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 (4.6mmx250mm) 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.1,3

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.3

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”.

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Separated molecules of small molecules as amino acids, carbohydrates, and fatty acids. However, affinity chromatographies (i.e., 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, ether, 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 DNA, RNA, proteins, amino acids, and small molecules as amino acids, carbohydrates, and fatty acids. However, gel filtration chromatography (TLC) 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 achieve a satisfactory separation within a suitable time interval. 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-pressure 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 through 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 pressure 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. [2]

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 reservoir. 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 reservoir and forces it through the system's column and detector. 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.

- **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.

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) = \( \frac{(t_2 - t_a)}{(t_1 - t_a)} \)

Theoretical plates \( n = 16 \left( \frac{t}{W} \right)_2 \)

Capacity factor \( K' = \frac{t_2}{t_a} - 1 \)

Resolution \( R = 2 \left( \frac{t_2 - t_1}{W_2 + W_1} \right) \)

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.
f = 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
W2 = Width of the base of component peak 2.
W1 = 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 reliably as determined.[1-3] 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.[10-12] 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.[14,23] 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.[25] 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 variation.\textsuperscript{[15,19,31]} ICH prescribes the reporting of standard deviation, relative standard deviation (coefficient of variety), and confidence interim of the information.\textsuperscript{[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).\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[41]}

Every quantitative result ought to be of high accuracy - there ought to be close to a ±2% variety in the examine framework.\textsuperscript{[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.\textsuperscript{[53-55]} 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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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).\textsuperscript{[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.\textsuperscript{[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).\textsuperscript{[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.\textsuperscript{[19-25,38]}

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

**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.\textsuperscript{[59,60]} Linearity ought to be assessed by visual examination of a plot of signs as an element of analyte fixation or substance.\textsuperscript{[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.\textsuperscript{[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)](image)

**Limit of Detection**

These cutoff points are regularly connected to related substances in the medication substance or medication item.\textsuperscript{[62-65]} 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.\textsuperscript{[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,
\[\sigma = \text{the standard deviation of the response}\]
\[S = \text{the slope of the calibration curve}\]

The slope S is estimated from the calibration curve of the analyte.

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 rundown 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RECOMMENDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity Factor (k')</td>
<td>The peak should be well-resolved from other peaks and the void volume, generally $k' &gt; 2.0$</td>
</tr>
<tr>
<td>Repeatability</td>
<td>RSD ≤ 2% for $N \geq 5$ is desirable.</td>
</tr>
<tr>
<td>Relative retention</td>
<td>Not essential as long as the resolution is stated.</td>
</tr>
<tr>
<td>Resolution (R_s)</td>
<td>$R_s &gt; 2$ between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.)</td>
</tr>
<tr>
<td>Tailing Factor (T)</td>
<td>$T \leq 2$</td>
</tr>
<tr>
<td>Theoretical Plates (N)</td>
<td>$N &gt; 2000$</td>
</tr>
</tbody>
</table>

**DRUG PROFILE**

**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 Structure](image)

**Chemical name:** 1-cyclohexyl-1-phenyl-3-(piperidin-1-yl) propan-1-ol hydrochloride
**Molecular formula:** C_{20}H_{32}ClNO
**Molecular Weight:** 337.927 g/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.

**Melting point:** 223-226 °C
**pKa (Strongest basic log p):** 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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug name</th>
<th>Label Claim</th>
<th>Brand name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl</td>
<td>2mg</td>
<td>Artane</td>
<td>INTAS</td>
</tr>
</tbody>
</table>

DRUG PROFILE (28-30)

Drug: Chlorpromazine
Synonym: Chlorpromazine
Drug category: Anticholinergic Agents
Structure:

![Chemical Structure](image)

Chemical name/ Nomenclature / IUPAC Name: 3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethylpropan-1-amine
Molecular Formula: C_{17}H_{19}ClN_{3}S
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 chlorpromazine 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 postsynaptic receptors—and 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 extrapyramidal 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 (antisymptomimetic 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 restless leg syndrome; before surgery; for acute intermittent porphyria; for the treatment of severe behavioral problems in 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, phenoxythiazine 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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug name</th>
<th>Label Claim</th>
<th>Brand name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorpromazine</td>
<td>25mg</td>
<td>Thorazine</td>
<td>SANDOZ</td>
</tr>
</tbody>
</table>

Combined Drug Formulation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drug name</th>
<th>Label Claim</th>
<th>Brand name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorpromazine HCL/ Trihexyphenidyl</td>
<td>100mg/2mg</td>
<td>Talentil Plus Tab</td>
<td>Talent India</td>
</tr>
</tbody>
</table>

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 a 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, 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.

