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# Development and Validation of High Performance Liquid Chromatographic Method for Simultaneous Estimation of Risperidone and Trihexyphenidyl in Combined Dosage form

**Komal Patel[1*], Mohan Sellepen[2]**

*1. Research Scholar, JJT University, Jhunjhunu, Rajasthan,India*

*2. Saraswati Institute of Pharmaceutical Sciences,Dhanap, Gandhinaagr-382355,India*

## ABSTRACT

A simple, rapid, accurate, precise and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of Risperidone and Trihexyphenidyl hydrochloride was determined using reversed-phase liquid chromatography method using ODS Hypersil $C_{18}$ column (250 mm × 4.6 mm id, 5µm as a stationary Phase and Methanol : Acetonitrile : Acetate Buffer (pH 4.0) (70 : 20 : 10, v/v/v) as a mobile phase pumped at a flow rate of 1.0 ml/min. Quantification was achieved with ultraviolet detection at 214 nm over concentration ranges of 2-20 µg/ml for Risperidone and 1-10 µg/ml for Trihexyphenidyl hydrochloride with mean accuracy 101.02 ± 0.19 and 101.3 ± 0.38 %, for Risperidone & Trihexyphenidyl hydrochloride respectively. The method was successively applied to tablet dosage forms as no chromatographic interferences from the tablet excipients were observed. The method retained its accuracy and precision when the standard addition technique was applied.

**Keywords:** Risperidone, Trihexyphenidyl, RP-HPLC

*Corresponding Author Email: mpharmkomalpatel19@gmail.com

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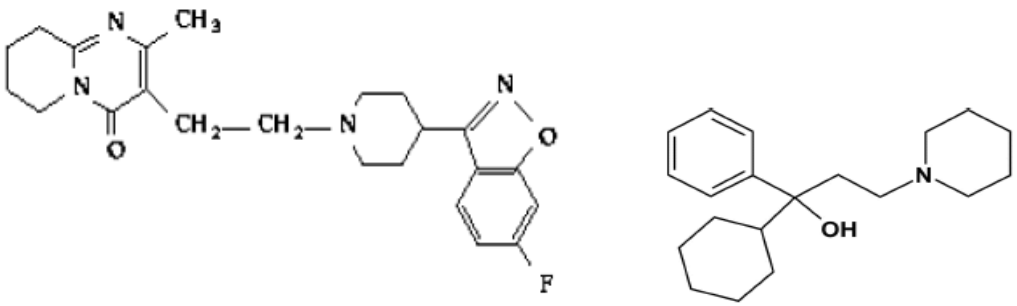
## INTRODUCTION

Risperidone (RIS) is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives. Chemically it is 3-[2-[4-(6-fluoro-1, 2-benzisoxazol-3-yl)-1-piperidinyl] ethyl]-6, 7, 8, 9-tetrahydro- 2-methyl-4H-pyrido [1, 2-a] pyrimidin-4-one.[1,2] It is indicated for the acute and maintenance treatment of schizophrenia in adolescents aged 13-17 years and also it is indicated for the short-term treatment of acute manic or mixed episodes associated with Bipolar Disorder in adults and in children and adolescents aged 10-17 years[3]. Trihexyphenidyl (THP) is an antidyskinetic and antiparkinson drug whose IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl)-1- propanol.[4,5] THP is official in IP. IP suggest a titrimetric assay method for THP. Literature survey revealed that HPLC, UV and HPTLC methods have been reported for the estimation of RIS and THP individually and with other drugs in pharmaceutical dosage forms. RIS and THP are formulated together in the form of a tablet. Literature survey revealed no method reported for simultaneous determination of the two drugs[6-18]. The present RP-HPLC method uses simple mobile phase ratio, higher sensitivity and analysis will complete before 6 min. Therefore the present study was to determine both drugs concurrently by sensitive, accurate, rapid and precise RP-HPLC method for routine analysis[19].




