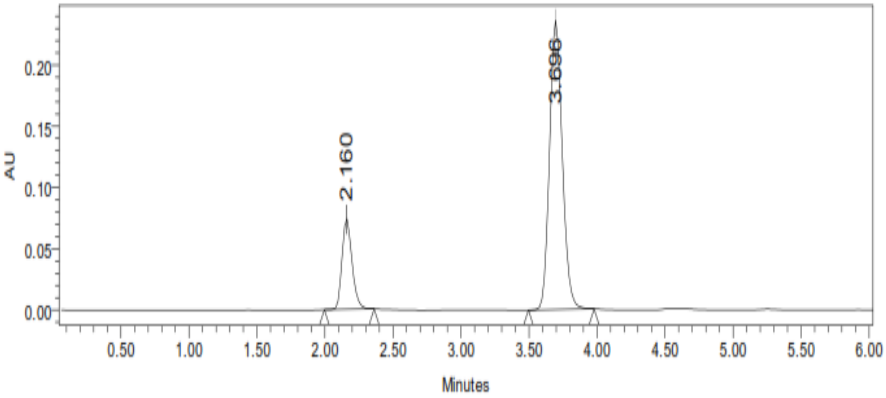


Fig: Chromatogram showing Day1 injection-5.

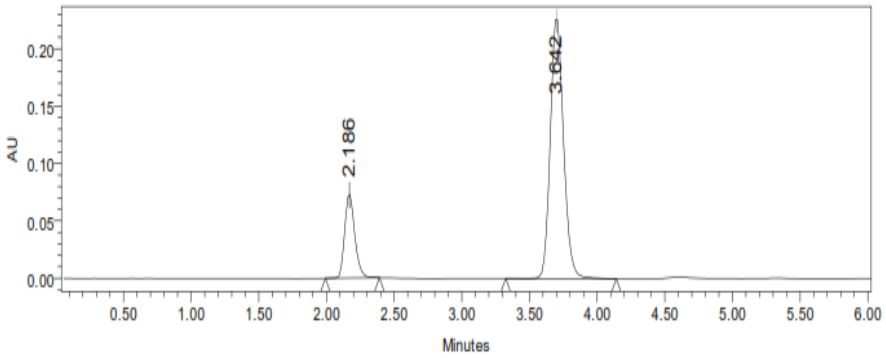


Fig: Chromatogram showing Day1 injection -6.

Table-: Results of Intermediate precision for Trihexyphenidyl HCL

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trihexyphenidyl HCL	2.198	57895	8586	6789	1.35
2	Trihexyphenidyl HCL	2.196	57498	8596	6759	1.36
3	Trihexyphenidyl HCL	2.160	57652	8547	6748	1.35
4	Trihexyphenidyl HCL	2.160	57254	8536	6785	1.36
5	Trihexyphenidyl HCL	2.160	57966	8554	6754	1.35
6	Trihexyphenidyl HCL	2.186	57854	8566	6759	1.35
Mean			57686.5			
Std. Dev.			273.2865			
% RSD			0.473744			

Acceptance criteria

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

Table: Results of Intermediate precision for Chlorpromazine HCL.

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Chlorpromazine HCL	3.623	101895	95869	8396	1.41	7.56
2	Chlorpromazine HCL	3.611	101986	95785	8346	1.42	7.56
3	Chlorpromazine HCL	3.696	101854	95842	8375	1.41	7.57
4	Chlorpromazine HCL	3.696	101698	95821	8354	1.42	7.56
5	Chlorpromazine HCL	3.696	101865	95641	8369	1.41	7.57
6	Chlorpromazine HCL	3.642	101989	95826	8374	1.42	7.57
Mean			101881.2				
Std. Dev.			107.1063				
% RSD			0.105129				

Acceptance criteria

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

Day 2

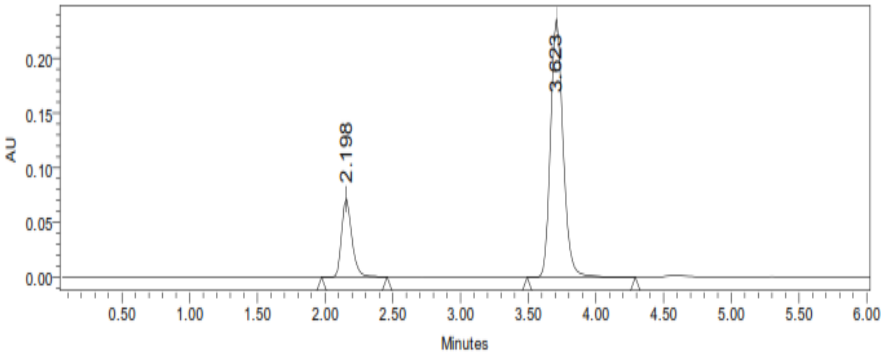


Fig: Chromatogram showing Day 2 injection -1.

