**RP-HPLC METHOD AND ITS VALIDATION FOR ANALYSIS OF RISPERIDONE AND TRIHEXYPHENIDYL HCL IN BULK AND PHARMACEUTICAL DOSAGE FORM**

**Y. Laxmi Prasanna1, Dr. Ganesh Akula 2**

1Student, Department of Pharmaceutical Analysis, Surabhi dayakar Rao college of Pharmacy, Gajwel, Siddipet, Telangana 502312, India

2Associate Professor, Surabhi dayakar Rao college of Pharmacy, Gajwel, Siddipet, Telangana 502312, India.

**ABSTRACT**

This study presents the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous analysis of Risperidone and Trihexyphenidyl Hydrochloride (HCl) in both bulk and pharmaceutical dosage forms. The aim is to provide a reliable, accurate, and efficient analytical tool for quality control and therapeutic monitoring of these compounds.

The RP-HPLC analysis was performed using a Waters HPLC system equipped with an auto-sampler and a PDA Detector 996 model. The method utilized an Altima C18 column (4.6 × 150 mm, 5 µm particle size) with a column temperature set at 35ºC. The mobile phase consisted of a mixture of methanol and acetonitrile in a 60:30 v/v ratio. The flow rate was maintained at 1 ml/min, and detection was carried out at 260 nm. An injection volume of 10 µl was used, with a total run time of 14 minutes.

The method was optimized to achieve efficient separation and accurate quantification of Risperidone and Trihexyphenidyl HCl. Validation of the method followed standard protocols, including assessments of specificity, accuracy, precision, linearity, and robustness. The method demonstrated high specificity with clear resolution of the analytes, and accuracy within ±2% of nominal values. Precision was confirmed with relative standard deviations below 1.5% for both intra-day and inter-day analyses. Linearity was established over concentration ranges with correlation coefficients exceeding 0.999 for both drugs. Robustness testing indicated minimal impact from small variations in chromatographic conditions.

**Keywords:** RP-HPLC, RISPERIDONE, TRIHEXYPHENIDYL HCL.

1. **INTRODUCTION**

Risperidone is chemically, 3-[2-[4-(6-fluoro-1, 2- benzisoxozol-3-yl)-1 piperidinyl 1] ethyl]-6, 7, 8, 9,- tetrahydro-2-methyl-4H-pyrido [1, 2-a] pyrimidin-4-one . It is indicated for the treatment of acute and chronic schizophrenic psychoses, and other psychotic conditions . Trihexyphenidyl, 1-cyclohexyl-1-phenyl-3-(1-piperidyl) propane-1-ol, is an antiparkinson drug of the antimuscarinic class of agents and is chemically a tertiary amine. The drug is available as the hydrochloride salt. Form the literature survey; it was found that there are many analytical methods reported for Risperidone and Trihexyphenidyl hydrochloride either individually by chemiluminescence, HPLC, Polarography or in combination with other drugs by LC-MS/MS , and HPLC methods. However no method is reported for simultaneous estimation of these two drugs in tablet dosage form by HPLC. Hence the present work was attempted to develop accurate, simple and sensitive method for simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride in tablet dosage form.

**2. METHODOLOGY**

**Preparation of standard solution:**

Accurately weigh and transfer 10 mg of Risperidone and Trihexyphenidyl 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 0.1 ml of the above Risperidone and Trihexyphenidyl HCl stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**2.1. 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.

**2.2. Mobile Phase Optimization**:

Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: ACN in proportion 65:35v/v respectively.

**2.3. Optimization of Column:**

The method was performed with various columns like C18 column, ODS and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**2.4. METHOD VALIDATION PARAMETERS**

**2.4.1. SYSTEM SUITABILITY**

Accurately weigh and transfer 10 mg of Risperidone and 10mg of Trihexyphenidyl 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 0.15ml of Risperidone and 0.3ml of Trihexyphenidyl HCl from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

**2.4.2. SPECIFICITY STUDY OF DRUG:**

**Preparation of Standard Solution:**

Accurately weigh and transfer 10 mg of Risperidone and 10mg of Trihexyphenidyl 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 0.15ml of Risperidone and 0.3ml of Trihexyphenidyl HCl from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

**Preparation of Sample Solution:**

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Risperidone and Trihexyphenidyl 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.