Risperidone Trihexyphenidyl

**Figure 1:Structre of Risperidone and Trihexyphenidyl**

## MATERIALS AND METHODS

### Instruments & Reagents

The chromatography was performed by a HPLC instrument (LC-2010CHT, Shimadzu, Japan) equipped with a UV-Visible detector and a photodiode array detector, manual injector with 20 µL loop, ODS Hypersil C18 column (250 mm × 4.6 mm id, 5 µm particle size) and LC-solution software were used. Digital pH meter (LI 361 pH analyzer, Systronic Ltd., Ahmedabad), Analytical balance (Sartorius CP224S) & Ultra sonic cleaner (Frontline FS 4, India) were also used. RIS and THP bulk powder was kindly gifted by reputed Pharmaceutical Company, Gujarat

(India), with 99.96% purity & market fixed dose combination product containing 4 mg RIS & 2 mg THP tablets were used. HPLC grade Methanol (Merck Ltd., Mumbai, India), HPLC grade Acetonitrile (Finar Chemicals Ltd., Mumbai, India), AR grade Glacial Acetic acid & NaOH (S.D Fine Chemicals Ltd, Mumbai, India), A nylon 0.45 $\mu$m – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) were used.

**Chromatographic condition**

The chromatographic estimation was performed using following conditions: Stationary phase: ODS Hypersil C18 column (250 mm × 4.6 mm id, 5µm particle size) was used at ambient temperature. Mobile Phase: Methanol : Acetonitrile : Acetate Buffer (pH 4.0) (70 : 20 : 10, v/v/v) Flow rate: 1.0 ml/min, Injection volume: 20 µL, Detection: At 214 nm with PDA detector. Calibration curves for both RIS and THP were plotted, and the corresponding regressions Equations were calculated.

**Preparation of stock solutions**

Accurately weighed RIS (20 mg) and THP (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard stock solutions having concentration RIS (200 µg/ml) and THP (100 µg/ml).

**Preparation of sample solution**

Powder from the mixed contents of 20 tablets, equivalent to 4 mg RIS and 2 mg THP, was transferred accurately to a 100 ml volumetric flask and diluted to volume with methanol. The content was mixed with methanol (50 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtering through a whatman filter paper no. 41. The volume was adjusted up to the mark with mobile phase. 1.0 ml from this solution was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with mobile phase. An aliquot of this solution (2.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with mobile phase. The separation was done on a C18 column.

**Preparation of mobile Phase**

Acetate buffer (PH 4.0); In 1000ml volumetric flask take 2.86ml of glacial acetic acid & 1.0ml of 50%w/v of NaOH. Make up the volume with water. The mobile phase pumped at a flow rate of 1.0 ml/min.

**Calibration curves**

Calibration curves were constructed by plotting peak areas Vs concentrations of RIS and THP, and the regression equations were calculated. Calibration curves were plotted over a concentration range 2-20 µg/ml for RIS and 1-10 µg/ml THP. Accurately measured standard

working solutions 0.1, 0.2, 0.4, 0.6, 0.8, & 1.0ml were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions.

## RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for RIS and THP was obtained with a mobile phase Methanol: Acetonitrile: Acetate buffer (pH 4.0) (70: 20: 10, v/v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 214 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure. 2). The Overlain absorption spectrum of standard and sample shows good correlation (Figure. 3). System suitability test parameters for RIS and THP for the proposed method are reported in Table 1.