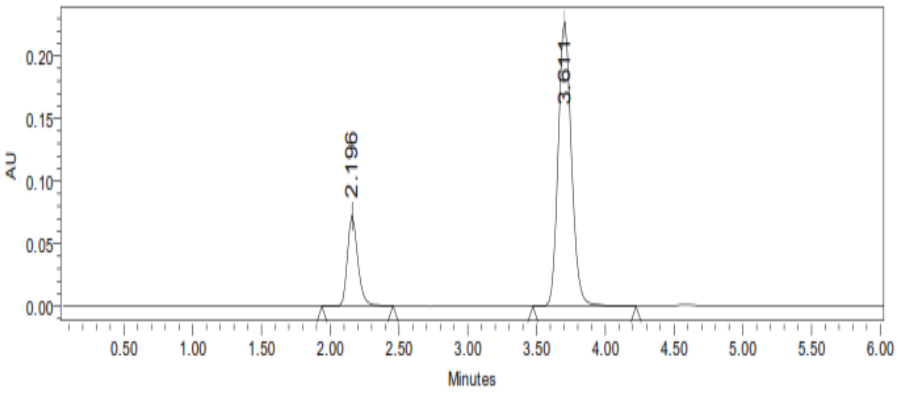


Fig-: Chromatogram showing Day 2 injection -2.

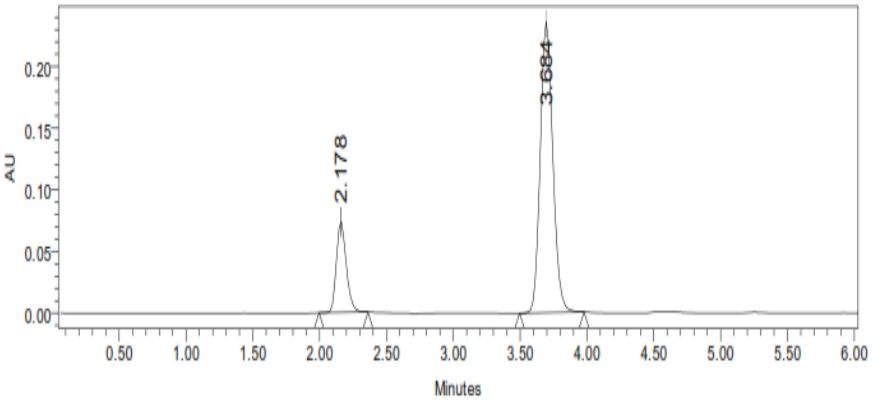


Fig-: Chromatogram showing Day 2 injection -3.

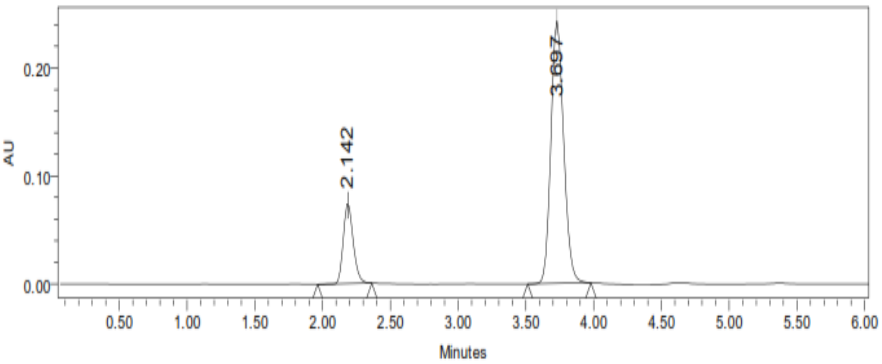


Fig-: Chromatogram showing Day 2 injection -4.

