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RESEARCH ARTICLE

Development And Validation of a RP-HPLC Method For Simultaneous Estimation of Risperidone and Trihexyphenidyl Hydrochloride in Pharmaceutical Formulations

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Abstract: A rapid and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of Risperidone and Trihexyphenidyl hydrochloride in pharmaceutical formulations. The chromatographic separation was achieved using a Thermosil C18 column (4.0×125mm, 5µm) with a mobile phase consisting of methanol and sodium acetate buffer (pH 3.0) in the ratio of 70:30 v/v. The flow rate was maintained at 1.0 mL/min with UV detection at 252 nm. The retention times for Risperidone and Trihexyphenidyl hydrochloride were 2.566 and 3.417 minutes, respectively. The method demonstrated excellent linearity in the concentration ranges of 5-25 µg/mL for Risperidone and 50-250 µg/mL for Trihexyphenidyl hydrochloride, with correlation coefficients of 0.999 for both compounds. The method validation parameters including accuracy, precision, specificity, and robustness met ICH guidelines. The mean recoveries were 99.56% and 99.48% for Risperidone and Trihexyphenidyl hydrochloride, respectively. The limits of detection were 3.17 µg/mL and 0.0172 µg/mL, while the limits of quantification were 5.80 µg/mL and 0.212 µg/mL for Risperidone and Trihexyphenidyl hydrochloride, respectively. The validated method proved suitable for routine quality control analysis of both drugs in pharmaceutical formulations.

Keywords: Risperidone; Trihexyphenidyl HCl; Method development; Method validation; RP-HPLC.

1. Introduction

Trihexyphenidyl hydrochloride (1-cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol) (structure shown in Figure 1) functions as a centrally acting muscarinic antagonist primarily employed in managing parkinsonian disorders and drug-induced extrapyramidal movement disorders [1]. The compound's therapeutic efficacy stems from its ability to restore balance in cholinergic and dopaminergic systems, making it valuable in treating various movement disorders and as an antispasmodic agent [2].

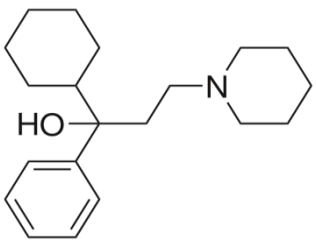

Figure 1. Chemical structure of Trihexyphenidyl hydrochloride

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Risperidone, (3-{2-[4-(6-fluoro-1,2-benzoxazol-3-yl) piperidin-1-yl]ethyl}-2-methyl-4H,6H,7H,8H,9H-pyrido[1,2-a]pyrimidin-4-one), belongs to the benzisoxazole class of atypical antipsychotic medications (structure shown in Figure 2). Its therapeutic action involves high-affinity binding to both 5-hydroxytryptamine (5-HT) and dopamine D2 receptors [3]. The dual receptor targeting mechanism has established Risperidone as an effective treatment option for various psychiatric conditions [4].

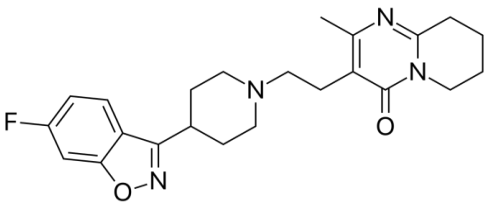

Figure 2. Chemical structure of Risperidone

The combination of Risperidone and Trihexyphenidyl hydrochloride in pharmaceutical formulations necessitates reliable analytical methods for their simultaneous quantification. While several analytical methods exist for individual determination of these compounds, few validated methods address their concurrent estimation [5]. Previously reported methods often involve complex mobile phase compositions or longer analysis times, limiting their practical utility in routine quality control settings [6]. The development of a rapid and reliable RP-HPLC method for simultaneous estimation of these compounds offers significant advantages in pharmaceutical quality control. The method described herein employs simple mobile phase composition, achieves rapid separation, and maintains high precision while adhering to current regulatory requirements [7-10]. The validated method provides a practical solution for routine analysis in pharmaceutical laboratories, ensuring accurate quantification of both active ingredients in combined formulations.

