ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Validated New Reverse Phase High Performance Liquid Chromatographic Method for the Determination of Risperidone in Bulk and Tablet Dosage Forms

Yunoos Mohammad*, B.Pragati Kumar, Subhani

* Nimra College of Pharmacy, Jupudi, Ibrahimpatnam, Vijayawada-521456 (A.P) India.

ABSTRACT

A simple, rapid, accurate, precise and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of Risperidone in bulk and tablet dosage forms. The quantification was carried out using cyberlab capcell pak, C_{18} column (ODS) 250 × 4.6mm i.d., 5µm particle size in a isocratic mode, with mobile phase comprising Acetonitrile and Methanol in the ratio 20:80 (%v/v).The flow rate was 1.2 ml/min and the detection was carried out at 239 nm. The retention time of the drug was found to be 4.51 min and the method produced linear response in the concentration range of 20-100 µg/mL (r~0.9997).The recovery studies were also carried out and % RSD from reproducibility was found to be 0.776.The proposed method was statistically evaluated and can be applied for routine quality control analysis of Risperidone in Tablets.

Key words: RP-HPLC, Risperidone, Tablets, validation.

*Corresponding author
Email: yunoos_vja@yahoo.co.in.
Telephone no.0866-2881854.

ISSN: 0975-8585

INTRODUCTION

Risperidone (Fig.1) 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. The molecular formula is $C_{23}H_{27}FN_4O_2$ and its molecular weight is 410.49.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 I Disorder in adults and in children and adolescents aged 10-17 years. It is a selective monoaminergic antagonist with high affinity for $5HT_2$, D_2 and H_1 histaminergic receptors [1].

Literature survey reveals that few LC/MS methods for the estimation of Risperidone in biological fluids such as plasma [2,3], visible spectrophotometric, few HPLC and chiral chromatography [4-6] methods for the determination of Risperidone in tablets were reported.

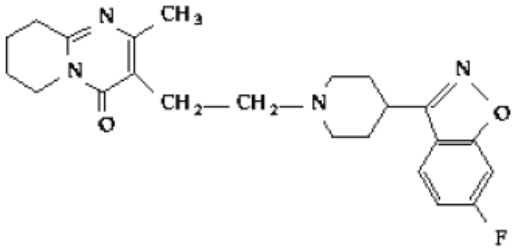

Fig.1: Structure of Risperidone.

The objective of this study is to develop a simple, fast, selective, accurate, precise and sensitive RP-HPLC-UV method for the determination of Risperidone in bulk and in pharmaceutical dosage forms (Tablets) suitable for routine quality control analysis.

MATERIALS AND METHODS

Reagents and Chemicals

Risperidone working standard was received as gift sample from Orchid Chemicals and Pharmaceuticals Ltd, Chennai, India. Don-2mg tablet, manufactured by Crescent Therapeutics Ltd.HPLC grade Methanol and HPLC grade Acetonitrile were purchased from Merck, Mumbai.

Instruments and Chromatographic conditions

The method development study was carried out isocratically on a high performance liquid chromatograph using Cyber lab LC-100 separation module equipped with a Rheodyne injector 7725i, Single pump, 20µL sample loop, 25µL Hamilton syringe and detection was carried out using Ultraviolet detector.Cyberlab Digital balance was used for weighing purpose.

Chromatographic separation was carried out at room temperature with Capcell Pak ODS C_{18} (250 × 4.6 mm with 5 µm particles) column. Mobile phase containing both Acetonitrile and Methanol (20:80%v/v), were filtered through 0.45 µ membrane filter and degassed in a sonicator for 10 min before use. The flow rate of mobile phase was maintained at 1.2 ml/min and detection was done using UV detector at 239 nm.The injection volume of both standards and samples were 20 µL (100µg/mL).

Preparation of standard

A standard stock solution containing 1 mg/ml of Risperidone was prepared by completely dissolving 100 mg of pure drug of Risperidone in 100 ml of methanol. A working standard solution containing 100 µg/ml was prepared by diluting 2.5 ml of standard stock solution (1000 µg/ml) into 25 ml of methanol.

ISSN: 0975-8585

Linearity and Calibration

Linearity solutions ranging of 20.40,60,80 and 100 µg/ml of Risperidone were prepared by diluting 0.2-1.0 ml of the above standard stock solution (1000 µg/ml) in 25 ml volumetric flask with methanol. Initially the mobile phase was pumped for 10 min to saturate the column there by to get the baseline corrected as shown in Fig.2.Then solutions prepared as above were filtered through 0.45 µ m membrane filter and then 20 µL of filtrate was injected each time into the column at a flow rate of 1.2 mL/min.Evaluation of the drug was performed with UV-Visible detector at 239 nm after the drug solution of 20 µg/ml in 0.1 N HCL was scanned in UV-Visible spectrophotometer SL-164 in the range of 200-350 nm against 0.1 N HCL as blank and found λ_{max} at 239 nm as show in Fig.3.Peak area was recorded for all the peaks. The plot of peak area vs. the respective drug concentration gives the calibration curve. The retention time of Risperidone standard was found to be 4.51 minutes as shown in Fig.4