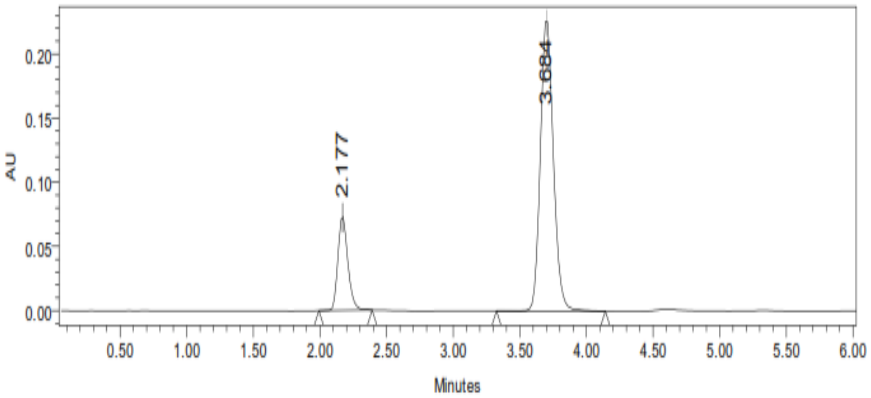


Fig: Chromatogram showing Day 2 injection -5.

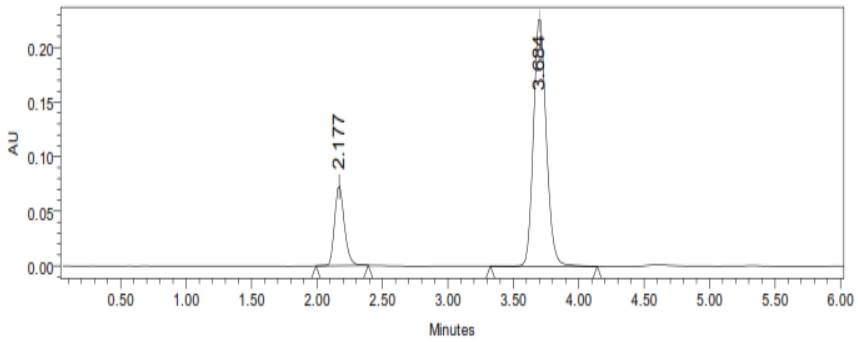


Fig: Chromatogram showing Day 2 injection 6.

Table: Results of Intermediate precision Day 2 for Trihexyphenidyl HCL.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Trihexyphenidyl HCL	2.198	59869	8759	6989	1.38
2	Trihexyphenidyl HCL	2.196	59668	8726	6952	1.39
3	Trihexyphenidyl HCL	2.178	59869	8795	6924	1.38
4	Trihexyphenidyl HCL	2.142	59898	8752	6935	1.39
5	Trihexyphenidyl HCL	2.177	59688	8796	6934	1.38
6	Trihexyphenidyl HCL	2.177	59789	8726	6975	1.39
Mean			59796.83			
Std. Dev.			99.15123			
% RSD			0.165814			

Acceptance criteria

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

Table: Results of Intermediate Precision Day 2 for Chlorpromazine HCL.

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Chlorpromazine HCL	3.611	103659	97859	8596	1.45	8.02
2	Chlorpromazine HCL	3.623	103854	97565	8574	1.44	8.01
3	Chlorpromazine HCL	3.684	103658	97645	8566	1.45	8.02
4	Chlorpromazine HCL	3.697	103587	97264	8534	1.45	8.01
5	Chlorpromazine HCL	3.684	103598	97862	8546	1.44	8.02
6	Chlorpromazine HCL	3.684	103524	97261	8519	1.45	8.01
Mean			103646.7				
Std. Dev.			113.352				
% RSD			0.109364				

