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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF RISPERIDONE AND TRIHEXYPHENIDYL HYDROCHLORIDE BY USING RP-HPLC METHOD

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ABSTRACT

A new method was established for simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Risperidone and Trihexyphenidyl hydrochloride by using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. HPLC instrument is Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Risperidone and Trihexyphenidyl hydrochloride was found to be 101.04% and 99.24% respectively. The system suitability parameters for Risperidone and Trihexyphenidyl hydrochloride such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Risperidone and Trihexyphenidyl hydrochloride was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively. Hence the suggested RP-HPLC.

Key Words:Thermosil C18 column, Risperidone and Trihexyphenidyl hydrochloride, RP-HPLC

INTRODUCTION

Chromatography was originally developed by the Russian botanist Michael Tswett in 1903 for the separation of colored plant pigments by percolating a petroleum ether extract through a glass column packed with powdered calcium carbonate. It is now, in general, the most widely used separation technique in analytical chemistry having developed into a number of related but quite different forms that enable the components of complex mixtures of organic or inorganic components to be separated and quantified. A chromatographic separation involves the placing of a sample onto a liquid or solid stationary phase and passing a liquid or gaseous mobile phase through or over it, a process known as elution. Sample components, or solutes, whose distribution ratios between the two phases differ will migrate (be eluted) at different rates, and this differential rate of migration will lead to their separation over a period of time and distance.

In the modern pharmaceutical industry, HPLC is a major analytical tool applied at all stages of drug discovery, development and production. Fast and effective development of rugged analytical HPLC methods is more efficiently undertaken with a thorough understanding of HPLC principles, theory and instrumentation.

Liquid Chromatography (LC), which is one of the forms of Chromatography, is an analytical technique that is used to separate a mixture in solution into its individual components. The separation relies on the use of two different "phases" or "immiscible layers," one of which is held stationary while the other moves over it. Liquid

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Chromatography is the generic name used to describe any chromatographic procedure in which the mobile phase is a liquid. The separation occurs because, under an optimum set of conditions, each component in a mixture will interact with the two phases differently relative to the other components in the mixture. HPLC is the term used to describe Liquid Chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. An HPLC instrument, therefore, consists of an injector, a pump, a column, and a detector.

HPLC Method Design and Development

Set the analytical objective first that may be quantification or qualitative identification or separation of two components / multicomponent mixtures or optimization of analysis time before starting HPLC. Method for analyzing drugs by HPLC demands primary knowledge about the nature of the sample, structure, polarity, volatility, stability and the solubility parameter. An exact recipe for HPLC cannot be provided because method development involves considerable trial and error procedures. The most difficult problem usually is where to start, with what kind of mobile phase.

Analytes are detected using absorbance mode. But if the analytes are not detected perfectly than it need change of column or mobile phase or need the help of pre or post chromatographic derivatization.

Optimization can be started only after a reasonable chromatogram which can be done by slight change in mobile phase composition. This leads to a reasonable chromatogram which has all the desired peaks in symmetry and well separated.

Risperidone is a second-generation antipsychotic (SGA) medication used in the treatment of a number of mood and mental health conditions including schizophrenia and bipolar disorder. It is one of the most widely used SGAs. Paliperidone, another commonly used SGA, is the primary active metabolite of risperidone (i.e. 9-hydroxyrisperidone). The primary action of risperidone is to decrease dopaminergic and serotonergic pathway activity in the brain, therefore decreasing symptoms of schizophrenia and mood disorders. Risperidone has a high binding affinity for serotonergic 5-HT2A receptors when compared to dopaminergic D2 receptors in the brain.2,3Risperidone binds to D2 receptors with a lower affinity than first-generation antipsychotic drugs, which bind with very high affinity. A reduction in extrapyramidal symptoms with risperidone, when compared to its predecessors, is likely a result of its moderate affinity for dopaminergic D2 receptors.

