World Journal of Pharmaceutical ReseaRch

Volume 3, Issue 2, 3230-3237. Research Article ISSN 2277 – 7105

SIMULTANEOUS ESTIMATION OF RISPERIDONE AND TRIHEXYPHENIDYL HYDROCHLORIDE BY USING ISOCRATIC REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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Article Received on
07 January 2014,
Revised on 13 January2014,
Accepted on 22 February 2014

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ABSTRACT

A simple, precise, fast Reversed phase High Performance Liquid Chromatographic (RP-HPLC) assay method by external standard method was developed for the determination of Risperidone and Trihexyphenidyl HCl in a solid dosage form. The chromatographic separation was performed using Phenomenex Bondalone 300 x 3.90 mm, 10 µL column. Analyses were resolved by using mobile phase 40:60 mixture of Ammonium acetate pH4.9: Acetonitrile at UV 220 nm as detection wavelength.

Keywords: RF-HPLC, simultaneous estimation, risperidone, trihexyphenidyl hydrochloride, isocratic.

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. Trihexyphenidyl Hydrochloride (THP) is an antidyskinetic and antiparkinson drug whose IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl-1-propanol[1]. Trihexyphenidyl is official in Indian Pharmacopoeia. Literature survey revealed that High Performance Liquid Chromatography, UV and High Performance Thin Layer Chromatography methods, have been reported for the estimation of Risperidone and Trihexyphenidyl Hydrochloride

individually and with other drugs in Pharmaceutical dosage forms, Risperidone and Trihexyphenidyl Hydrochloride are formulated together in the form of tablet. Literature Survey revealed that very few methods are reported for simultaneous determination of two drugs. Determinations of risperidone and 9-hydroxy risperidone, an active metabolite, in biological material were accomplished using LC/MS method.[5-8,10-13] In the present work, a specific stability indicating RP-HPLC method is described for simultaneous estimation of the two drugs from solid dosage form. Both the drugs were subjected to stress studies to ensure that the degradation products formed were separated from the drug peak. The proposed validated method can be used for routine analysis for assay determination.

EXPERIMENTAL

Chemicals

Risperidone and Trihexyphenidyl Hydrochloride are received from RPG Life sciences. HPLC grade acetonitrile from Merck, Germany and the double distill (HPLC grade) water.

Instrument

High Performance Liquid Chromatographic System of WATERS with Breeze software, which is equipped with isocratic 1515 HPLC pump, 20 µL fixed loop and Waters 2487 Dual λ Absorbance UV detector.

METHOD DEVELOPMENT

The UV absorption of Risperidone and Trihexyphenidyl Hydrochloride is carried at different wavelengths. Risperidone and Trihexyphenidyl Hydrochloride are showing optimum UV absorption at 220nm. Therefore wavelength 220nm is selected for the study and determination of Risperidone and Trihexyphenidyl Hydrochloride.

SELECTION OF STATIONARY PHASE

Chromatographic Conditions

The mobile phase used was Acetonitrile and 0.1% Ammonium Acetate solution pH 4.9 in the ratio 60:40 (Isocratic). The mobile phase was mixed thoroughly & filtered through 0.45 µ membrane filter paper and degassed. The column used was Phenomenex Bondalone RP C18 (300 x 3.90 mm, 10 µL). The elution was carried out with flow rate of 1.0 mL/min. The detector wavelength selected for the method was 220 nm. The Injection volume was 20uL. Under these specified chromatographic conditions Risperidone is eluted first followed by Trihexyphenidyl Hydrochloride.

Working Standard Solution

Standard solution was prepared by dissolving 30 mg of Risperidone working standard in 100mL volumetric flask. Add 60 mL diluent, sonicate for 2 minutes with intermittent swirling, cool, and make up to volume with 100 mL (solution 1). 20 mg of Trihexyphenidyl Hydrochloride working standard in 100 mL volumetric flask. Add 60 mL diluents, sonicate for 2 minutes with intermittent swirling, cool, and make up to volume with100 mL (Solution 2). Mix of 5 mL of the stock solution1and 5 mL stock solution 2 is further diluted l to 50 mL with diluents.