Acceptance criteria

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

6.3.4: ACCURACY

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Accuracy 50%

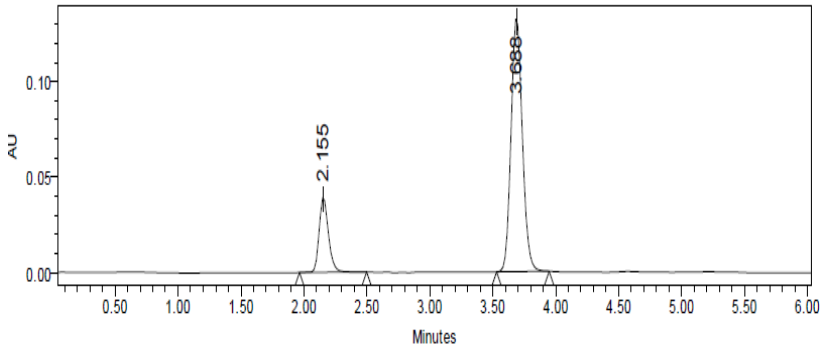


Fig: Chromatogram showing accuracy-50% injection-1.

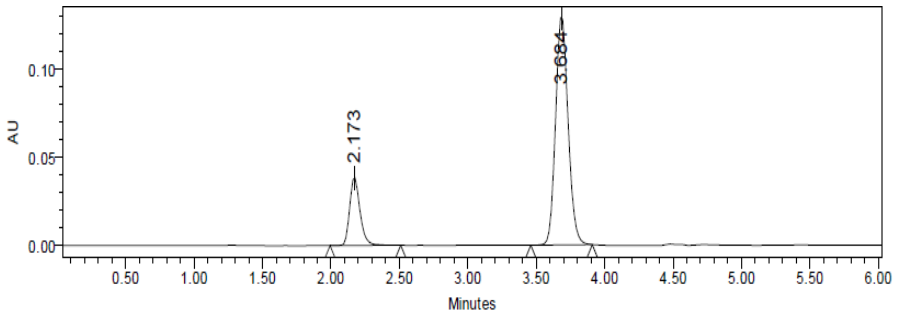


Fig-: Chromatogram showing accuracy-50% injection-2.

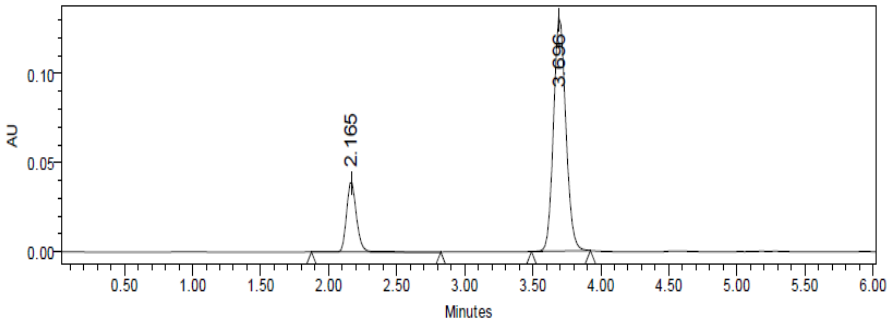


Fig-: Chromatogram showing accuracy-50% injection-3.

Table: Results of Accuracy for concentration-50%.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trihexyphenidyl HCL	2.165	30695	5698	1.06	5895	1
2	Chlorpromazine HCL	3.696	54289	6859	1.16	6547	1
3	Trihexyphenidyl HCL	2.155	30789	5648	1.07	5965	2
4	Chlorpromazine HCL	3.684	54189	6925	1.16	6659	2
5	Trihexyphenidyl HCL	2.173	30698	5635	1.07	5879	3
6	Chlorpromazine HCL	3.688	54234	6924	1.17	6598	3

Accuracy 100%

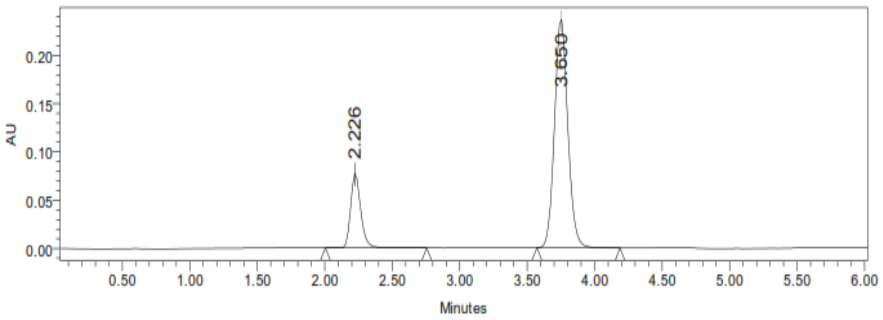


Fig: Chromatogram showing accuracy-100% injection-1.