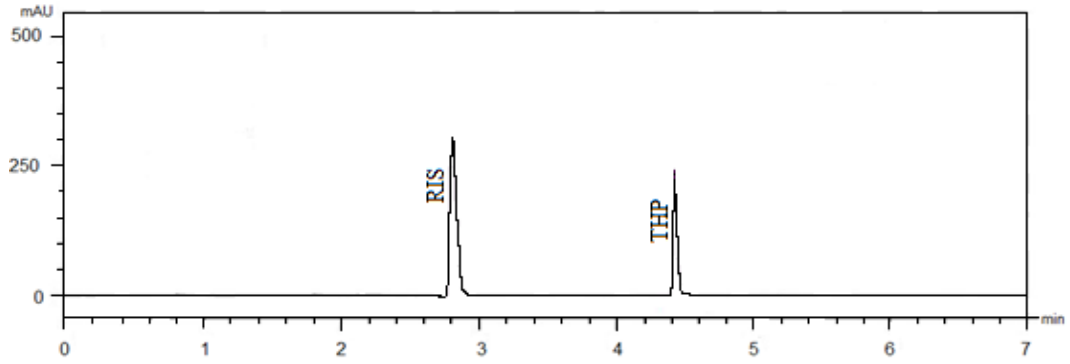



**Figure 2: RP-HPLC Chromatogram of RIS and THP**

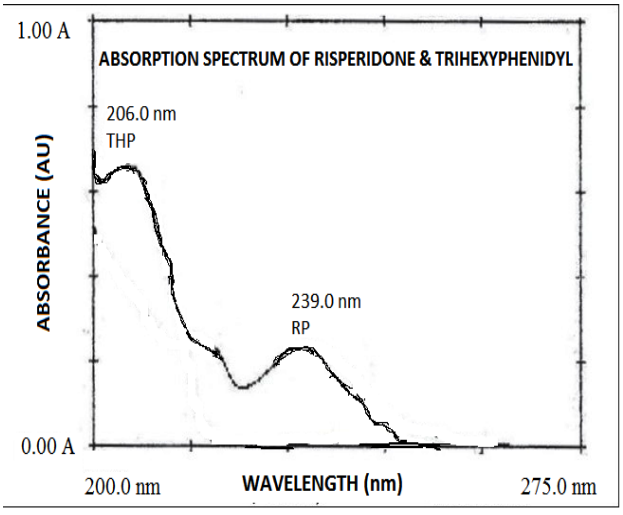



**Figure 3: Overlain Absorption Spectra of RIS and THP for Standard and Marketed Formulation**

**Table 1: System Suitability Parameters of Chromatogram for RIS and THP**

| Parameters | RIS ± RSD (n = 6) | THP ± RSD (n = 6) |
|---|---|---|
| Retention time (min) | 2.70 ± 1.39 | 4.44 ± 0.097 |
| Tailing factor | 1.03 ± 0.84 | 1.19 ± 0.219 |
| Theoretical plates | 3577 ± 0.997 | 4109 ± 1.22 |
| Resolution | 8.52 ± 0.54 | |

**Validation of the proposed method**

**Linearity**

Linear correlation was obtained between peak areas versus concentrations of RIS and THP in the ranges of 2-20 µg/ml for RIS and 1-10 µg/ml THP. Regression parameters are mentioned in Table 2 and the calibration curves of RIS and THP at 214 nm, respectively are shown in Figure.4 & Figure.5.