Estimation of Risperidone and Trihexyphenidyl Hydrochloride from Tablets

Weighed and crushed 10 tablets. Transferred powder (equivalent to 30 mg of Risperidone and 20 mg of Trihexyphenidyl Hydrochloride) into 50 mL volumetric flask. Added about 40 mL of diluents & sonicated for 15 minute with intermittent swirling. Cooled and diluted up to the mark with diluent and mixed. Filtered the solution through 0.45 µ filter paper and further diluted 5 mL to 50 ml with diluents and injected. The details of parameter for the peak are given in table-1.

TABLE-1

Parameters	Risperidone	Trihexyphenidyl Hydrochloride
Tailing Factor	1.16	1.0
Resolution between Risperidone and Trihexyphenidyl HCl	---	4.82
Retention time	5.1min	7.7 min
RSD of Replicate Injections	0.25	0.21
Correlation coefficient	0.9991	1.0

Specificity

i. **Interference of Blank**:- In the blank chromatograms, No peaks observed at the retention time of Risperidone and Trihexyphenidyl Hydrochloride indicates that no interference of any Blank peak. Hence the method is specific.

ii. **Degradation study:-** Risperidone and Trihexyphenidyl Hydrochloride are individually subjected for stress study. During the study both the compounds are treated with acid, base & Oxidation. Risperidone and Trihexyphenidyl Hydrochloride are also subjected Thermal, humidity & UV degradation. The degradation found in Risperidone. The degradation peak/s is not co-eluting with main peak Trihexyphenidyl Hydrochloride. The

degradation found in Trihexyphenidyl Hydrochloride. The degradation peak/s is not co-eluting with main peak Risperidone indicating that the method is specific.

Linearity and Range

Six different concentrations of Risperidone and Trihexyphenidyl Hydrochloride were prepared for linearity studies. Each concentration was injected in duplicate & the responses were measured as peak areas. The calibration curve obtained by plotting peak areas against concentration showed linearity in the concentration ranges 0.006 mg/mL to 0.036 mg/mL for Risperidone and 0.004 mg/mL to 0.024 mg/mL for Trihexyphenidyl Hydrochloride. The correlation coefficients were 0.9991 and 1.000 for Risperidone and Trihexyphenidyl Hydrochloride respectively.