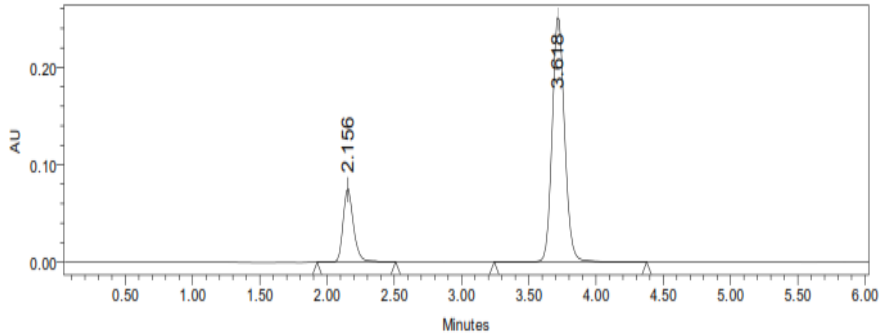


Fig: Chromatogram showing accuracy-100% injection-2.

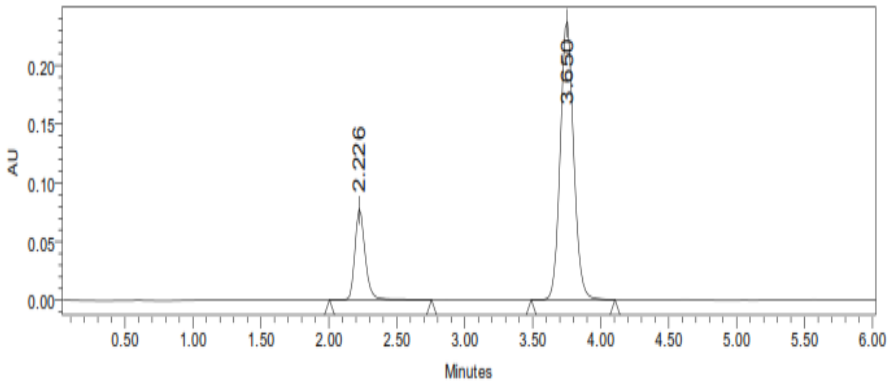


Fig: Chromatogram showing accuracy-100% injection-3.

Table: Results of Accuracy for concentration-100%

S.No.	Name	RT	Area	Heigh	USP Tailing	USP Plate Count	Injection
1	Trihexyphenidyl HCL	2.156	60986	8758	1.37	6985	1
2	Chlorpromazine HCL	3.618	107498	96598	1.43	8545	1
3	Trihexyphenidyl HCL	2.226	60998	8699	1.38	6899	2
4	Chlorpromazine HCL	3.650	107359	96678	1.44	8594	2
5	Trihexyphenidyl HCL	2.226	60854	8795	1.38	6935	3
6	Chlorpromazine HCL	3.650	107265	96854	1.44	8574	3

Accuracy 150%

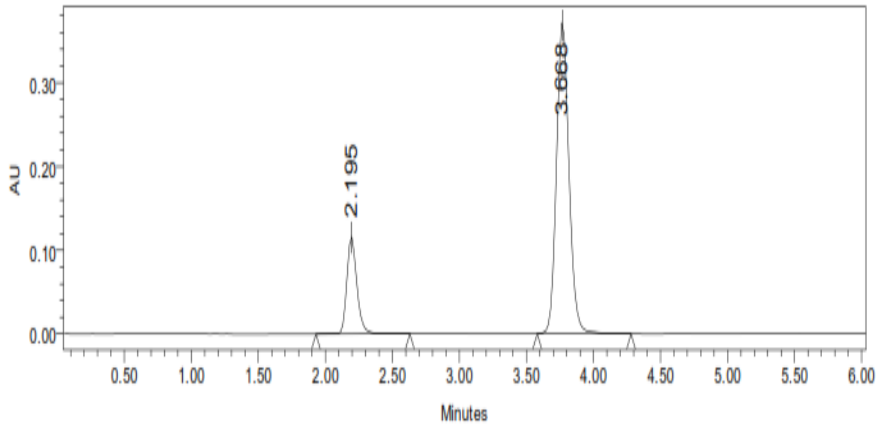


Fig: Chromatogram Showing Accuracy-150% Injection-1.