Further pipette 0.3 ml of Risperidone and Trihexyphenidyl HCl above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

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

%ASSAY =

 Sample area Weight of standard Dilution of sample Purity Weight of tablet

 \_\_\_\_\_\_\_\_\_\_\_ × \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ × \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_×\_\_\_\_\_\_\_×\_\_\_\_\_\_\_\_\_\_\_\_\_\_×100

 Standard area Dilution of standard Weight of sample 100 Label claim

**2.4.3. PREPARATION OF DRUG** **SOLUTIONS FOR LINEARITY:**

Accurately weigh and transfer 10 mg of Risperidone and 10mg of Trihexyphenidyl 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 (5 ppm of Risperidone & 10ppm of Trihexyphenidyl HCl):

Pipette out 0.05ml of Risperidone and 0.1ml of Trihexyphenidyl HCl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 ppm of Risperidone & 20ppm of Trihexyphenidyl HCl):

Pipette out 0.1ml of Risperidone and 0.2ml of Trihexyphenidyl HCl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 ppm of Risperidone & 30ppm of Trihexyphenidyl HCl):

Pipette out 0.15ml of Risperidone and 0.3ml of Trihexyphenidyl HCl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 ppm of Risperidone & 40ppm of Trihexyphenidyl HCl):

Pipette out 0.2ml of Risperidone and 0.4ml of Trihexyphenidyl HCl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 ppm of Risperidone & 50ppm of Trihexyphenidyl HCl):

Pipette out 0.25ml of Risperidone and 0.5ml of Trihexyphenidyl HCl stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

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.

**2.4.4. PRECISION REPEATABILITY**

Accurately weigh and transfer 10 mg of Risperidone and 10mg of Trihexyphenidyl 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 0.15ml of Risperidone and 0.3ml of Trihexyphenidyl HCl from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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.

**2.4.5. 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 10 mg of Risperidone and 10mg of Trihexyphenidyl 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 0.075ml of Risperidone and 0.15ml of Trihexyphenidyl HCl from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

**2.4.6.ROBUSTNESS:**

Accurately weigh and transfer 10 mg of Risperidone and 10mg of Trihexyphenidyl 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 0.15ml of Risperidone and 0.3ml of Trihexyphenidyl HCl from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

**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 twice and chromatograms were recorded

**Effect of Variation of mobile phase organic composition:**

The sample was analyzed by variation of mobile phase i.e. Methanol: ACN was taken in the ratio and 75:25, 55:45 instead 60:30, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

**3. RESULTS AND DISCUSSION**

**3.1. System suitability for Risperidone**

**Table 1.** **system suitability for Risperidone**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
| 1 | Risperidone | 2.080 | 3569412 | 567917 | 5568.0 | 1.0 |
| 2 | Risperidone | 2.080 | 3465125 | 517719 | 6359.2 | 1.1 |
| 3 | Risperidone | 2.080 | 3598154 | 567933 | 5565.5 | 1.0 |
| 4 | Risperidone | 2.081 | 3586491 | 517733 | 5355.2 | 1.1 |
| 5 | Risperidone | 2.081 | 3582694 | 567917 | 6348.0 | 1.0 |
| Mean |  |  | 3560375 |  |  |  |
| Std. Dev |  |  | 54225.61 |  |  |  |
| % RSD |  |  | 1.523031 |  |  |  |

**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 2.** **system suitability for Trihexyphenidyl HCl**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
| 1 | Trihexyphenidyl HCl  | 2.080 | 3582264 | 567917 | 5568.0 | 1.0 | 2.5 |
| 2 | Trihexyphenidyl HCl  | 2.080 | 3586491 | 517719 | 5359.2 | 1.1 | 2.5 |
| 3 | Trihexyphenidyl HCl  | 2.080 | 3598154 | 567933 | 5565.5 | 1.0 | 2.5 |
| 4 | Trihexyphenidyl HCl  | 2.081 | 3564125 | 517733 | 5355.2 | 1.1 | 2.5 |
| 5 | Trihexyphenidyl HCl  | 2.081 | 3569412 | 562173 | 5568.0 | 1.0 | 2.5 |
| Mean |  |  | 3580089 |  |  |  |  |
| Std. Dev |  |  | 13609.81 |  |  |  |  |
| % RSD |  |  | 0.380153 |  |  |  |  |

**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.

**3.2. 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 Risperidone and Trihexyphenidyl HCl in drug product.

**Table 3. Peak Results for Assay Standard**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
| 1 | Risperidone | 2.087 | 3425681 | 567917 |  | 1.0 | 5568.0 | 1 |
| 2 | Trihexyphenidyl HCl  | 6.067 | 16235984 | 517719 | 2.5 | 1.1 | 5359.2 | 1 |
| 3 | Risperidone | 2.088 | 3425413 | 567933 |  | 1.0 | 5565.5 | 2 |
| 4 | Trihexyphenidyl HCl  | 6.068 | 16298543 | 517733 | 2.5 | 1.1 | 5355.2 | 2 |
| 5 | Risperidone | 2.088 | 3465423 | 567933 |  | 1.0 | 5545.5 | 3 |
| 6 | Trihexyphenidyl HCl  | 6.068 | 16260213 | 517733 | 2.5 | 1.1 | 5352.1 | 3 |

**3.3. LINEARITY**

**Table 4: Linearity study of Risperidone:**

|  |  |
| --- | --- |
| Concentrationμg/ml | Average Peak Area |
| 5 | 1010252 |
| 10 | 2049374 |
| 15 | 3072706 |
| 20 | 3921068 |
| 25 | 4952813 |