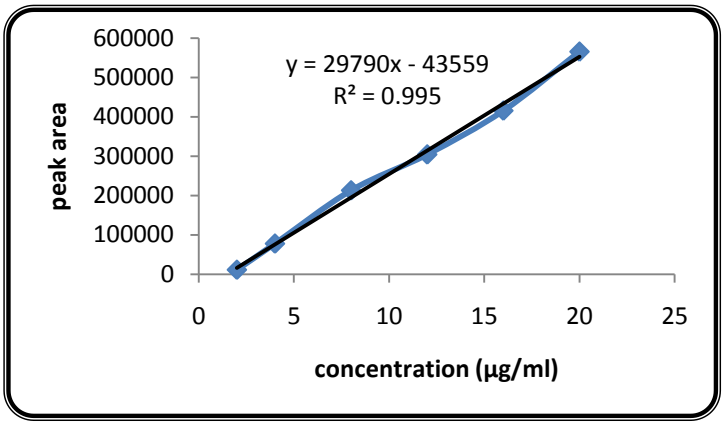



**Figure 4: Calibration Curve of RIS**

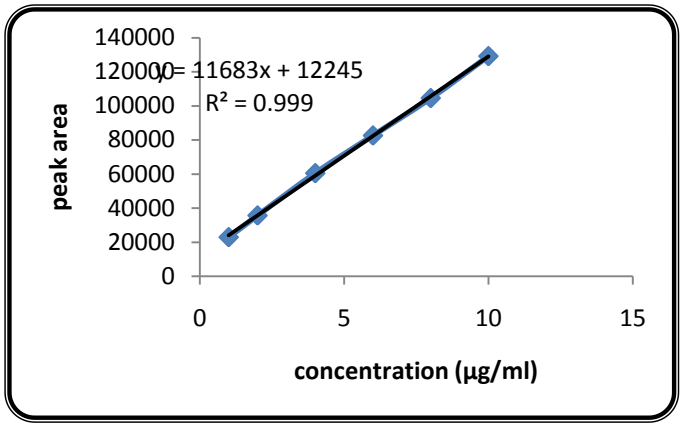



**Figure 5: Calibration Curve of THP**

**Accuracy**

The recovery experiment was performed by the standard addition method. The recoveries obtained were 101.02 ± 0.19% and 101.3 ± 0.38% for RIS and THP, respectively (Table 2). The

low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3.

**Method Precision (% Repeatability)**

The RSD values for RIS and THP were found to be 0.19 % and 0.37 %, respectively (Table 2). The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.

**Intermediate Precision (Reproducibility)**

The low RSD values of interday (0.18-1.60 % and 0.27 – 0.70 %) and intraday (0.10-0.60 % and 0.24 – 0.38 %) variations for RIS and THP, respectively, reveal that the proposed method is precise (Table 2).

**Table 2: Regression Analysis Data and Summary of Validation Parameter for the proposed Method**

| **Parameters** | **RIS** | **THP** |
|---|---|---|
| Detection wavelength (nm) | 230 | 230 |
| Concentration range (µg/ml) | 2 – 20 | 1 – 10 |
| Slope | 29790 | 11683 |
| Intercept | 43559 | 12245 |
| Correlation coefficient ($r^2$) | 0.9957 | 0.9994 |
| LOD (µg/ml) | 0.11 | 0.21 |
| LOQ (µg/ml) | 0.33 | 0.62 |
| % Recovery ( Accuracy, n = 6) | 101.02 ± 0.19 | 101.3 ± 0.38 |
| Repeatability (RSD, n = 6), % | 0.19 | 0.37 |
| Precision (RSD), % | - | - |
| Interday (n = 6) | 0.18-1.60 | 0.27 – 0.70 |
| **Intraday (n = 6)** | **0.10-0.60** | **0.24 – 0.38** |

**Table 3: Recovery Data for the proposed Method**

| **Drug** | **Level** | **Amount of sample taken (µg/ml)** | **Amount of standard spiked (%)** | **Mean % Recovery ± SD*** |
|---|---|---|---|---|
| **RIS** | I | 8 | 50 % | 101.34 ± 0.62 |
| | II | 8 | 100 % | 100.42 ± 0.67 |
| | III | 8 | 150 % | 100.46 ± 0.63 |
| **THP** | I | 4 | 50 % | 101.16 ± 0.53 |
| | II | 4 | 100 % | 100.6 ± 1.11 |
| | III | 4 | 150 % | 101.12 ± 0.60 |

* Mean % Recovery ± SD of six observations.

**LOD and LOQ**

LOD values for RIS and THP were found to be 0.11µg/ml and 0.06µg/ml, respectively and LOQ values for RIS and THP were found to be 0.34µg/ml and 0.19µg/ml, respectively (Table 2). These data show that the proposed method is sensitive for the determination of RIS and THP.

**Robustness**

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %. The low value of % RSD indicates robustness of the proposed method.

## CONCLUSION

In this proposed method the linearity is observed in the concentration range of 2 - 20 µg/ml & 1-10 µg/ml with co-efficient of correlation, ($r^2$) = 0.9957 and ($r^2$) = 0.9994 for RIS and THP, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the RIS and THP in combined dosage form without any interference of the excipients.