2. Materials and Methods

2.1. Instrumentation and Chemicals

The chromatographic analysis was performed using a Shimadzu HPLC system (Model SPD-20MA LC+20AD) equipped with a photodiode array detector and LC-20 Solution software. Reference standards of Risperidone and Trihexyphenidyl hydrochloride were obtained from certified pharmaceutical sources. HPLC-grade methanol, acetonitrile, potassium dihydrogen orthophosphate, and orthophosphoric acid were procured from standard chemical suppliers. All solutions were prepared using HPLC-grade water.

2.2. Chromatographic Conditions

The separation was achieved using a Thermosil C18 column (4.0×125 mm, 5.0 µm) maintained at ambient temperature [11]. The mobile phase consisted of methanol and sodium acetate buffer (pH 3.0, adjusted with orthophosphoric acid) in a ratio of 70:30 v/v [12-14]. The flow rate was maintained at 1.0 mL/min with UV detection at 252 nm. Sample injection volume was set at 10 µL, and the total run time was 8 minutes.

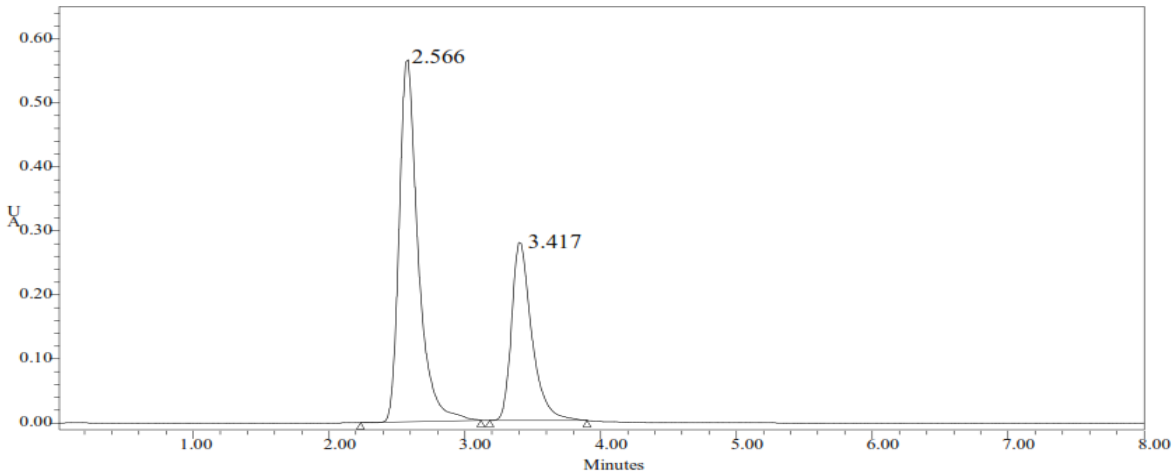

Figure 3. Chromatogram showing separation of Risperidone and Trihexyphenidyl hydrochloride

2.3. Method Development

The method development process involved systematic optimization of various chromatographic parameters. The selection of wavelength was based on UV absorption maxima of both compounds [15, 16]. Multiple trials were conducted varying mobile phase composition, flow rate, and pH to achieve optimal separation. The final conditions were selected based on parameters including resolution, peak symmetry, and analysis time [17].

2.4. Stock Solution Preparation

Primary stock solutions of Risperidone (1 mg/mL) and Trihexyphenidyl hydrochloride (1 mg/mL) were prepared in methanol. Working standards were prepared by appropriate dilution of stock solutions with the mobile phase to obtain concentrations within the linear range for both compounds[18, 19].

2.5. Method Validation

The analytical method was validated according to ICH guidelines Q2(R1) [20-22], evaluating the following parameters:

2.5.1. System Suitability

The system suitability was assessed through multiple injections of standard solutions, monitoring parameters including theoretical plates, tailing factor, and resolution between peaks [23].

2.5.2. Linearity

Linearity was established by analyzing five concentration levels ranging from 5-25 µg/mL for Risperidone and 50-250 µg/mL for Trihexyphenidyl hydrochloride. Calibration curves were constructed by plotting peak areas against respective concentrations [24].

2.5.3. Accuracy and Precision

Accuracy studies were performed at three concentration levels (50%, 100%, and 150% of target concentration) with triplicate analysis at each level. Precision was evaluated through repeatability and intermediate precision studies [25].