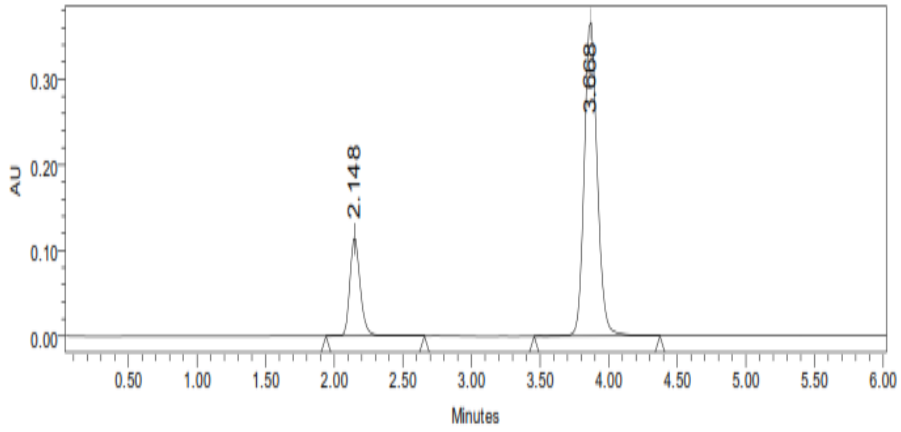


Fig: Chromatogram showing accuracy-150% injection-2.

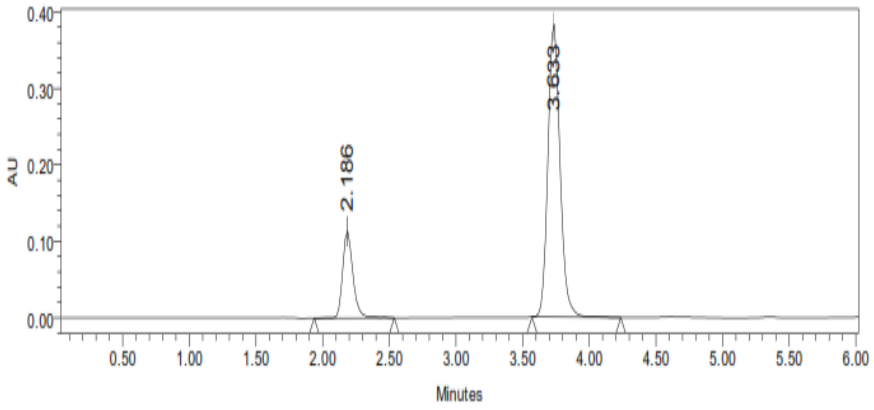


Fig: Chromatogram showing accuracy-150% injection-3.

Table: Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Trihexyphenidyl HCL	2.148	91258	9895	1.58	8598	1
2	Chlorpromazine HCL	3.668	160385	102568	1.69	9985	1
3	Trihexyphenidyl HCL	2.195	91165	9985	1.59	8641	2
4	Chlorpromazine HCL	3.633	160452	103698	1.70	9965	2
5	Trihexyphenidyl HCL	2.186	91285	9965	1.59	8597	3
6	Chlorpromazine HCL	3.668	160582	102856	1.69	9928	3

Table-: The accuracy results for Trihexyphenidyl HCL

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	30727.33	25	25.114	100.456%	100.23%
100%	60946	50	50.068	100.136%	
150%	91236	75	75.080	100.106%	

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).

Table: The accuracy results for Chlorpromazine HCL

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	54237.33	50	50.204	100.408%	100.20%
100%	107374	100	100.145	100.145%	
150%	160473	150	150.050	100.033%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION

The detection limit 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.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Trihexyphenidyl Hcl

Result:

= 1.3µg/ml

Chlorpromazine Hcl

Result:

= 1.9µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

TRIHEXYPHENIDYL HCL

Result: ==3.9µg/ml

CHLORPROMAZINE HCL

Result

=5.7µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Trihexyphenidyl HCL and Chlorpromazine HCL. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Trihexyphenidyl HCL and Chlorpromazine HCL were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count.

