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

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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.

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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:

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

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)

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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			

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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:

Concentration µg/ml	Average Peak Area
5	1010252
10	2049374
15	3072706
20	3921068
25	4952813

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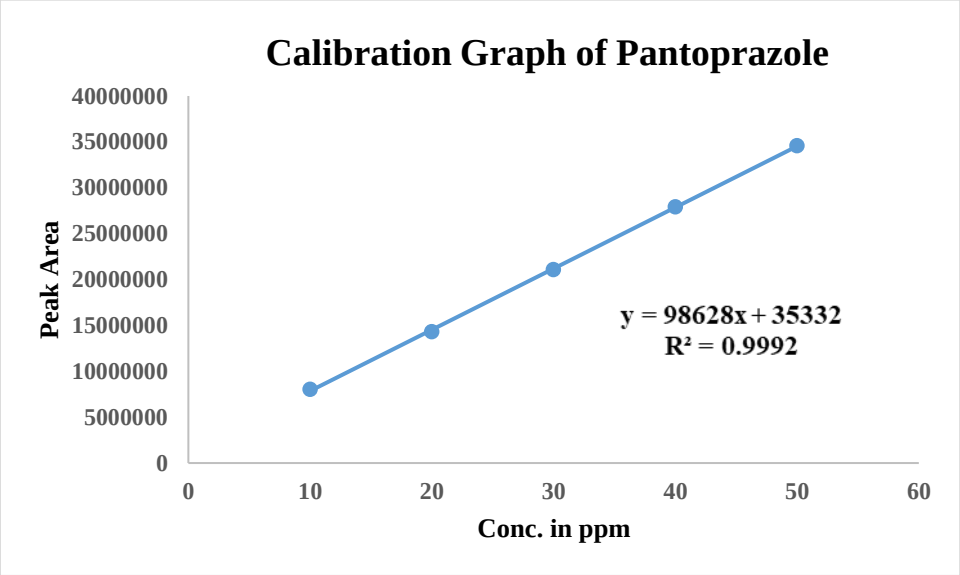

Figure1: Calibration graph for Risperidone

Table 4: Linearity study of Trihexyphenidyl HCl

Concentration µg/ml	Average Peak Area
10	8040807
20	14318417
30	21087985
40	27913928
50	34584741

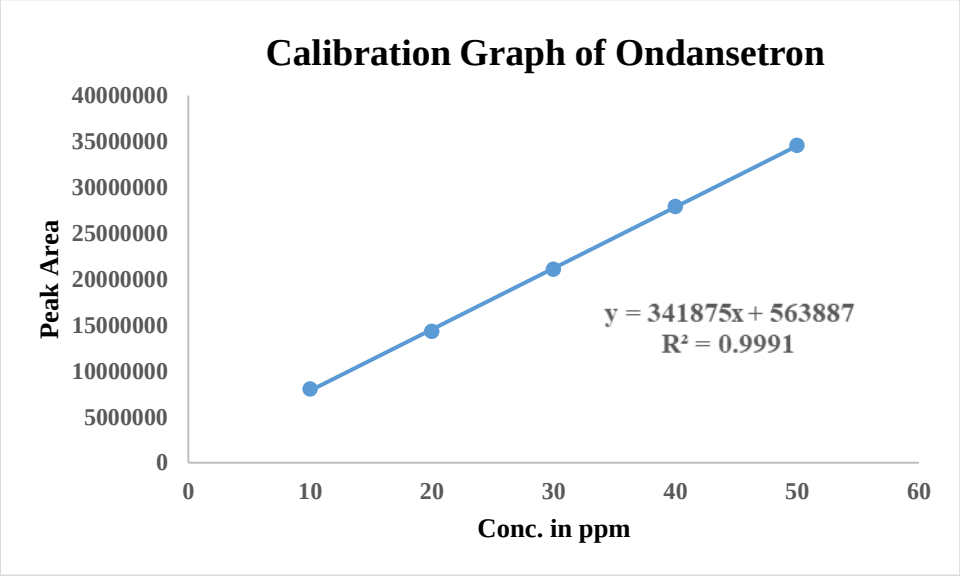

Figure2: Calibration graph for Trihexyphenidyl HCl

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

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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:

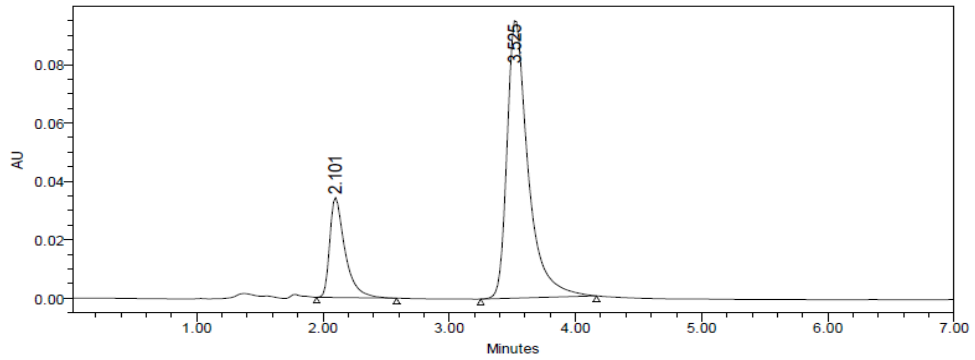

Fig-3: Chromatogram showing accuracy-50% injection

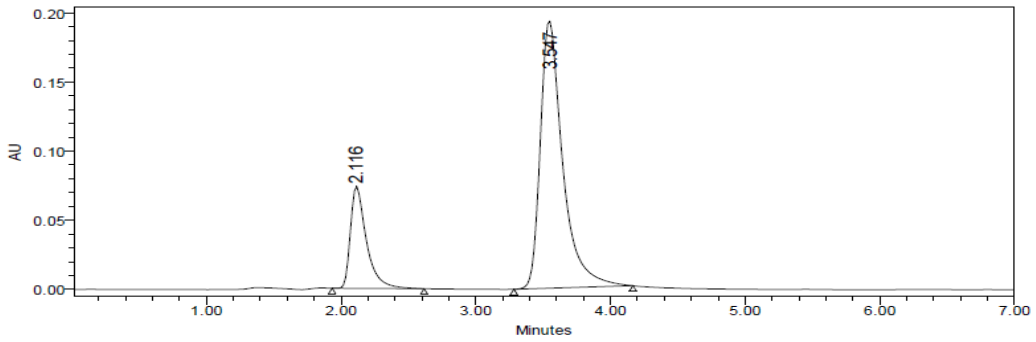

Fig 4: Chromatogram showing accuracy-100% injection

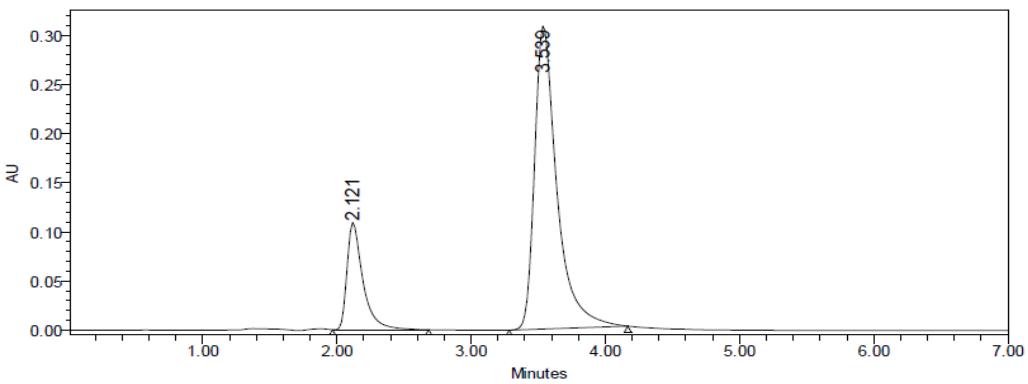

Fig-5: Chromatogram showing accuracy-150% injection

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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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