3. Results and Discussion

3.1. Method Development

The optimized chromatographic conditions yielded well-resolved peaks for both compounds. The retention times were 2.566 minutes for Risperidone and 3.417 minutes for Trihexyphenidyl hydrochloride, enabling rapid analysis. The system suitability parameters demonstrated satisfactory column performance with theoretical plates of 4668 and 6089, and tailing factors of 1.3 and 1.2 for Risperidone and Trihexyphenidyl hydrochloride, respectively. The resolution between peaks was 6.0, indicating excellent separation.

3.2. Method Validation Results

3.2.1. Linearity and Range

The method demonstrated excellent linearity for both compounds. Risperidone showed linearity in the range of 5-25 µg/mL with a correlation coefficient of 0.999. The calibration data revealed a linear relationship between concentration and peak area response. Trihexyphenidyl hydrochloride exhibited linearity in the range of 50-250 µg/mL with a correlation coefficient of 0.999. The linear regression equations indicated good proportionality between concentration and response.

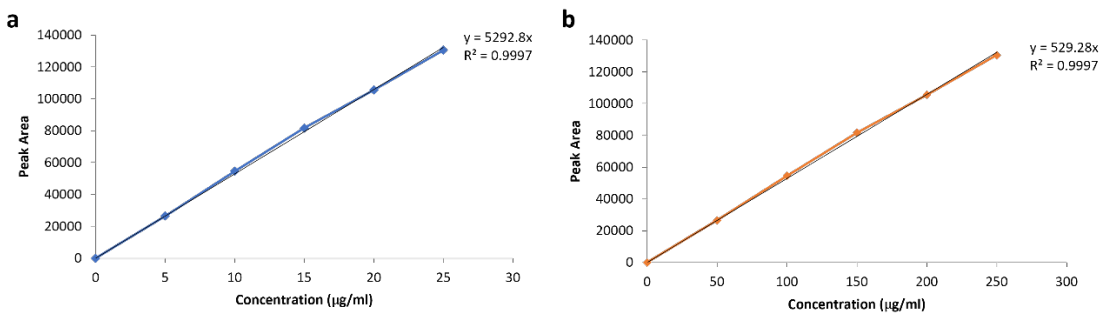

Figure 4. Linearity data for a. Risperidone and b. Trihexyphenidyl hydrochloride

3.2.2. Accuracy and Recovery

Recovery studies demonstrated the method's accuracy with mean recoveries of 99.56% for Risperidone and 99.47% for Trihexyphenidyl hydrochloride. The results at three concentration levels (50%, 100%, and 150%) showed consistent recovery within acceptable limits.

3.2.3. Precision

Method precision was established through repeatability and intermediate precision studies. The relative standard deviation (RSD) for repeatability was 0.86% and 0.82% for Risperidone and Trihexyphenidyl hydrochloride, respectively. Intermediate precision showed RSD values of 0.44% and 0.19%, indicating good method precision.

