Research Article

RP-HPLC Method Development and Validation for Estimation of Risperidone in Bulk & Dosage Forms.

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ABSTRACT

A simple, rapid, sensitive, accurate and specific reverse phase high performance liquid chromatographic method was development and validation for estimation of Resperidone in bulk and dosage forms. The method was validated as per ICH guidelines. A phenomenex C18 (250mm×406mm,5µm) was used with mobile phase water 35: Methanol 65 (PH 5.5 aq.phase) & detection was carried out in 276nm. The analysis was performed with retention time 3.79min at a flow rate 0.9ml/min. A good linear relationship (r^2= 0.999) was observed between the concentration of RSPD and mean peak areas. The method was validated for precision, limit of detection, limit of quantitaion, linearity, robustness and ruggedness. The limit of quantitation and limit of detection was found to be 7.53µg/ml and 2.48µg/ml respectively. Recovery of RSPD was found to contain average 96.13-104.57% w/w. Proposed HPLC method was sensitive and reproducible for the analysis of RSPD in pharmaceutical dosage form (tablet) with short analysis time.

Keywords: RP-HPLC, Risperidone, Validation, Bulk & Dosage forms.

INTRODUCTION

Resperidone chemical name is 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. Risperidone is an atypical antipsychotic drug used for the treatment of schizophrenia, the mixed and manic states associated with bipolar disorder, and irritability in children with autism. It has high affinity for D_2 dopaminergic receptors. It has actions at several 5-HT (serotonin) receptor subtypes. These are 5-HT_{2C}, linked to weight gain, 5-HT_{2A} linked to its antipsychotic action and relief of some of the extrapyramidal side effects experienced with the typical neuroleptics through action at 5-HT_{1A}. Like other 5-HT_2 antagonists, Risperidone also binds at alpha (1)-adrenergic receptors and, to a lesser extent, at histamine H-1 and α-2 adrenergic receptors. The purpose of this investigation was to develop and validate a method using simple, sensitive, rapid, accurate and specific reverse phase RP-HPLC assay.

MATERIALS AND METHODS

Instrumentation

Qunatitative HPLC was performed on high performance liquid chromatography equipped water™ 486 with UV detector.

Chromatographic conditions

Chromatographic analysis was performed on Phenomenex C18 (250mm×4.6mm) and particle size is 5µm. The mobile phase used in this study was water:methanol (35:65,aq.phase) and PH adjusted to 5.5 with ortho-phosphoric acid. The mobile phase was filtered through 0.45µm whatman filter paper. The elute monitored at 276nm using UV detector. The retention time of the drug was found to be 3.799min.

Preparation of standard stock solution

Accurately weighed 10mg of Risperidone and transferred to 10ml volumetric flask containing a mixture of Water:Methanol (35:65). The volume was made up to the mark using same mixture of mobile phase. The resulting stock solution (1000µg/ml) was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min.

Preparation of working solution

Aliquot solution of 0.5ml was pipette out from the above standard stock solution and transferred to 10ml volumetric flask. It was then diluted up to the mark using mobile phase to obtain resultant solution of 50µg/ml. This working solution was sonicated for three cycles each of 10 min.

System suitability

The solution was prepared as per procedure of working solution. Six repeated injections were made at following chromatographic conditions. The results were recorded for retention time, area, theoretical plates and tailing factors and evaluated for criteria for system suitability testing as per ICH guideline Q2R1.

Method Validation

Linearity

From stock solution aliquots of 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml were pipetted out and diluted up to 10 ml to obtain 40, 50, 60, 70, 80, 90 and 100µg/ml resultant solutions respectively. Calibration curve was constructed between concentrations versus peak area. Results were recorded for equation of line, correlation coefficient and intercept were determined.

Precision

From the calibration range three QC standard were decided viz. 45, 75 and 95µg/ml as LQC, MQC and NQC respectively. The solutions for QC standards were prepared by diluting stock solution of 0.45, 0.75, and 0.95ml solutions up to 10ml. Area of each QC standard was recorded for intraday and inter day precision in three replicates as per ICH guidelines Q2R1. Results were recorded to calculate mean, SD, %RSD.