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

MATERIAL AND METHODS

Chemicals and standards used

Table1: List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	Sodium acetate Buffer(CH3COONa)	Merck	G.R
8.	0. 22µ Nylon filter	Advanced lab	HPLC grade
9.	0.45µ filter paper	Millipore	HPLC grade
10.	Risperidone and Trihexyphenidyl hydrochloride	In - House	In- House

Instruments used

Table2: List of instruments used

S.No	Instrument name	Model number	Soft ware	ManufacturersName
1	HPLC instrument is Shimadzu	SPD-20MA LC+20AD,	LC-20 Solution	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance(sensitivity 5mg)	ER 200A	-	Ascoset
4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Method development for the simultaneous estimation of RisperidoneandTrihexyphenidyl hydrochloride by using RP-HPLC

1. Selection of mobile phase
2. Selection of detection wavelength
3. Selection of column
4. Selection of solvent delivery system
5. Selection of flow rate
6. Selection of column temperature
7. Selection of diluent
8. Selection of test concentration and injection volume

Preparation of phosphate buffer

6.8 grams of sodium acetete was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 50 ml (50%) and 50 ml of Methanol (HPLC grade-50%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual Trihexyphenidylhydrochloride standard preparation

10 mg ofTrihexyphenidylhydrochloride working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Risperidone standard preparation

10 mg of Risperidone working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to

Dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Assay

Assay preparation of the Risperidone and Trihexyphenidyl hydrochloride standard and sample solution

Sample solution preparation:

1mg of Risperidone and 10 mg Trihexyphenidyl hydrochloride tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

1mg Risperidone and 10 mg Trihexyphenidylhydrochloride in working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

10µL of the blank, standard and sample were inje cted into the chromatographic system and areas for the Risperidone andTrihexyphenidyl hydrochloride the peaks were used for calculating the % assay by using the formulae.

System suitability

- Tailing factor for the peaks due to Risperidone and Trihexyphenidyl hydrochloride in standard solution should not be more than 1.5.
- Theoretical plates for the Risperidone and Trihexyphenidyl hydrochloride peaks in standard solution should not be less than 2000.

Analytical Method Validation

Validation parameters

Specificity

- Linearity
- Range
- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Detection Limit
- Quantitation Limit
- Robustness

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity

Preparation of stock solution

1 mg of Risperidone and 10 mg of Trihexyphenidyl hydrochloride working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (5ppm of Risperidone and 50 ppm of Trihexyphenidyl hydrochloride)

0.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – II (10ppm of Risperidone and 100ppm of Trihexyphenidyl hydrochloride)

1 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – III (15ppm of Risperidone and 150ppm of Trihexyphenidyl hydrochloride)

1.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – IV (20 ppm of Risperidone and 200ppm of Trihexyphenidyl hydrochloride)

2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – V (25 ppm of Risperidone and 250ppm of Trihexyphenidyl hydrochloride)

2.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Procedure

Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated.

Acceptance criteria

- Correlation coefficient should be not less than 0.999.

Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 5µg/ml-25µg/ml and 50µg/ml-250µg/ml of Risperidone and Trihexyphenidylhydrochloride respectively.

Accuracy

Preparation of standard stock solution

1mg of Risperidone and 10mg of Trihexyphenidyl hydrochloride working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1 ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

0.5mg of Risperidone and 5 mg of Trihexyphenidyl hydrochloride working standard were accurately weighed and transferred into a 10 ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).Further pipette out 10 ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 100% solution (with respect to target assay concentration)

1 mg of Risperidone and 10 mg of Trihexyphenidyl hydrochloride working standards were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

For preparation of 150% solution (with respect to target assay concentration)

2 mg of Risperidone and 15 mg of Trihexyphenidyl hydrochloride working standards into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for RisperidoneandTrihexyphenidyl hydrochloride and calculate the individual % recovery and mean % recovery values.

Acceptance criteria

- The % recovery for each level should be between 98.0 to 102.0%

Precision

Repeatability

Preparation of stock solution

1mg of Risperidone and 10 mg of Trihexyphenidyl hydrochloride working standard were

accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted 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.

Acceptance criteria

- The % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution

1 mg of Risperidone and 10 mg of Trihexyphenidyl hydrochloride working standard were accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted 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.

Acceptance criteria

- The % RSD for the area of five sample injections results should not be more than 2%.

Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\mathbf{LOD = 3.3\ X\ \frac{\sigma}{S}}$$

Where

σ - Standard deviation (SD)

S - Slope

Limit of quantification

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\mathbf{LOD = 10\ X\ \frac{\sigma}{S}}$$

Where

σ - Standard deviation

S - Slope

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 15ppm of Risperidoneand 150 ppm of Trihexyphenidyl hydrochloride was prepared and analysed using the varied flow rates along with method flow rate.
b) The organic composition in the mobile phase was varied from 65% to75 %standard solution 15 µg/ml of Risperidoneand 150 µg/ml of Trihexyphenidyl hydrochloride were prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

System suitability

1mg ofRisperidone and 10 mg of Trihexyphenidylhydrochloride working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of Risperidone and Trihexyphenidyl hydrochloride from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Risperidone andTrihexyphenidyl hydrochloride by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Risperidone andTrihexyphenidyl hydrochloride by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Risperidone andTrihexyphenidyl hydrochloride was obtained and the isobestic point of Risperidone and Trihexyphenidyl hydrochloride showed absorbance's maxima at 252 nm. The spectrums are shown in Fig.

Figure 1: Spectrum showing overlapping spectrum of Risperidone and Trihexyphenidyl hydrochloride

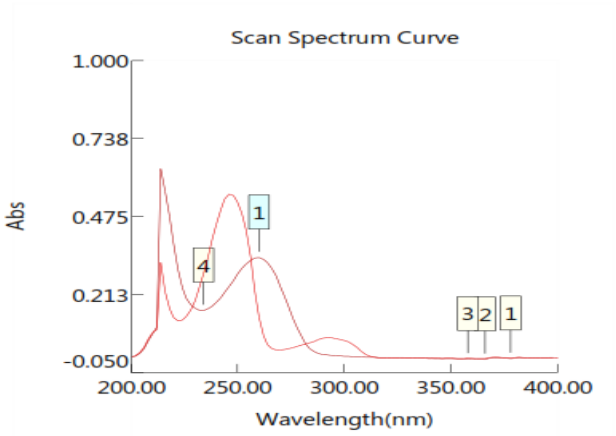


Figure 2: Spectrum showing wavelength of Risperidone

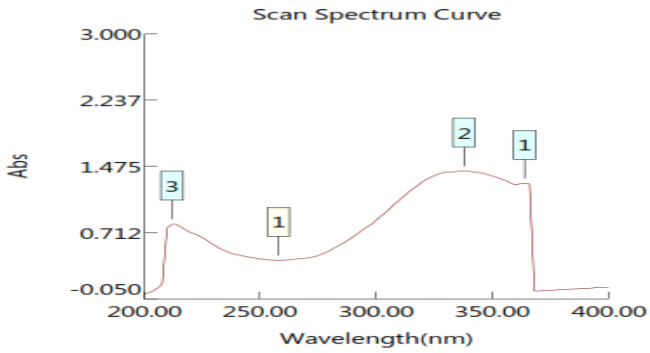
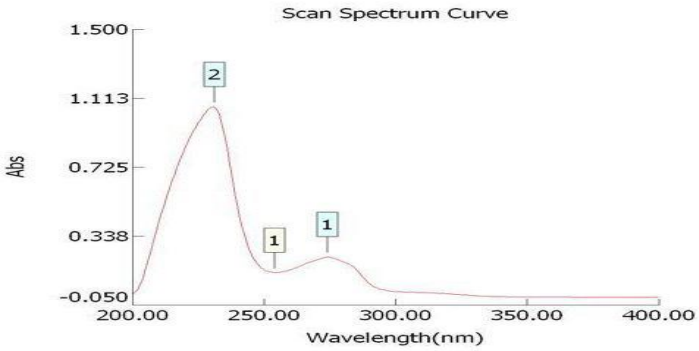


Figure 3: Spectrum showing wavelength of Trihexyphenidyl hydrochloride



The chromatographic method development for the simultaneous estimation of Risperidone and Trihexyphenidylhydrochloride were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Risperidone and Trihexyphenidyl hydrochloride in API and pharmaceutical dosage form by RP-HPLC method.Assay calculation for Risperidone andTrihexyphenidyl hydrochloride

The assay study was performed for the Risperidone and Trihexyphenidyl hydrochloride. Each

three injections of sample and standard were injected into chromatographic system.