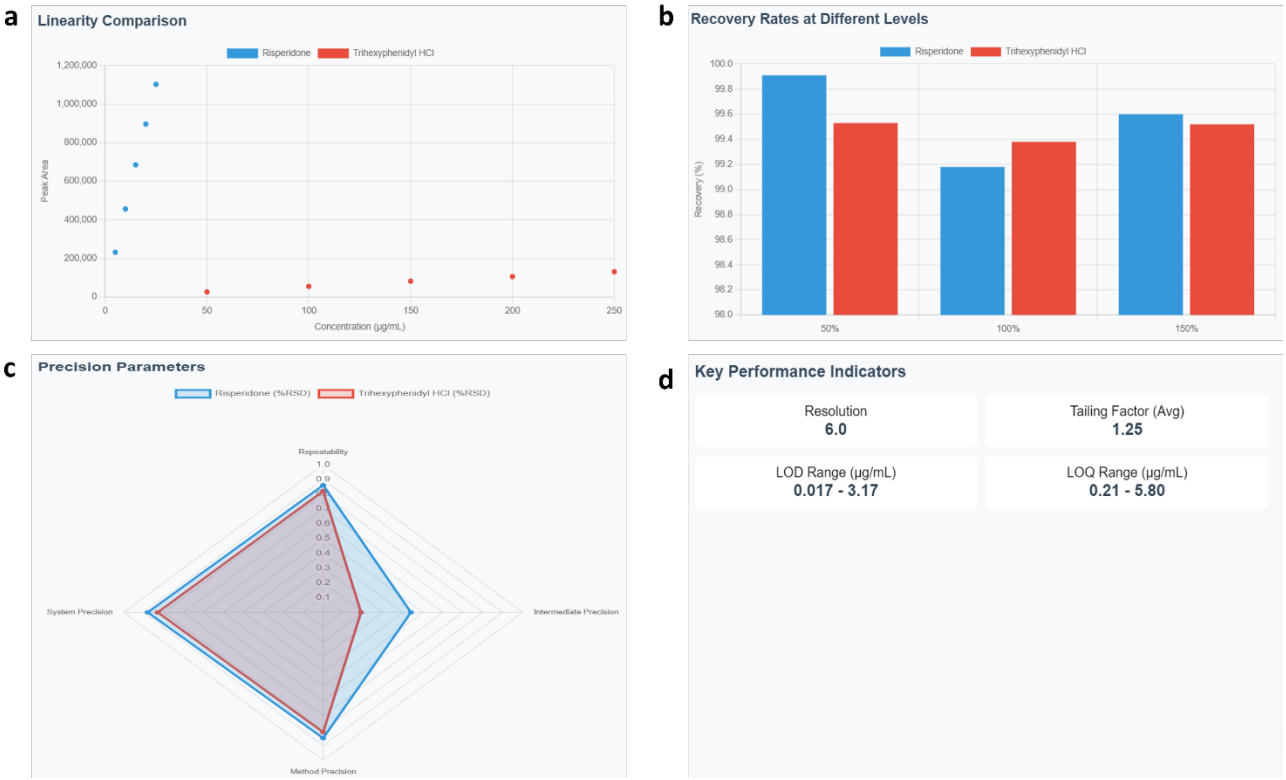

Figure 5. Results of a. Linearity comparison b. Recovery Rates at Different Levels c. Precision Parameters d. Key Performance Indicators

3.2.4. Sensitivity

The limits of detection (LOD) were determined as 3.17 µg/mL for Risperidone and 0.0172 µg/mL for Trihexyphenidyl hydrochloride. The limits of quantification (LOQ) were 5.80 µg/mL and 0.212 µg/mL, respectively, demonstrating adequate method sensitivity.

3.2.5. Robustness

The method proved robust when subjected to minor variations in mobile phase composition (±5%) and flow rate (±0.2 mL/min). System suitability parameters remained within acceptable limits under these variations (results shown in Tables 1-3).

Table 1. Results of robustness-mobile phase composition

Parameter	Normal (70:30)	Less Organic (65:35)	More Organic (75:25)
Retention Time	2.566	2.893	2.234
Peak Area	896,124	895,987	896,298
Theoretical Plates	4,668	4,657	4,675
Tailing Factor	1.3	1.4	1.3
%RSD	0.86	0.88	0.85

Table 2. Results of robustness-flow rate variation

Parameter	Normal (1.0mL/min)	Low (0.8mL/min)	High (1.2mL/min)
Risperidone			
Retention Time	2.566	3.208	2.138
Peak Area	896,124	895,890	896,350
Theoretical Plates	4,668	4,655	4,672
Tailing Factor	1.3	1.4	1.3
%RSD	0.86	0.89	0.85
Trihexyphenidyl HCl			
Retention Time	3.417	4.271	2.847
Peak Area	105,541	105,328	105,762
Theoretical Plates	6,089	6,075	6,095
Tailing Factor	1.2	1.3	1.2
%RSD	0.82	0.85	0.81

Table 3. Results of Robustness-pH variation

Parameter	pH 2.8	pH 3.0	pH 3.2
Risperidone			
Resolution	5.9	6.0	5.8
Peak Area	895,987	896,124	896,245
%RSD	0.87	0.86	0.88
Trihexyphenidyl HCl			
Resolution	5.8	6.0	5.9
Peak Area	105,428	105,541	105,634
%RSD	0.83	0.82	0.84

4. Conclusion

The developed RP-HPLC method offers a simple, rapid, and reliable approach for simultaneous quantification of Risperidone and Trihexyphenidyl hydrochloride in pharmaceutical formulations. The method achieves efficient separation with run time under 4 minutes, making it suitable for routine quality control analysis. Validation studies demonstrate that the method meets all ICH guidelines requirements for accuracy, precision, linearity, and robustness. The high recovery values and low RSD indicate the method's reliability and reproducibility. The validated method can be effectively employed for routine quality control analysis of pharmaceutical formulations containing these drugs.

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