

## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RISPERIDONE AND TRIHEXYPHENIDYL HYDROCHLORIDE IN TABLET DOSAGE FORMS

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### ABSTRACT

A binary mixture of Risperidone (RIS) and Trihexyphenidyl hydrochloride (THP) was determined using reversed-phase liquid chromatography method using methanol: acetonitrile: 0.05 M phosphate buffer (pH 3.7) (60:30:10, v/v/v) pumped at a flow rate of 1.0 ml/min. Quantification was achieved with ultraviolet detection at 230 nm over concentration ranges of 0.5-5.0 and 1.0-10.0 µg/ml; mean accuracies were 100.55±0.64 and 100.75±0.81%, 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 hydrochloride, RP-HPLC.

### 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<sup>1-2</sup>. 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<sup>3-4</sup>. Trihexyphenidyl (THP) is an antidyskinetic and antiparkinson drug whose IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl)-1-propanol<sup>1</sup>. THP is official in IP. IP suggest a titrimetric assay method for THP. Literature survey revealed that HPLC, UV and HPTLC methods<sup>5-28</sup> 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. 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.

### MATERIALS AND METHODS

The chromatography was performed on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010 CHT) equipped with PDA detector, Phenomenex (Torrance, CA) C18 column (250×4.6 mm id, 5 µm particle size) was used as stationary phase. Standard samples of RIS and THP and Market samples of Sizdon fortin tablets (Sun Pharma), each tablet contained 4mg RIS and 2 mg THP were used. Triple distilled water, methanol, acetonitrile (HPLC grade, S. D. Fine Chemical, Ahmedabad, India), dihydrogen

phosphate (AR grade, S. D. Fine Chemical, Ahmedabad, India) were used.

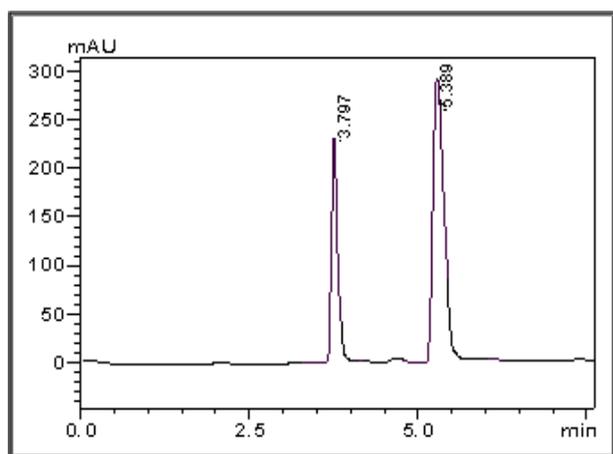
RIS and THP stock solutions (50 µg/ml and 100 µg/ml, respectively) were prepared by weighing accurately 2.5 mg RIS and 5.0 mg THP powder into 2 separate 50 ml volumetric flasks; 25 ml methanol was added, shaken for a few minutes, and diluted to volume with methanol. From these solutions (2.5 ml) were transferred into 2 separate 10 ml volumetric flasks and diluted to the mark with methanol to give final concentrations of 12.5 and 25.0 µg/ml, respectively. Accurate aliquots equivalent to 0.5-5.0 µg RIS from its working solution (12.5 µg/ml) and aliquots equivalent to 1.0-10.0 µg THP from its working solution (25 µg/ml) were transferred into 2 separate sets of 5 ml volumetric flasks and diluted to volume with methanol. Powder from the mixed contents of 20 tablets, equivalent to 4 mg RIS and 2 mg THP, was transferred accurately to a 50 ml volumetric flask and diluted to volume with methanol. The solution was diluted to the same concentrations of working standard solutions and treated according to the linearity for the RP-HPLC method. The separation was done on a C18 column using methanol: acetonitrile: 0.05 M phosphate buffer (pH 3.7) (60:30:10, v/v/v) the mobile phase pumped at a flow rate of 1.0 ml/min. The chromatogram was recorded under the following instrumental parameters: 10 µl injection volume, flow rate, 1.0 ml/min at 40°C temperature and the eluent monitored at 230 nm. Calibration curves for both RIS and THP were plotted, and the corresponding regressions Eqns were calculated.

