CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.7986524

Available online at: http://www.iajps.com ***Research Article***

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF ANTIPSYCHOTIC DRUG IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

Hirachand Narayankar[1],V M Waghulkar[2], A W Baitule[3]

Vidyabharati College of Pharmacy, C. K. Naidu road, Amravati, Maharashtra, India-444602

Abstract:

Analytical method was developed for the estimation of Risperidone drug substance by High Performance liquid chromatography. The chromatographic separation was achieved on C8 column (Zodiac 100, 150×4.6mm, 5um) at ambient temperature, the separation achieved employing a mobile phase consists of 0.1%v/v Ammonium formate in water: Methanol (30:70). The flow rate was 1.0 ml/ minute and ultra violet detector at 240nm. The average retention time for Risperidone found to be 3.52 min the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. Antipsychotic drug tend to block dopamine D2 receptor in dopaminergic pathways of brain this means that dopamine released in these pathway has less effect. Excess release of dopamine in the mesolimbic pathway has been linked to psychotic experience decreased dopamine released in other pathway are associated with psychotic episodes in schizophrenia and bipolar disorder.

Keywords:- *Risperidone, HPLC, C8, Ammonium formate, Validation etc.*

Corresponding author:

Hirachand Narayankar,

Vidyabharati College of Pharmacy,
C. K. Naidu road, Amravati, Maharashtra, India
444602.E-mail ID : hiranarayankar97@gmail.com

QR code

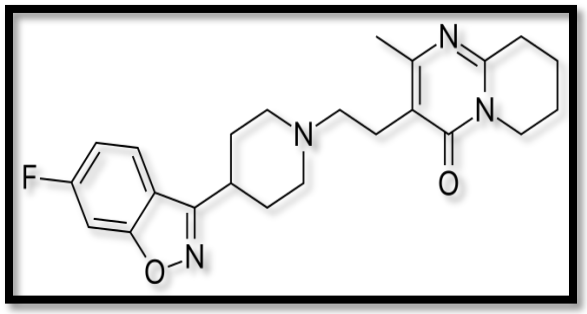
Please cite this article in press Hirachand Narayankar et al, ***Analytical Method Development And Validation For Quantitative Estimation Of Antipsychotic Drug In Pharmaceutical Dosage Form By RP-HPLC Method****., Indo Am. J. P. Sci, 2023; 10 (05).*

INTRODUCTION:

Risperidone is an antipsychotic medication. Its main mechanism of action involved in reduction of dopaminergic neurotransmitter in the mesolimbic pathway. Risperidone is used to treat the symptoms of schizophrenia. It is also used together with other medications to treat major depressive disorder in adults. Risperidone is a novel SGA with high 5-HT2A/D2 ratio.

Risperidone is chemically designated as 3-[2-[4-(6-fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9tetrahydropyrido[1,2-a]pyrimidin-4-one. Its molecular formula is C23H27FN4O2 and its molecular weight is 410.485. Risperidone is a white-to-off white powder. It is freely soluble in methanol and practically insoluble in water.

Structure of Risperidone



EXPERIMENT :-

Equipment :

The chromatographic technique performed on LC Solution as Software and UV detector, reversed phase C8 column (Zodiac-100 5µ, 150 mm × 4.6 mm) as stationary phase ,(9L250H, PCI) Sonicator, (ME-205, Mettler-Toledo) analytical balance ,Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

Material:-

Pharmaceutically pure sample of Risperidone were obtained as gift samples from Yarrow chemical Product. HPLC-grade Methanol was from Merck Life sciences pvt ltd. Ammonium formate (AR grade) was from sd fine chem, Mumbai.

Chromatographic Condition:-

The sample separation was achieved on a C8 (5 µ, 15 cm X 4.6 mm i.d.) Zodiac -100 column, aided by mobile phase mixture of 0.1%v/v Ammonium formate in water: Methanol (30:70). The flow rate was 1.0 ml/ minute and ultra violet detector at 240nm that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of Mobile phase :-

Buffer Preparation:

Take accurately 1ml of Ammonium formate in 1000mL of water

Mobile phase:

Then add 30 volumes of buffer and 70 volumes of Methanol mixed well and sonicated for 5 min.