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.

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

CHEMICALS USED

Table: Chemicals used.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical</th>
<th>Brand names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL (Pure)</td>
<td>Sura labs</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL (Pure)</td>
<td>Sura labs</td>
</tr>
<tr>
<td>3</td>
<td>Water and Methanol for HPLC</td>
<td>LICHROSOLV (MERCK)</td>
</tr>
<tr>
<td>4</td>
<td>Acetonitrile for HPLC</td>
<td>Merck</td>
</tr>
</tbody>
</table>

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 C18 columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6x250mm) 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: 38°C
Column: Hypersil ODS C18 (4.6mmx250mm) 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 sonicator 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

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

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

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)
Pipe out 0.5ml 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)
Pipe 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)
Pipe 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)
Pipe 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)
Pipe 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

Table: Peak Results for Trail 1.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak Name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP Plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>3.118</td>
<td>8569852</td>
<td>98698</td>
<td>0.96</td>
<td>985</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL</td>
<td>8.077</td>
<td>58645</td>
<td>652</td>
<td>0.98</td>
<td>967</td>
</tr>
</tbody>
</table>

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

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

Figure: Chromatogram for Trail 2.
Table: Peak Results for Trail 2.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>1.383</td>
<td>12698544</td>
<td>65984</td>
<td>0.99</td>
<td>2658</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL</td>
<td>1.751</td>
<td>52652</td>
<td>4521</td>
<td>0.93</td>
<td>1235</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak name</th>
<th>R&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.497</td>
<td>854784</td>
<td>96587</td>
<td>1.06</td>
<td>1365</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL</td>
<td>5.382</td>
<td>75847</td>
<td>4526</td>
<td>0.92</td>
<td>1748</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak name</th>
<th>R_t</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>5.035</td>
<td>568955</td>
<td>4547</td>
<td>2.06</td>
<td>1526</td>
</tr>
</tbody>
</table>

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: 40°C
- 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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak name</th>
<th>R_t</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.610</td>
<td>185986</td>
<td>45265</td>
<td>2.01</td>
<td>3879</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL</td>
<td>3.248</td>
<td>46528</td>
<td>3658</td>
<td>1.14</td>
<td>2659</td>
</tr>
</tbody>
</table>
**Observation**
In this above trial it shows more tailing and less plate count in the chromatogram. More trails required to obtained proper peaks.

**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>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP Plate Count</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.133</td>
<td>58985</td>
<td>8654</td>
<td>1.36</td>
<td>6859</td>
<td>7.54</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL</td>
<td>3.692</td>
<td>102569</td>
<td>96578</td>
<td>1.42</td>
<td>8475</td>
<td></td>
</tr>
</tbody>
</table>

**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)**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Tail</th>
<th>USP Plate Count</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.166</td>
<td>59865</td>
<td>8759</td>
<td>1.38</td>
<td>6985</td>
<td>8.52</td>
</tr>
<tr>
<td>2</td>
<td>Chlorpromazine HCL</td>
<td>3.629</td>
<td>104568</td>
<td>97856</td>
<td>1.43</td>
<td>8598</td>
<td></td>
</tr>
</tbody>
</table>

**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.
Tabbasum et al. European Journal of Pharmaceutical and Medical Research

Fig: Chromatogram showing injection-3.

Fig: Chromatogram showing injection -4.

Fig: Chromatogram showing injection-5.

Table: Results of system suitability for Trihexyphenidyl HCL.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak Name</th>
<th>RT</th>
<th>Area (µV*sec)</th>
<th>Height (µV)</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.152</td>
<td>58986</td>
<td>8659</td>
<td>6859</td>
<td>1.36</td>
</tr>
<tr>
<td>2</td>
<td>Trihexyphenidyl HCL</td>
<td>2.157</td>
<td>58798</td>
<td>8645</td>
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<td>1.37</td>
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<tr>
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<td></td>
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</table>
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.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak Name</th>
<th>RT (µV*sec)</th>
<th>Area (µV)</th>
<th>Height (µV)</th>
<th>USP Plate Count</th>
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<td>7.62</td>
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</table>

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)

![Chromatogram showing assay of standard injection -1.](image)

Fig: Chromatogram showing assay of standard injection -1.