Variation in flow

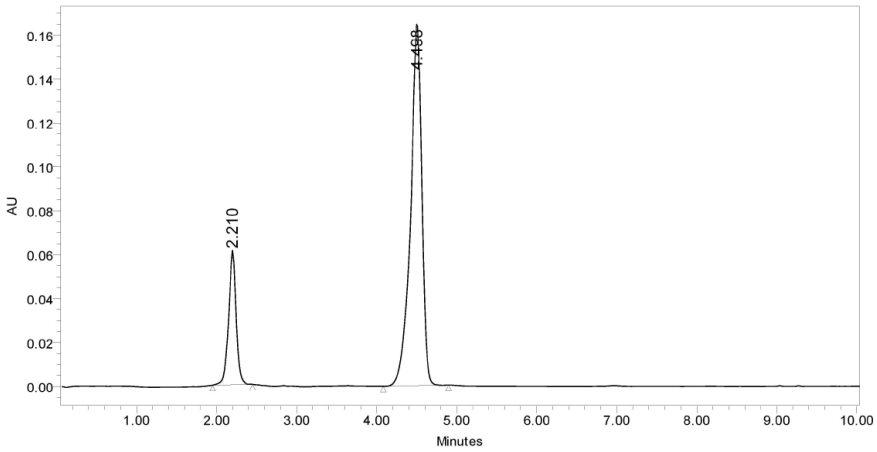


Figure: Chromatogram showing less flow of 0.9ml/min.

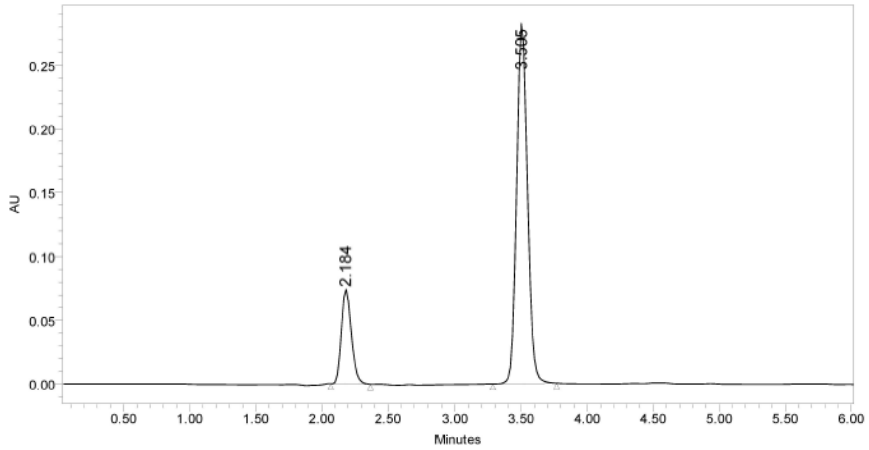


Figure: Chromatogram showing more flow of 1.1 ml/min.

Variation of mobile phase organic composition

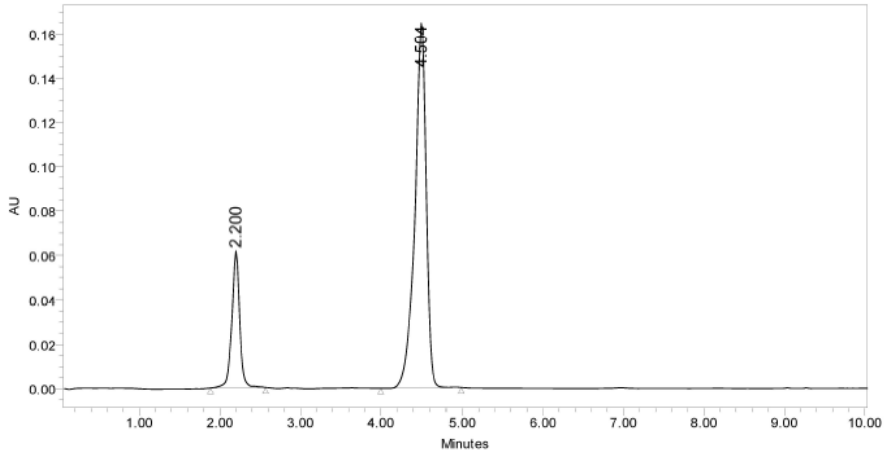


Figure-: Chromatogram showing less organic composition.