Figure 4: Chromatogram showing assay of sample injection-1, 2

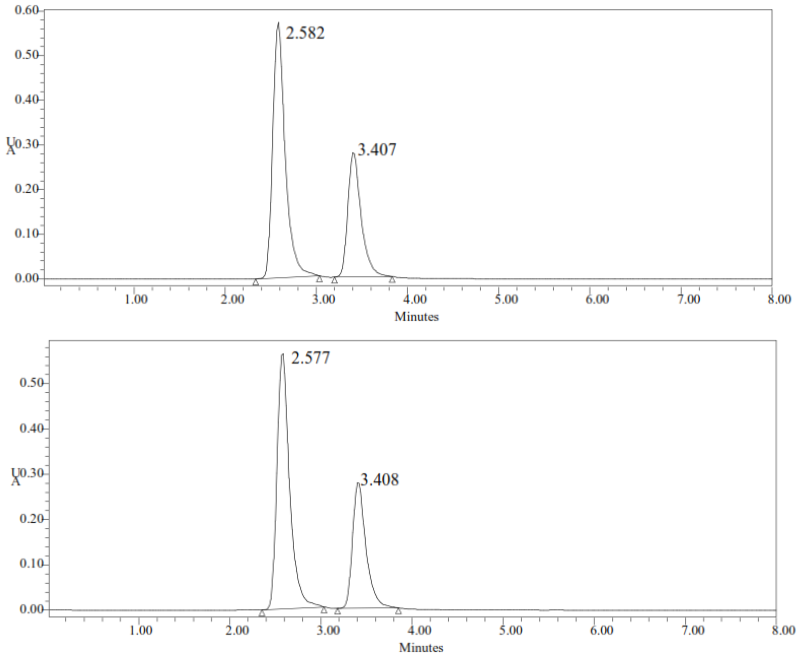


Figure 5: Chromatogram showing assay of Standared injection -1,2.

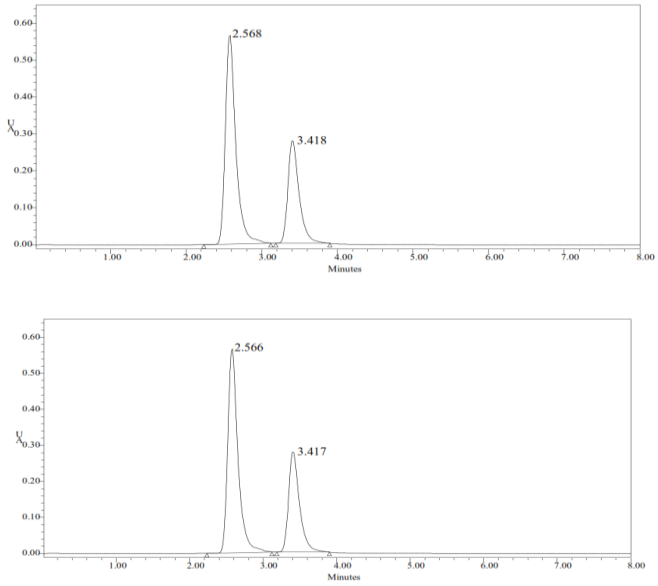


Table 3: Showing assay results

S. No	Name of compound	Label claim(mg)	Amount taken(mg)	%purity
1	Trihexyphenidyl hydrochloride	60	510.6	101.04
2	Risperidone	100	1012	99.24

The retention time of Risperidone and Trihexyphenidyl hydrochloride was found to be 2.566 mins and 3.417 mins respectively. The system suitability parameters for Risperidone and Trihexyphenidylhydrochloride such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089, 1.2. Resolution was 6.0 the % purity Risperidone and

Trihexyphenidylhydrochloride in pharmaceutical dosage form was found to be 99.24 and 101.04% respectively.

Validation Report

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

Linearity

The linearity study was performed for the concentration of 50 ppm to 250 ppm and 5ppm to 25 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient.

Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Risperidone andTrihexyphenidyl hydrochloridesuccinate. Each level was injected in triplicat e into chromatographic system. The area of each level was used for calculation of % recovery.

Precision

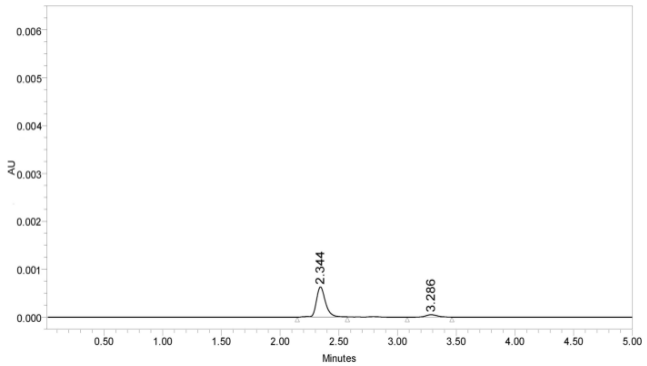
- ❖ Repeatability
- ❖ Intermediate Precision

Repeatability

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/Ruggedness

Figure 6: Results of Lod



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.

Repeatability

The precision study was performed for five injections of Risperidoneand Trihexyphenidylhydrochloride . Each standard injection was injected into chromatographic system. The area of each Standard injection was used for calculation of % RSD.

Intermediate precision/Ruggedness

The intermediate precision study was performed for five injections of Risperidone andTrihexyphenidyl hydrochloride . Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

Detection limit

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\text{LOD} = 3.3 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation (SD)

S - Slope

Table 4: Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Risperidone	373625.50	581075863	3.17
Trihexyphenidyl hydrochloride	5772.40	476579210	0.0172

The LOD was performed for Risperidone and Trihexyphenidyl hydrochloride was found to be 3.17and 0.0172 respectively.

Quantitation limit

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\text{LOQ} = 10 \text{ X } \frac{\sigma}{S}$$

Where

σ - Standard deviation

S - Slope

Figure 7: Results of Loq

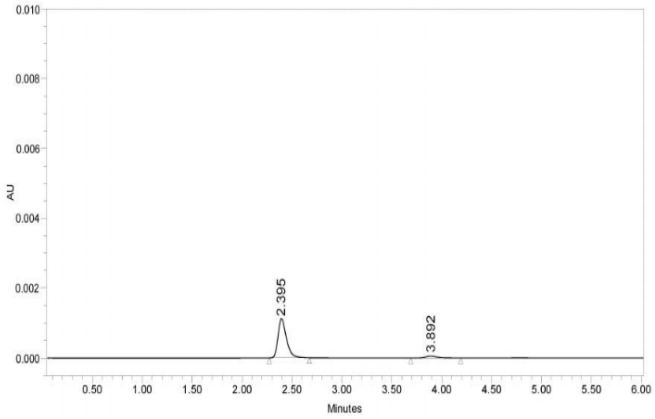


Table 5: Showing results for Limit of Quantitation

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Risperidone	372727.80	574265980	5.80
Trihexyphenidyl hydrochloride	5761.30	478828490	0.212

The LOQ was performed for Risperidone and Trihexyphenidyl hydrochloride was found to be 5.80 and 0.212 respectively.

Robustness

The robustness was performed for the flow rate variations from 0.4ml/min to 0.6ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Risperidone and Trihexyphenidyl hydrochloride. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

CONCLUSION

A new method was established for simultaneous estimation of Risperidone and Trihexyphenidyl hydrochloride by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Risperidone and Trihexyphenidyl hydrochloride Sby using Thermosil C18 column (4.0×125mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Sodium acetate buffer pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 252nm. HPLC instrument is Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. The retention times were found to be 2.566 mins and 3.417 mins. The % purity of Risperidone and Trihexyphenidyl hydrochloride was found to be 101.04% and 99.24% respectively. The system suitability parameters for Risperidone and Trihexyphenidyl hydrochloride such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Risperidone and Trihexyphenidyl hydrochloride was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Risperidone and Trihexyphenidyl hydrochloride in API and Pharmaceutical dosage form

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