## REFERENCES

1. Isaacson EI, Block JH, Beale JM. Central Nervous System Depressant. In, Wilson & Gisvold's (Ed). Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th Edition. Lippincott Williums & Wilkins publisher, 2004; 501.
2. Extra pharmacopoeia, Sweetman SC. Martindale- The complete Drug Reference, 36th edition. London (U.K.), Pharmaceutical Press, 2009; 1024-1025.
3. Rang HP, Dale MM, Ritter JM, Flower RJ. Antipsychotic (Risperidone). In, Rang and Deale's (Ed). Pharmacology, 6th Edition. Edinburgh Churchill Livingstone publisher, 2007; 539-540.
4. Cocolas GH, Culter SJ, Block JH, Beale JM. Cholinergic drugs & Related Agents (Trihexyphenidyl), In, Wilson & Gisvold's (Ed). Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th Edition. Lippincott Williums & Wilkins publisher, 2004; 584.
5. Extra pharmacopoeia, Sweetman SC. Martindale- The complete Drug Reference, 36th edition. London (U.K.), Pharmaceutical Press, 2009; 820-821.
6. Baldaniya SL, Bhatt KK, Mehta RS, Shah DA. RP-HPLC Estimation of Risperidone in tablet dosage forms. Ind J of Pharm Sci 2008; 70(4): 494-7.
7. Charde M, Sheikh I, Dhole S, Kasture AV. RP-HPLC Method for Simultaneous Estimation of Benzhexol Hydrochloride and Trifluoperazine Hydrochloride in their combined Dosage Form. J Pharm Res 2010; 3(7): 1616.
8. Dedania Z, Dedania R, Sheth N, Patel J, Patel B. Stability Indicating HPLC Determination of Risperidone in Bulk Drug and Pharmaceutical Formulations. Intern J Anal Chem 2011; 1-6.

9. Kulkarni S, Chhabra G, Shivani M. Development and validation of uv spectrophotometric method for the determination of risperidone in bulk and tablets formulation. International J Pharm Chem Res 2012; 1(1):1-5.
10. M. Sravan Kumar. Development of Analytical Method for Risperidone by UV Spectrophotometry. Int J Pharma Sci Res 2010: 1(2), 122-6.
11. Mahadik KR, Aggarwal H, Kaul N. Development and validation of HPLC method for simultaneous estimation of trihexyphenidyl hydrochloride and chlorpromazine hydrochloride from tablet dosage form. Indian drugs 2002; 39(8): 441-5.
12. Mohammad Y, Pragatikumar B, Subhani. Development and Validated New Reverse Phase High Performance Liquid Chromatographic Method for the Determination of Risperidone in Bulk and Tablet Dosage Forms. Res J Pharm, Bio Chem Sci 2002; 1(2), 165-71.
13. Patel D, Patel J. Development & Validation of RP-HPLC method for simultaneous estimation of Risperidone & Trihexyphenidyl HCl in tablet dosage forms. Int J Pharm Sci Res 2010; 4 (3), 85-88.
14. Patel S, Vidyasagar G. Development and validation of analytical method for simultaneous Estimation of Trihexyphenidyl HCl and Trifluoperazine HCl in combined solid dosage form. Int J Univ Pharm life Sci 2009; 2(3); 693-702.
15. Santhosam S, Kannan S. An HPLC method for the simultaneous estimation of Risperidone and Trihexyphenidyl HCl from bulk and dosage forms.Hygeia J Drugs Med 2011; 3(1), 29-33.
16. Schatz DS, Saria A**.** Simultaneous Determination of Paroxetine, Risperidone and 9-Hydroxyrisperidone in Human Plasma by High-Performance Liquid Chromatography with Coulometric Detection, Pharmacology 2000; 60: 51–56.
17. Vladimir C, Yan X, Yong HC. Stereoselective determination of trihexyphenidyl in human serum by LC–ESI–MS, J of Pharm Bio Anal 1999; 21(3): 507-17.
18. Zhenghua S, Changna W. Sensitive chemiluminescence assay for risperidone in pharmaceutical preparations, J of Pharm Bio Anal 1999; 36(3): 491-94.
19. ICH Q2a, Validation of analytical procedure methodology, in. proc. Int. con. Harmonization, 2004, Geneva.