Accuracy

% Accuracy was determined from the observations of precision study using following formula. Limit for % accuracy is NMT 5% RSD.

% Accuracy = (Mean measured concentration/Nominal Concentration) x 100

Robustness

50µg/ml solution was selected for robustness study for the parameters like variation in aqueous phase pH and temperature etc.

%Recovery

$$\% \text{ Recovery} = \frac{Sample\ Area}{Standard\ Area} \times \frac{Standard\ Concentration}{Sample\ Concentration} \times 100$$

LOD and LOQ

Limit of detection (LOD) and Limit of quantitation (LOQ) was determined from the following formulae.

$$LOD = \frac{3.3 * STEYX}{Slope}$$

$$LOQ = \frac{10 * STEYX}{Slope}$$

Where, STEYX = Standrad error of Y and X axis.

RESULTS AND DISCUSSION

System suitability

Table 1: Data obtained for different system suitability parameters

Sr. No.	Parameter	Mean observations	SD	%RSD	Acceptance criteria	Inference
1	Peak Area	814530.50	14307.59	1.76	< 2	Pass
2	Retention time	3.79	0.01	0.16	< 0.5	Pass
3	Number of Theoretical plates	5247	--	--	> 2000	Pass
4	Tailing factor	1.12	--	--	< 2	Pass

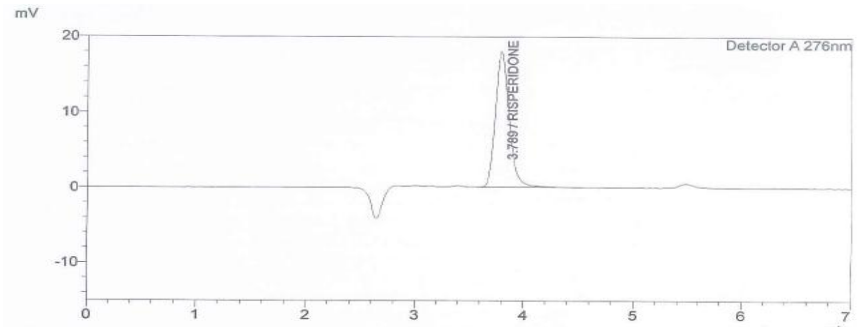

Figure 1: RSPD chromatogram obtained for system suitability testing

Linearity

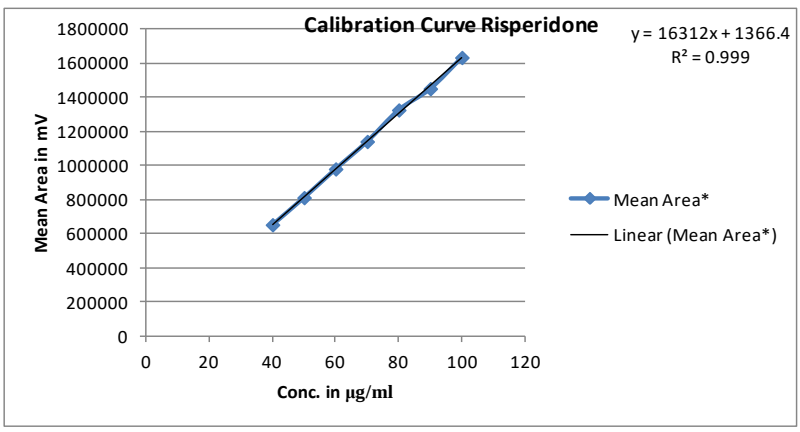

Figure 2: Calibration curve of RSPD indicating linearity of the method

Precision

Table 2: Results obtained for inter and intra-day precision study

Conc. (µg/ml)	Intra-day precision			Inter-day precision		
	Mean area ± SD	% RSD	Inference	Mean area ± SD	% RSD	Inference
45	732450.00 ± 5460.62	0.75	45	732450.00 ± 5460.62	0.75	45
75	1237866.67 ± 17105.44	1.38	75	1237866.67 ± 17105.44	1.38	75
95	1581450.33 ± 12181.26	0.77	95	1581450.33 ± 12181.26	0.77	95