Preparation of Standard Stock Solution:-

A 7mg of pure Risperidone were weighed and transferred to 50 ml of volumetric flask and dissolved in 7ml of acetonitrile: water: methanol (2:1:4). The flask was shaken and volume was made up to mark with methanol to give a primary stock solution containing 1000µg/ml (1000ppm). From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 100µg/ml (100ppm) of Risperidone.

Preparation of Sample Solution:-

A 7mg of pure Risperidone were weighed and transferred to 50 ml of volumetric flask and dissolved in 7ml of acetonitrile: water: methanol (2:1:4). The flask was shaken and volume was made up to mark with methanol to give a primary stock solution containing 1000µg/ml (1000ppm). From the above solution 1ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 100µg/ml (100ppm) of Risperidone.

RESULT AND DISCUSSIONS:-

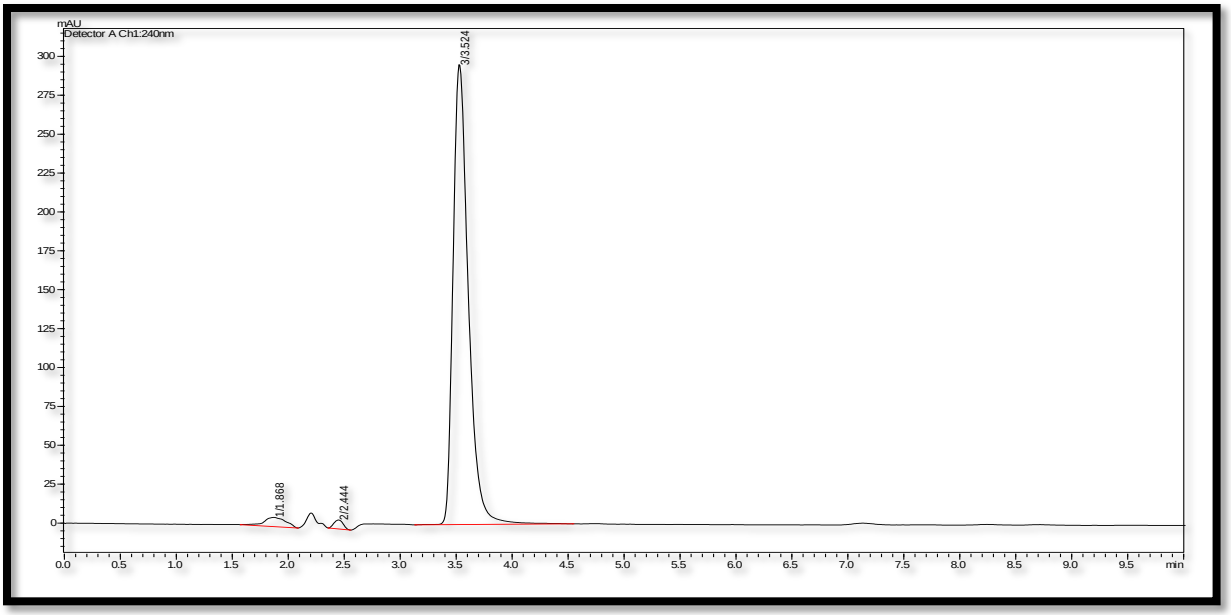
Determination of working wavelength (λmax)

7mg of the Risperidone standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml.

The above prepared solution is scanned in uv between 200-400 nm using methanol as blank. The λmax was found to be 240nm

After several initial trails with mixtures of acetonitrile, methanol, water, and buffers in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Ammonium formate as sovant B in water: Methanol (30:70). The flow rate was 1.0 ml/ minute brought sharp peaks.

The chromatogram was shown in Figure-1.



Peak No..	Peak Name	R.T.	Area	%Area	T.Plate	T.F.
1	Risperidone	3.52	2813768	96.14	3160.52	1.47

Figure No.1 Chromatogram of Risperidone

METHOD VALIDATION

Linearity:-

Linearity was studied by analyzing five standard solutions covering the range of 50-3.12 µg/ml of Risperidone. From the primary stock solution 1ml of solution pipette into 10 ml volumetric flasks individually and made up to the mark with methanol to give a concentrations of 50µg/mL , 25µg/mL ,12.25µg/mL ,6.25µg/mL and 3.12µg/mL of Risperidone.