![Chromatogram showing assay of standard injection-2.](image)

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.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP Plate Count</th>
<th>Injection</th>
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Table: Peak Results for Assay Standard of Chlorpromazine HCL.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP Plate Count</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>3.646</td>
<td>102658</td>
<td>96859</td>
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</table>

Assay (Sample)

Fig: Chromatogram Showing Assay of Sample Injection-1.

Fig: Chromatogram Showing Assay of Sample Injection-2.
Table: Peak results for Assay sample of Trihexyphenidyl HCL.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
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<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP Plate Count</th>
<th>Injection</th>
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<tbody>
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</table>

Table: Peak results for Assay sample of Chlorpromazine HCL.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>USP Tailing</th>
<th>USP Plate Count</th>
<th>Injection</th>
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<tbody>
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<td>1</td>
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<td>97268</td>
<td>1.43</td>
<td>8569</td>
<td>3</td>
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</tbody>
</table>

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

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

**LINEARITY**
Fig: Chromatogram showing linearity level-2.

Fig: Chromatogram showing linearity level-3.

Fig: Chromatogram Showing Linearity Level-4.
Chromatographic Data for Linearity Study of Trihexyphenidyl HCL.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Average Peak Area</th>
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</thead>
<tbody>
<tr>
<td>30</td>
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<td>60</td>
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</tr>
<tr>
<td>70</td>
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</tr>
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</table>

**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.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Average Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>65784</td>
</tr>
<tr>
<td>80</td>
<td>86185</td>
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<tr>
<td>100</td>
<td>107569</td>
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<tr>
<td>120</td>
<td>128544</td>
</tr>
<tr>
<td>140</td>
<td>148985</td>
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</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak name</th>
<th>Retention time</th>
<th>Area (µV*sec)</th>
<th>Height (µV)</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.157</td>
<td>58987</td>
<td>8659</td>
<td>6895</td>
<td>1.36</td>
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<tr>
<td>2</td>
<td>Trihexyphenidyl HCL</td>
<td>2.159</td>
<td>58963</td>
<td>8625</td>
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<td>1.35</td>
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<td>3</td>
<td>Trihexyphenidyl HCL</td>
<td>2.186</td>
<td>58694</td>
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<td>4</td>
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<td>5</td>
<td>Trihexyphenidyl HCL</td>
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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.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak name</th>
<th>Retention time</th>
<th>Area (µV*sec)</th>
<th>Height (µV)</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
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<tbody>
<tr>
<td>1</td>
<td>Chlorpromazine HCL</td>
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</table>

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak Name</th>
<th>RT</th>
<th>Area (µV*sec)</th>
<th>Height (µV)</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
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</tbody>
</table>

Acceptance criteria

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

Table: Results of Intermediate precision for Chlorpromazine HCL

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak Name</th>
<th>Rt</th>
<th>Area (µV*sec)</th>
<th>Height (µV)</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
<th>Resolution</th>
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<td>Chlorpromazine HCL</td>
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<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td></td>
<td>0.105129</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Acceptance criteria

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

Day 2

Fig: Chromatogram showing Day2 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.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Peak Name</th>
<th>RT</th>
<th>Area (µV*sec)</th>
<th>Height (µV)</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trihexyphenidyl HCL</td>
<td>2.198</td>
<td>59869</td>
<td>8759</td>
<td>6989</td>
<td>1.38</td>
</tr>
<tr>
<td>2</td>
<td>Trihexyphenidyl HCL</td>
<td>2.196</td>
<td>59668</td>
<td>8726</td>
<td>6952</td>
<td>1.39</td>
</tr>
<tr>
<td>3</td>
<td>Trihexyphenidyl HCL</td>
<td>2.178</td>
<td>59869</td>
<td>8795</td>
<td>6924</td>
<td>1.38</td>
</tr>
<tr>
<td>4</td>
<td>Trihexyphenidyl HCL</td>
<td>2.142</td>
<td>59898</td>
<td>8752</td>
<td>6935</td>
<td>1.39</td>
</tr>
<tr>
<td>5</td>
<td>Trihexyphenidyl HCL</td>
<td>2.177</td>
<td>59688</td>
<td>8796</td>
<td>6934</td>
<td>1.38</td>
</tr>
<tr>
<td>6</td>
<td>Trihexyphenidyl HCL</td>
<td>2.177</td>
<td>59789</td>
<td>8726</td>
<td>6975</td>
<td>1.39</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>59796.83</td>
<td></td>
<td>99.15</td>
<td>0.165814</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td></td>
<td></td>
<td>99.15123</td>
<td></td>
<td>RSD</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td></td>
<td>0.165814</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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%.