### RESULTS AND DISCUSSION

The aim of this work was to develop sensitive, accurate, precise and rapid analytical method for the simultaneous determination of RIS and THP. This was achieved using RP-HPLC method. To optimize the proposed RP-HPLC method, all of the experimental conditions were



investigated. For the choice of the stationary phase, reversed-phase separation was preferred due to the drawbacks of the normal phase, e.g., hydration of silica with water that can cause peak tailing. To optimize the mobile phase, different systems were tried for chromatographic separation of the two components by combining homogenous design and solvent polarity optimization. The best resolution was achieved using a mobile phase consisting of methanol: acetonitrile: 0.05 M phosphate buffer (pH 3.7) (60:30:10, v/v/v), which gave good resolution and sensitivity of both drugs (fig. 1).



**Figure 1:** RP-HPLC chromatogram of RIS and THP at 230 nm

System suitability testing of the RP-HPLC method gave good relative retention time = 1.592; theoretical plates = 4253.8 and 6293.98; asymmetry factor (A) = 1.09 and 1.20; and tailing factor (T) = 1.15 and 1.31 for RIS and THP, respectively (Table 1).

**Table 1:** System suitability test parameters for RIS & THP for proposed RP-HPLC method

| Parameters  | Proposed methods        |                         |
|---|-------------------------|-------------------------|
|   | RP-HPLC                 |                         |
|   | RIS± % RSD <sup>a</sup> | THP± % RSD <sup>a</sup> |
| Retention time, min                               | 3.797±0.11              | 5.389± 0.18             |
| Tailing factor                                    | 1.15±0.16               | 1.31±0.52               |
| Asymmetry factor                                  | 1.09±0.51               | 1.20±0.18               |
| Theoretical plates                                | 4253.8±0.64             | 6293.98±1.34            |
| Repeatability of measurement (n <sup>b</sup> = 6) | 1.82                    | 1.49                    |

<sup>a</sup> RSD is a Relative standard deviation, <sup>b</sup> n is number of determinations

A linear relation was obtained between peak area and the concentration of the two drugs in the range of 0.5-5.0 and 1.0-10.0 µg/ml for RIS and THP, respectively. The linear regressions Eqns were computed as: Y=154926 X + 11501, r= 0.9995 and Y=126789 X+ 45913, r= 0.9979, where Y is the area under the peak, X is the concentration in µg/ml, and r is the correlation coefficient. Results obtained by applying the RP-HPLC procedure showed that RIS and THP can be simultaneously analyzed in the prepared mixtures with mean recoveries of 100.55±0.64 and 100.75±0.81 %, respectively. The proposed method has been applied to assay RIS and THP in tablets without any interference from the additives (Table 2). The limit of detection for RIS and THP were found to be 0.05µg/ml and 0.5µg/ml, respectively; the limit of quantification for RIS and THP were found to be 0.5µg/ml and 1.0 µg/ml, respectively by visual method. The low % CV values of intra-day (0.15-1.41 for RIS and 0.46-1.38 for THP) and inter-day (0.19-1.67 for RIS and 0.37-1.62 for THP) precision reveal that the proposed method is precise. Thus, the proposed procedure can be used in routine analysis.

**Table 2:** Assay results for tablets using the proposed methods (RP-HPLC)

| Mix. | Amount of drug added (mg) |     | Amount of drug found (mg) |      | % Amount found (n <sup>a</sup> =3) ± SD <sup>b</sup> |            |
|------|---------------------------|-----|---------------------------|------|--|------------|
|      | RIS                       | THP | RIS                       | THP  | RIS  | THP        |
| 1    | 4                         | 2   | 4.02                      | 1.99 | 100.5±0.19   | 99.5±0.16  |
| 2    | 4                         | 2   | 3.97                      | 2.01 | 99.25±0.44   | 100.5±0.21 |

<sup>a</sup> n is number of determinations, <sup>b</sup> SD is a Standard deviation, RIS is Risperidone, THP is Trihexyphenidyl HCl