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

The mean regression equations were found as y=30616x+27326

R^2 =0.9992

y=mx ±c

Where,

y= the peak area of drug

m= the slope

Table No.1:- Linearity data of risperidone

Name of Drug**: risperidone**		
S. No.	Concentration (µg.mL^{-1})	Area
1	50	1545708
2	25	811745
3	12.5	429776
4	6.25	205497
5	3.125	123307
6	1.5625	61653.5
Regression Equation		y=30616x + 27326
Correlation coefficient (R^2)		0.9992
Std. error of intercept		10102.02409
Std. Dev. Of intercept		24744.80439
LOQ		8.08 µg/ml
LOD		2.42 µg/ml

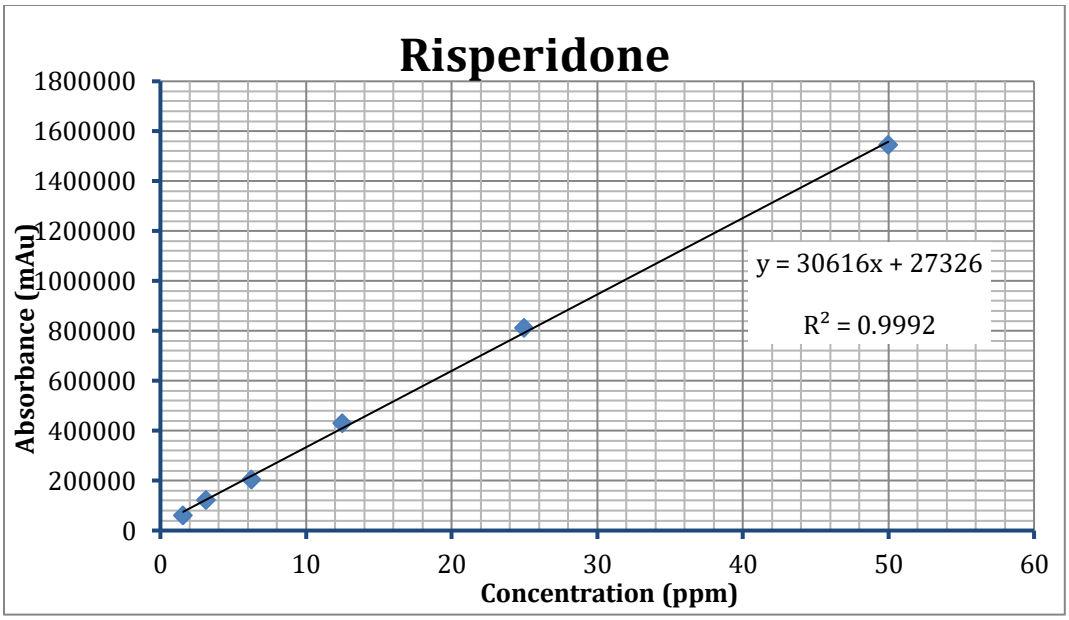


Figure No. 2 Linearity Graph of Risperidone

Repeatability:-

Implementing the procedure mentioned under experimental section , the standard reference sample of risperidone was tested for six injections within the same day. The % RSD was calculated and found it is less than 2%. It represents the proposed method accepted all basic characteristics of ICH guidelines

The standard Deviation and % Relative Standard Deviation obtained are listed below table no.

Table No.2; Repeatability data of risperidone

S. No.	Drug Name; risperidone
	Peak Area; Conc. 100 ppm
1	2813768
2	2701589
3	2771991
4	2807891
5	2771191
6	2829629
Mean	**2782677**
STD. DEV.	**46104.81301**
RSD (%)	**1.66**

Precision:-

The precision of the instrument was checked by repeated injections and measurement of peak areas, Standard Deviation and % Relative Standard Deviation of solutions can be calculated by Intra-day and Inter-day Precision (n = 6) for, 100 µg/ml of Risperidone without changing the parameter of the proposed chromatographic method.

Table No.3; Intraday precision data of Risperidone

Drug Name: Risperidone					
S. No.	**Concentration (ppm)**	**Area**	**Average**	**Std. Deviation**	**%RSD**
1	100 ppm	2813768	2809850	3393.09	0.12
	100 ppm	2807891			
	100 ppm	2807891			
2	100 ppm	2701589	2748257	40417.65	1.47
	100 ppm	2771991			
	100 ppm	2771191			
3	100 ppm	2701589	2724263	42550.21	1.56
	100 ppm	2697851			
	100 ppm	2773348			
	Range of % RSD				**0.12-1.56**