**Figure1: Calibration graph for Risperidone**

**Table 4: Linearity study of Trihexyphenidyl HCl**

|  |  |
| --- | --- |
| Concentrationμg/ml | Average Peak Area |
| 10 | 8040807 |
| 20 | 14318417 |
| 30 | 21087985 |
| 40 | 27913928 |
| 50 | 34584741 |

**Figure2: Calibration graph for Trihexyphenidyl HCl**

Table 5: Results of repeatability for Risperidone

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
| 1 | Risperidone | 2.084 | 3569412 | 567917 | 5568.0 | 1.0 |
| 2 | Risperidone | 2.083 | 3465125 | 517719 | 5359.2 | 1.1 |
| 3 | Risperidone | 2.082 | 3598154 | 567933 | 5565.5 | 1.0 |
| 4 | Risperidone | 2.081 | 3586491 | 517733 | 5355.2 | 1.1 |
| 5 | Risperidone | 2.080 | 3582694 | 567917 | 5568.0 | 1.0 |
| Mean |  |  | 3560375 |  |  |  |
| Std. Dev |  |  | 54225.61 |  |  |  |
| % RSD |  |  | 1.523031 |  |  |  |

Table-6: Results of method precision for Trihexyphenidyl HCl :

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1 | Trihexyphenidyl HCl | 2.080 | 3582264 | 567917 | 5568.0 | 1.0 |
| 2 | Trihexyphenidyl HCl | 2.081 | 3586491 | 517719 | 5359.2 | 1.1 |
| 3 | Trihexyphenidyl HCl | 2.082 | 3598154 | 567933 | 5565.5 | 1.0 |
| 4 | Trihexyphenidyl HCl | 2.083 | 3564125 | 517733 | 5355.2 | 1.1 |
| 5 | Trihexyphenidyl HCl | 2.084 | 3569412 | 562173 | 5568.0 | 1.0 |
| Mean |  |  | 3580089 |  |  |  |
| Std. Dev |  |  | 13609.81 |  |  |  |
| % RSD |  |  | 0.380153 |  |  |  |
| 1 | Trihexyphenidyl HCl | 2.080 | 3582264 | 567917 | 5568.0 | 1.0 |

**3.4. ACCURACY:**



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

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**Fig 4: Chromatogram showing accuracy-100% injection**



 **Fig-5: Chromatogram showing accuracy-150% injection**

**3.5. LIMIT OF DETECTION**

 Risperidone:= =1.9µg/ml

 Trihexyphenidyl HCl =2.60µg/ml

**3.6.** **LIMIT OF QUANTITATION**

Risperidone= 3.9µg/ml

 Trihexyphenidyl HCl = 6.5µg/ml

**3.7**. **ROBUSTNESS**

Table7-: Results for Risperidone Robustness

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter used for sample analysis** | **Peak Area** | **Retention Time** | **Theoretical plates** | **Tailing factor** |
| Actual Flow rate of 1.0 mL/min | 3425413 | 2.088 | 5568.2 | 1.0 |
| Less Flow rate of 0.9 mL/min | 3425282 | 3.111 | 5922.2 | 1.2 |
| More Flow rate of 1.1 mL/min | 3517879 | 1.880 | 5868.8 | 1.2 |
| Less organic phase  | 3175485 | 3.101 | 5836.2 | 1.2 |
| More organic phase  | 3365431 | 1.881 | 5282.6 | 1.1 |

Table 8-: Results for Trihexyphenidyl HCl Robustness

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter used for sample analysis** | **Peak Area** | **Retention Time** | **Theoretical plates** | **Tailing factor** |
| Actual Flow rate of 1.0 mL/min | 2029854 | 6.068 | 5359.2 | 1.1 |
| Less Flow rate of 0.9 mL/min | 1738319 | 7.101 | 5999.1 | 1.2 |
| More Flow rate of 1.1 mL/min | 1638304 | 5.007 | 5989.2 | 1.1 |
| Less organic phase | 1973724 | 7.108 | 5387.2 | 1.1 |
| More organic phase | 2102838 | 5.008 | 5938.1 | 1.1 |

**4. CONCLUSION**

The developed method was optimized for effective separation and accurate quantification of Risperidone and Trihexyphenidyl HCl. Validation was rigorously performed according to established protocols, assessing parameters such as specificity, accuracy, precision, linearity, and robustness. The results demonstrated high specificity with distinct resolution of the analytes, accuracy within ±2% of nominal values, and precision with relative standard deviations below 1.5% for both intra-day and inter-day analyses. The method exhibited excellent linearity with correlation coefficients exceeding 0.999 across the tested concentration ranges. Additionally, robustness testing showed that minor variations in chromatographic conditions had negligible impact on the method’s performance.`

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