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

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%

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

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%

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

Table: The accuracy results for Trihexyphenidyl HCL

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (ppm)</th>
<th>Amount Found (ppm)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>30727.33</td>
<td>25</td>
<td>25.114</td>
<td>100.456%</td>
<td>100.23%</td>
</tr>
<tr>
<td>100%</td>
<td>60946</td>
<td>50</td>
<td>50.068</td>
<td>100.136%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>91236</td>
<td>75</td>
<td>75.080</td>
<td>100.106%</td>
<td></td>
</tr>
</tbody>
</table>

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

Table: The accuracy results for Chlorpromazine HCL

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (ppm)</th>
<th>Amount Found (ppm)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>54237.33</td>
<td>50</td>
<td>50.204</td>
<td>100.408%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>107374</td>
<td>100</td>
<td>100.145</td>
<td>100.145%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>160473</td>
<td>150</td>
<td>150.050</td>
<td>100.033%</td>
<td></td>
</tr>
</tbody>
</table>

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.

\[
\text{LOD} = 3.3 \times \sigma / S
\]

Where
- \(\sigma\) = 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.

\[
\text{LOQ} = 10 \times \sigma / S
\]

Where
- \(\sigma\) = 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 1.1 ml/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

![Chromatogram showing less flow of 0.9ml/min.](image1)

![Chromatogram showing more flow of 1.1 ml/min.](image2)

Variation of mobile phase organic composition

![Chromatogram showing less organic composition.](image3)
Figure: Chromatogram showing more organic composition.

Table:- Results for Robustness

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

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

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

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.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Observation</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>System suitability</td>
<td></td>
<td>Not less than 2000</td>
</tr>
<tr>
<td></td>
<td>Theoretical plates</td>
<td>6859</td>
<td>Not more than 2</td>
</tr>
<tr>
<td></td>
<td>Tailing %RSD</td>
<td>1.36</td>
<td>Not more than 2.0%</td>
</tr>
<tr>
<td>2</td>
<td>Specificity %Assay</td>
<td>99.75%</td>
<td>98-102%</td>
</tr>
<tr>
<td>3</td>
<td>Method Precision (%RSD)</td>
<td>0.349</td>
<td>Not more than 2.0%</td>
</tr>
<tr>
<td>4</td>
<td>Linearity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>30-70ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient(r²)</td>
<td>1211</td>
<td>≤0.99</td>
</tr>
<tr>
<td></td>
<td>Mean % recovery</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Accuracy</td>
<td>100.23</td>
<td>98 - 102%</td>
</tr>
<tr>
<td>6</td>
<td>Robustness</td>
<td></td>
<td>All the system suitability parameters are within the limits.</td>
</tr>
<tr>
<td></td>
<td>Flow rate variation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic phase variation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary of validation data for Chlorpromazine HCL.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Observation</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>System suitability</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Theoretical plates</td>
<td>8475</td>
<td>Not less than 2000</td>
</tr>
<tr>
<td></td>
<td>Tailing %RSD</td>
<td>1.42</td>
<td>Not more than 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>Not more than 2.0%</td>
</tr>
<tr>
<td>2</td>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%Assay</td>
<td>99.75%</td>
<td>98-102%</td>
</tr>
<tr>
<td>3</td>
<td>Method Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(%RSD)</td>
<td>0.19</td>
<td>Not more than 2.0%</td>
</tr>
<tr>
<td>4</td>
<td>Linearity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>60-140ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient(r²)</td>
<td>1064</td>
<td>≤0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Accuracy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean % recovery</td>
<td>100.20</td>
<td>98 - 102%</td>
</tr>
<tr>
<td>6</td>
<td>Robustness</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flow rate variation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Organic phase variation</td>
<td></td>
<td></td>
</tr>
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<td>All the system suitability parameters are within the limits.</td>
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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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