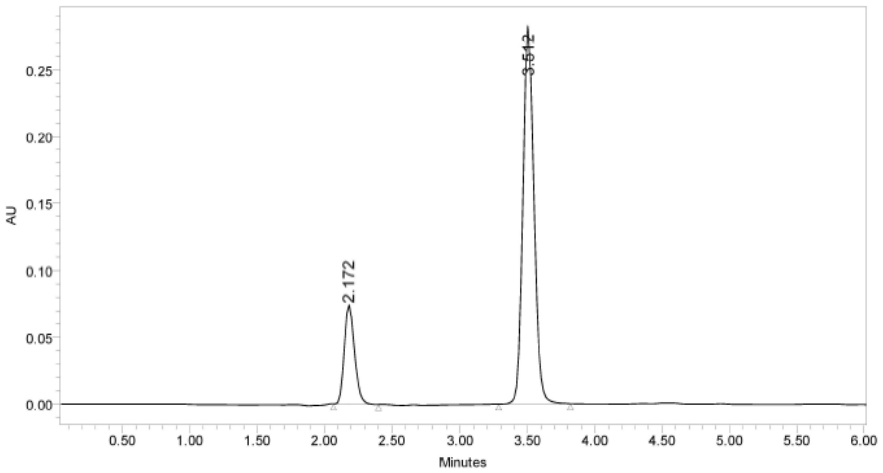


Figure: Chromatogram showing more organic composition.

Table-: Results for Robustness

TRIHEXYPHENIDYL HCL

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	58985	2.133	6859	1.36
Less Flow rate of 0.9 mL/min	63528	2.210	6256	1.32
More Flow rate of 1.1 mL/min	57859	2.184	6358	1.34
Less organic phase	56985	2.200	6793	1.35
More Organic phase	55898	2.172	6429	1.31

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CHLORPROMAZINE HCL

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	102569	3.692	8475	1.42
Less Flow rate of 0.9 mL/min	109658	4.498	8265	1.41
More Flow rate of 1.1 mL/min	101255	3.505	8149	1.40
Less organic phase	103568	4.504	8068	1.42
More organic phase	101548	3.512	8365	1.43

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY

Summary of validation data for Trihexyphenidyl HCL.

S.No.	Parameter	Observation	Acceptance criteria
1	System suitability Theoretical plates Tailing %RSD	 6859 1.36 0.9	 Not less than 2000 Not more than 2 Not more than 2.0%
2	Specificity %Assay	 99.75%	 98-102%
3	Method Precision (%RSD)	0.349	Not more than 2.0%
4	Linearity Slope Correlation coefficient(r^2)	30-70ppm 1211 0.999	 ≤0.99
5	Accuracy Mean % recovery	 100.23	 98 - 102%
6	Robustness Flow rate variation Organic phase variation	All the system suitability parameters are within the limits.	

Summary of validation data for Chlorpromazine HCL.

S.No.	Parameter	Observation	Acceptance criteria
1	**System suitability** Theoretical plates Tailing %RSD	 8475 1.42 0.1	 Not less than 2000 Not more than 2 Not more than 2.0%
2	**Specificity** %Assay	 99.75%	 98-102%
3	**Method Precision** (%RSD)	0.193	Not more than 2.0%
4	**Linearity** Slope Correlation coefficient(r^2)	60-140ppm 1064 0.999	 ≤0.99
5	**Accuracy** Mean % recovery	100.20	98 - 102%
6	**Robustness** Flow rate variation Organic phase variation	All the system suitability parameters are within the limits.	

CONCLUSION

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

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

Trihexyphenidyl hydrochloride is soluble in water. It dissolves in methanol at 50 mg/ml to yield a clear to hazy, colorless solution. It is very slightly soluble in ether and benzene. Chlorpromazine was found to be Soluble in 100% ethanol, methanol or water (50mg/ml), soluble in chloroform; practically insoluble in ether, benzene and soluble in DMSO, and dimethyl formamide. Acetonitrile: Phosphate Buffer (0.05M) (pH-3.6) (28:72% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

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

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

This method can be used for the routine determination of Trihexyphenidyl HCL and Chlorpromazine HCL in bulk drug and in Pharmaceutical dosage forms.

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