**Table 3:** Application of the standard addition technique to the analysis of RIS and THP in tablets by the proposed methods

| Proposed methods | Amount of drug taken (µg/ml or ng/spot) |     | Amount of drug added (µg/ml or ng/spot) |     | Amount of drug found (µg/ml or ng/spot) |      | % Recovery (n <sup>a</sup> =3) ± SD <sup>b</sup> |             |
|------------------|---|-----|---|-----|---|------|--|-------------|
|                  | RIS                                     | THP | RIS                                     | THP | RIS                                     | THP  | RIS  | THP         |
| RP-HPLC          | 1                                       | 2   | 0.5                                     | 2   | 1.504                                   | 4.04 | 100.31±0.50                                      | 100.72±0.79 |
|                  | 1                                       | 2   | 1                                       | 4   | 2.014                                   | 6.01 | 100.7±1.16                                       | 100.79±0.58 |
|                  | 1                                       | 2   | 1.5                                     | 6   | 2.516                                   | 8.51 | 100.66±0.22                                      | 100.63±1.07 |

<sup>a</sup> n is number of determinations, <sup>b</sup> SD is a Standard deviation

## CONCLUSION

We have successfully developed a new simple RP-HPLC method for the simultaneous estimation of Risperidone and Trihexyphenidyl HCl combination in mixture using simple mobile phase buffer, methanol and acetonitrile. Rapidity and capability of qualifying very low concentration of respective drugs, made them useful for variety of analyses, including pure drug analysis, assay of formulations and stability studies analysis. The proposed method did not utilize any extraction step for recovering the drug from the formulation excipient matrixes and their by decreased the degree of error, time in estimation of the drugs and the overall cost of the analysis. The method was validated and found to be simple, sensitive, accurate, precise and economical. The proposed method could be applied for routine analysis in quality control laboratories.

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## REFERENCES

1. The Merck Index, 13th ed, Merck Research Laboratories division of Merck and company, NJ, USA, 2001, 1627, 1651.
2. British Pharmacopoeia, Vol. I, 4th Edn., 2002, 1, 1500.
3. Hardman, G., Limbid, L.E. and Gilman, A.G. The Pharmacological Basis of Therapeutics. Edn 10, McGraw Hill, 2001, 279.
4. Tripathi K.D. Essentials of Medical Pharmacology. Edn 5, Jaypee Brothers Medical publishers, New Delhi, 747, 150, 388, 391, 394, 396, 397.
5. Baldaniya SL, Bhatt KK, Mehta RS, Shah DA. RP-HPLC estimation of Risperidone in tablet dosage form. *Indian J. of Pharm. Sci* 2008; 70 (4): 494- 497.
6. Singhvi I, Goyal A. Visible spectrophotometric determination of Risperidone in tablet formulations accessed on [www.pharmainfo.net](http://www.pharmainfo.net) on 25/04/08
7. Huang MZ, Shentu J Z, Chen J C, Liu J, Zhou H. Determination of Risperidone in human plasma by HPLC-MS and its application to a pharmacokinetic study in Chinese volunteer. *J Zhejiang Univ Sci B* 2008; 9(2): 114-120.
8. Zhou Z, L xin, Kunyan L, Zhihong X, Zeneng C, Wenxin P, Wang F, Zhu R, Huande L. Simultaneous determination of clozapine, olanzapine, risperidone and quetiapine in plasma by high-performance liquid chromatography- electrospray ionization mass spectrometry. *J. Chromatogr B* 2004; 802 (2): 257-262.
9. Bartlett MG, Zhang G, Terry Jr. A V. Simultaneous determination of five antipsychotic drugs in rat plasma by high performance liquid chromatography. *J. Chromatogr B* 2007; 856(1-2): 20-28.
10. Subbaiah G, Singh S, Bhatt J. Liquid chromatography/tandem mass spectrometry method for simultaneous determination of risperidone and its active metabolite 9-hydroxyrisperidone in human plasma. *Rapid Communications in Mass Spectrometry* 2006; 20(14): 2109-2114.
11. Danel C, Barthelemy C, Azarzar D, Robert H, Bonte J P, Odou P, Vaccher C. Analytical and semipreparative enantioseparation of 9-hydroxyl risperidone, the main metabolite of RIS, using high-performance liquid chromatography and capillary electrophoresis. Validation and determination of enantiomeric purity. *J. Chromatogr A* 2007; 1163(1-2): 228-36.
12. Joshi A, Jeyaseelan C, Jugade R. Differential pulse polarographic studies of risperidone in pharmaceutical formulations. *Croat. Chem. Acta* 2006; 79(4): 541-544.
13. Song Z, Wang C. Sensitive chemiluminescence assay for Risperidone in pharmaceutical preparations. *J. Pharm. Biomed. Anal.*, 2004; 36(3): 491-494.
14. 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.
15. Handan HE, Gordon M, Bill W, Kamal K. Development and application of a specific and sensitive radioimmunoassay for trihexyphenidyl to a pharmacokinetic study in humans. *Journal of Pharmaceutical Sciences* 2006; 84(5): 561 – 567.
16. Mahadik KR, Aggarwal H and Kaul N. Simultaneous HPTLC estimation of trifluoperazine HCL, trihexyphenidyl HCL and chlorpromazine HCL in tablet dosage form. *J. Indian Drugs* 2003; 40(6): 340-341.
17. Mahadik KR, Aggarwal H and Kaul N. HPLC method for simultaneous estimation of Trihexyphenidyl hydrochloride and chlorpromazine hydrochloride from tablet dosage form. *J. Indian Drugs* 2002; 39(8): 441-445.
18. Pradas TN, Sivakumar M. HPLC quantification of a tri-component psychiatric formulation containing chlorpromazine, trifluoperazine and trihexyphenidyl. *J. Pharmazie* 1992; 47(3): 231.
19. Capka V, Xu Y and Chen YH. Stereoselective determination of trihexyphenidyl in human serum by LCESI-MS. *J. Pharm Biomed Anal.* 1999; 21(3): 507-17.