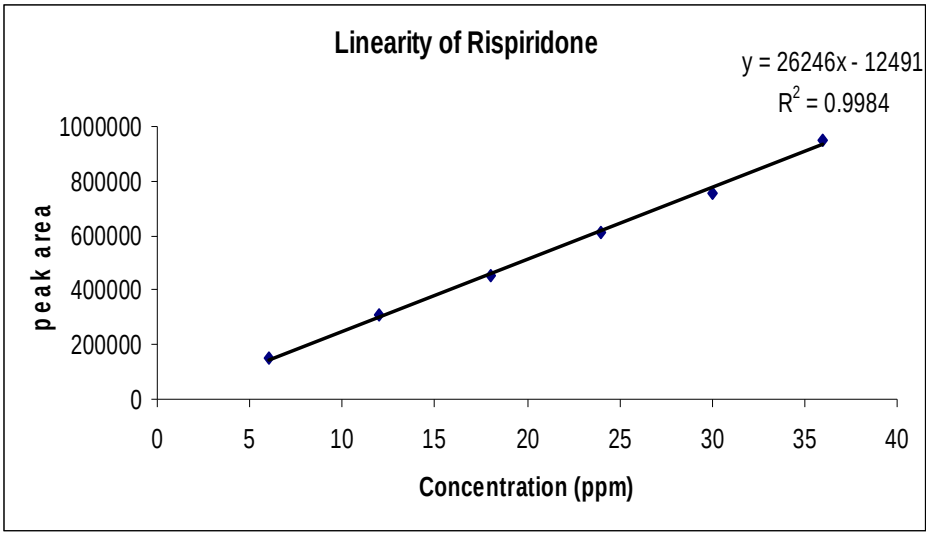

Fig. 1 Linearity chart of Risperidone

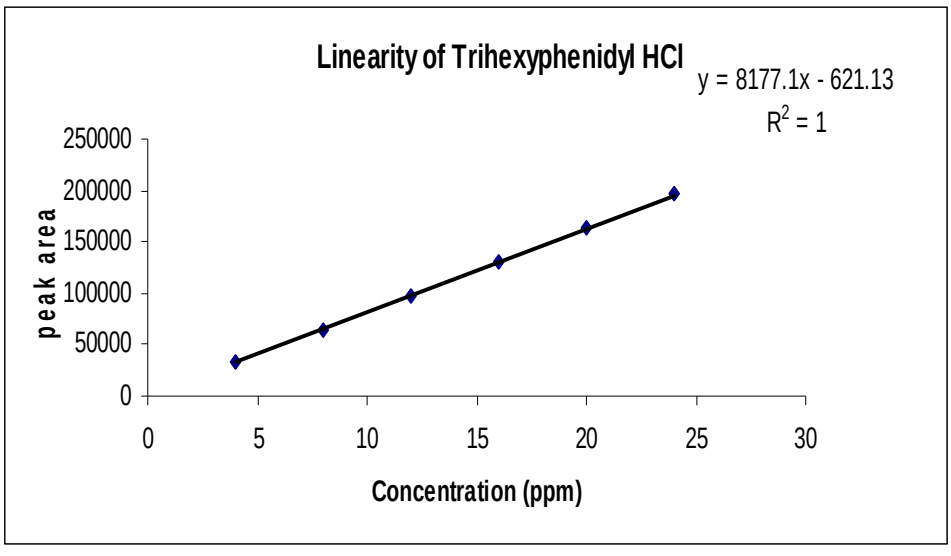

Fig.2 Linearity chart of Trihexyphenidyl Hydrochloride

Precision

The precision of the method was established by injecting six standard preparations using the proposed method. % RSD of Repeatability and Repeatability 1 for Risperidone is 0.25% and For Trihexyphenidyl HCl is 1.02 %.

Repeatability

The repeatability of the method was established by injecting six test preparations using the proposed method. The result obtained is shown in table 2.

TABLE 2.

Exp. No.	Content of Risperidone	Content of Trihexyphenidyl HCl
	% of label claim	% of label claim
1	98.29	99.90
2	98.90	100.20
3	99.58	99.38
4	100.03	100.65
5	100.30	100.61
6	100.25	100.26
Mean	99.56	100.17
SD	0.81	0.48
%RSD	0.82	0.47

Repeatability I

The Repeatability I is the intraday variation determined on successive day. The Related standard deviation for the assay of Risperidone and Trihexyphenidyl Hydrochloride is given in table 3.

TABLE 3

Sr. No.	Contents in % Risperidone	Contents in% Trihexyphenidyl HCl .
REP.	99.56	100.17
INT.DAY	99.21	101.63
Mean	99.39	100.90
SD	0.25	1.03
%RSD	0.25	1.02

Accuracy

The known amount of standard was added at three different levels (40%, 60% & 80%) in the test preparation. Each determination was performed in triplicate. The results of recovery analysis are presented in table 4 and 5.

TABLE 4

	Amount of Standard added (%)	Amount of Standard Recovered (%)	Recovery of RSP(%)
Spike I (n=3)	40	40.60	101.51
Spike II(n=3)	60	59.97	99.96
Spike III(n=3)	80	79.66	99.58

Mean Recovery = 100.35 %

TABLE 5

	Amount of Standard added (%)	Amount of Standard Recovered (%)	Recovery of THP(%)
Spike I (n=3)	40	39.99	99.99
Spike II(n=3)	60	59.80	99.67
Spike III(n=3)	80	79.11	98.89

Mean Recovery = 99.52 %

The mean accuracy For Risperidone is 100.35% and For Trihexyphenidyl HCl is 99.52%.

Stability of solution

Sample and standard is stable for 24 hours and RSD between the two values (Initial & after 24hours) of test samples is less than 2.0 %.

Robustness

The following alterations in the chromatographic conditions were carried out

a. change in composition of the mobile phase by ± 1 % with respect to organic phase
b. Change in the wavelength by± 2 nm
c. Change in the flow rate 1.0 ±0.1 mL/min.

The difference between the results obtained in accordance with the method and analysis by altered method was calculated and the system suitability test criteria were evaluated for each condition. The result of test samples shows %RSD within 1.0%. There is no impact on the system suitability criteria due to alteration of parameters in the method.

CONCLUSION

The proposed RP-HPLC method is Rapid. The method is precise, Linear and accurate for the simultaneous determination of **Risperidone and Trihexyphenidyl Hydrochloride** in solid dosage. Hence it can be adopted for the routine quality control analysis.

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