Table No.4; Interday precision data of Risperidone

Drug Name: Risperidone					
S. No.	**Concentration (ppm)**	**Area**	**Average**	**Std. Deviation**	**%RSD**
Day 1	100 ppm	2813768	2809850	3393.09	0.12
	100 ppm	2807891			
	100 ppm	2807891			
Day 2	100 ppm	2702316	2730453	28758.95	1.05
	100 ppm	2759796			
	100 ppm	2729248			
Day 3	100 ppm	2796921	2745124	53186.08	1.94
	100 ppm	2690650			
	100 ppm	2747802			
	Range of % RSD				**0.12-1.94**

Accuracy:-

The accuracy of the method was determined by calculating the recoveries of Risperidone by analyzing solutions containing approximately 80%, 100% and 120% of the working strength of Risperidone.

Also by using accuracy data of Risperidone data from API to calculate the accuracy of Marketed Formulation of drug.

The percentage recovery results obtained are listed in Table 5

Table No 5; Accuracy data of risperidone

Drug Name: risperidone			**Drug content: 25 mg**		**Marketed formulation: Dovopine-25 Tablet**		
Std. conc. (%)	**Std. (ppm)**	**Peak area**	**Drug (%)**	**Drug (ppm)**	**Peak area**	**Avg. peak area**	**Drug Rec. (%)**
100%	100 ppm	2771191	80	80	2204090	2203210	99.38%
				80	2202330		
			100	100	2697851	2735599.5	98.72%
				100	2773348		
			120	120	3608002	3611376	108.60%
				120	3614750		
			Drug recovery Range (%) as per ICH = 100±10%				98.72 % - 108.60%

Robustness:-

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.1 ml/min.

The results were shown in (Table no.

Table No. 6; Robustness data of risperidone

Variables	**Risperidone**			
	t_R (min)	**k'**	**T_f**	**N**
Flow rate (+0.2 mL.min^{-1})	3.21	0.89	1.43	3271
Flow rate (-0.2 mL.min^{-1})	3.9	0.89	1.46	3548
CH3OH (+2%)	3.36	0.77	1.47	3295
CH3OH (-2%)	3.56	0.93	1.41	3787
Temperature (+2˚C)	3.51	0.89	1.46	3403
Temperature (-2˚C)	3.51	0.89	1.46	3388
Mean ± S.D.	3.51±0.23	0.88±0.05	1.45±0.02	

Limit of Detection and Limit of Quantification :-

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 δ/S ……. (1)

LOQ =10 δ/S …….. (2)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table No. 7: LOD and LOQ Values Calculated From Calibration Curve

Sr.No.	**Parameter**	**Calculated Value**
1.	LOQ	8.08
2.	LOD	2.42

SUMMARY:-

Analytical Method development and validation parameters was successfully applied for estimation of Risperidone. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence, the method was found to be simple, accurate, precise, economic and reproducible.

So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Risperidone in bulk drug as well as in formulations.

CONCLUSION :-

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Risperidone was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries and approved testing laboratories.

REFERENCE:-

1. Ashutosh Kar, Pharmaceutical Analysis-I, New International Ltd. Publishers, 1st Edition, 2020.
2. Tripathi Medical Pharmacology, 8th edition New Delhi jaypee brothers medical Published 2019: 462-464.
3. Sharma B. K., Instrumental Methods of Chemical Analysis, 19th Edition, Meerut: Krishna Prakashan Media (P) Ltd., 1 January 2014, Page No. 1-4.
4. Kenneth A. Connors, A Textbook of Pharmaceutical Analysis. 3rd Edition, Published by John Wiley & Sons, 1967, Page No.1-3.
5. Chatwal G. And Anand K., Instrumental Method of Chemical Analysis, 5th Edition Himalaya Publishing House Mumbai, 2007.
6. S Koog, D. A. Holler, F.J. Timonthy, A. Nieman, Principle of Instrumentation Analysis, 5th Edition, Sauders College Publication, London 2004, Page No. 621-659.
7. Aguilar M-1, editor, HPLC of Peptides and Proteins. Est ed. New Jersey: Humana Press; 2004; 9-22 p.
8. Ahuja S. Rasmussen H, editors. Hplc Method Development For Pharmaceuticals. 2007th ed. Elsevier Inc.: 2007, P. 1-11.
9. https://pubchem.ncbi.nlm.nih.gov/compound/Risperidone
10. https://en.wikipedia.org/wiki/risperidone