20. Vargas G, Havel J, Babackova L and Patocka J. Determination of drugs used as anti-Parkinson's disease drugs in urine and serum by capillary electrophoresis. *J. Capillary Electrophor* 1998; 5(3-4): 153-158.
21. Desage M, Rousseau-Tsangaris M, Lecompte D and Brazier JL, Quantitation of trihexyphenidyl from plasma using a mass-selective detector and electron impact ionization. *J. Chromatogr* 1991; 571(1-2): 250-256.
22. Owen JA, Sribney M, Lawson JS, Delva N and Letemendia F. Capillary gas chromatography of trihexyphenidyl, procyclidine and cycrimine in biological fluids. *J. Chromatogr* 1989; 494: 135-142.
23. Kintz P, Godelar B, Mangin P, Chaumont AJ and Lugnier AA. Identification and quantification of trihexyphenidyl and its hydroxylated metabolite by gas chromatography with nitrogen phosphorus detection. *J. Anal Toxicol.* 1989; 13(1): 47-49.
24. Lugnier A, Kintz P, Tracqui A, Ludes B, Mangin P, and Chaumont AJ. The determination of trihexyphenidyl and its hydroxyl metabolite by gas chromatography. *Acta Med Leg Soc.*1989; 39(1): 389-391.
25. Bargo E. GLC determination of trihexyphenidyl hydrochloride dosage forms. *J. Pharm Sci.* 1979; 68(4): 503-505.
26. Lovejoy DJ. A rapid gas chromatographic method for the determination of benzhexol hydrochloride. *J Chromatogr.*1971; 57(1): 137-138.
27. Papke E. Gravimetric methods for determination of Parkopan, Spasman and Dispasmol. On the analysis of some spasmolytic. *Pharmazie* 1968; 23(3): 148-150.
28. Bozsai G and Vastagh G. The polarographic determination of primidone and trihexyphenidyl (Benzhexol). *J. Pharm Zentralhalle Dtschl.* 1964; 103: 403-408.

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