Robustness

Table 3: Results obtained in Robustness experiment for pH variation

pH	Standard Conc. (µ)	Mean peak area*	Mean measured conc. (µg/ml)	% Assay	Inference (Compendial standard 90-110 %w/w)
5.00	50	888459	54.38	108.76	Pass
5.5	50	838827	51.34	102.68	Pass
6.0	50	759361	46.46	92.93	Pass

Table 4: Results obtained in Robustness experiment for pH variation

Temperature	Standard Conc. (µ)	Mean peak area*	Mean measured conc. (µg/ml)	% Assay	Inference (Compendial standard 90-110 %w/w)	Temperature
20°C	50	843842	51.64	103.29	Pass	20°C
25°C	50	804194	49.21	98.43	Pass	25°C
30°C	50	778430	47.63	95.27	Pass	30°C

Recovery

Table 5: Results obtained for percent recovery experiment

% Recovery Level	Conc. of standard spiked (µg/ml)	Conc. of sample (µg/ml)	Mean peak Area*	Amount recovered (µg/ml)	% Recovery	Inference (Standards 90-110%w/w)
80	50	40	1437748	38.23	96.13	Pass
100	50	50	1615472	49.12	98.77	Pass
120	50	60	1832607	62.43	104.57	Pass

LOD & LOQ

Table 6: Results obtained for LOD and LOQ

Standard Drug Solution	LOD (µg/ml)	LOQ (µg/ml)
Risperidone	2.48	7.53

CONCLUSION

Eventually it was concluded that the proposed HPLC method was sensitive and reproducible for the analysis of Risperidone in pharmaceutical dosage form (tablet) with short analysis time. Also, the method proved to be economic as it involved the simple and monetary mobile phase.

REFERENCES

1. Anthony G.K., Ramya Sri.S., Development et al.and validation of RP-HPLC method for simultaneous estimation of risperidone and haloperidol in tablet dosage forms, IJPDA, Vol.2(9), 2014,pp. 666-671.
2. Sanka Krishna, Divan Prakash V.et al, Method development and validation for estimation of risperidone in novel liquidsolid formulation by RP-HPLC, Der pharmacia letter, vol.6(1), 2014, pp-166-174.

3. Ashour Safwan, Kattan Nuha, Sensitive et al, Method for the quantitative determination of risperidone in tablet dosage form by HPLC using chlordiazepoxide as internal std, Int.j.biomed sci, vol.9(2), 2013, pp-91-97.
4. Cheng-tung chen, Cheng-wei cheng et al, Development and validation of RP-UPLC method for determination of related substance in risperdal consta, chromatogr.sep.tech, vol.6, 2015, pp-6.
5. Bhusnure O.G., Shinde N.G, Analytical method development and validation of risperidone and benzoic acid in oral solution,Int.J.of pharma and Bio science, vol.6(3), 2015, pp-33-41.
6. K.Rambhau, A reverse phase HPLC method development and validation for the determination of paliperidone in pure and dosage forms, chem. Sci. trans vol.2(1), 2013, pp-41-46.
7. Kumar Sravan, Development of analytical method for risperidone by UV spectrophotometry, International journal of pharma science and research, vol.1(2), 2010, pp-122-126.
8. Suthar A.P., Dubey S.A., Determination of risperidone and forced degradation behavior by HPLC in tablet dosage form, Int.J.pharmaTech res.,vol.1(3), 2009, pp-568-574.
9. http://www.chromacademy.com/lms/sco2/Theory_Of_HPLC_Chromatographic_Parameters. pdf cited on 23/05/2018.

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