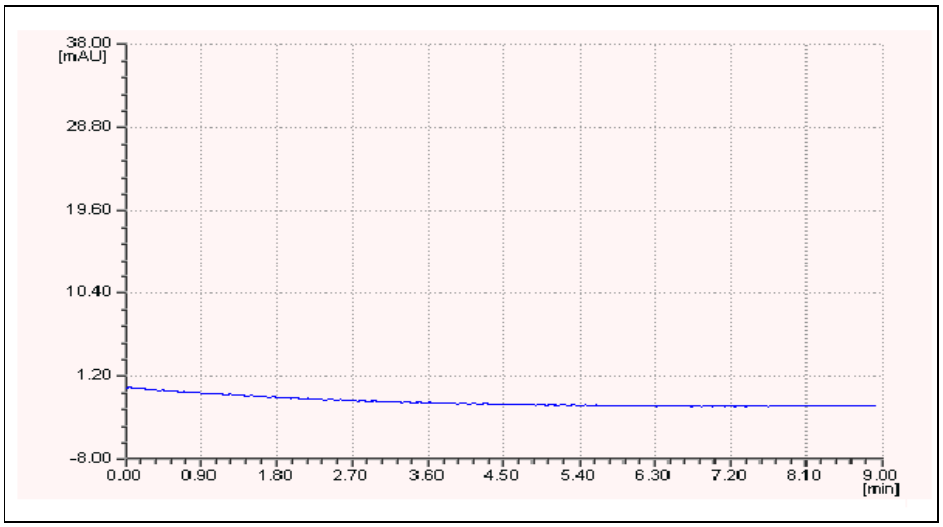

Fig.2.Chromatogram of Risperidone blank

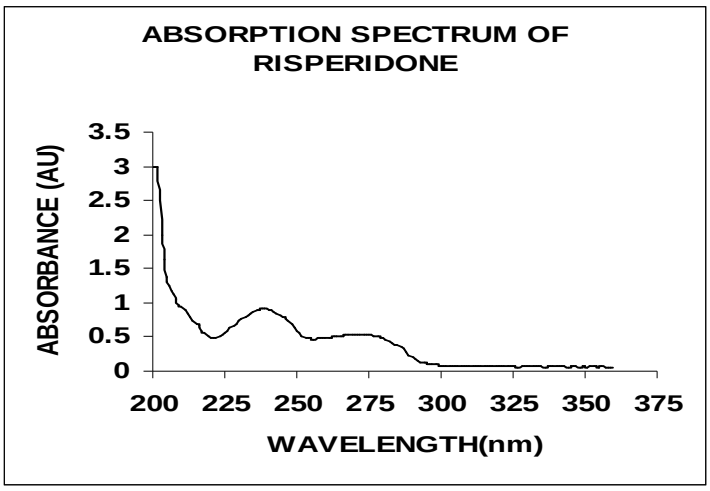

Fig.3.Absorption spectrum of Risperidone (20 µg/ml) in 0.1N HCL

ISSN: 0975-8585

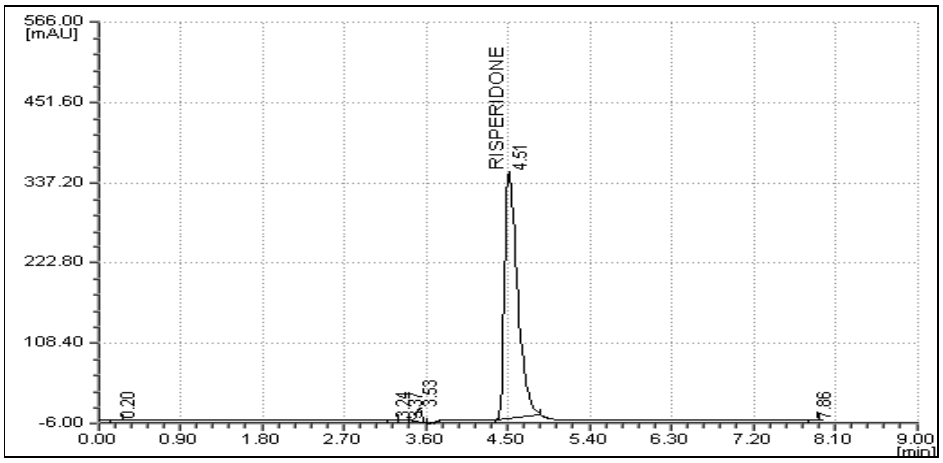

Fig.4.Chromatogram of Risperidone standard

Analysis of Risperidone in tablet dosage forms

Twenty tablets each containing 2 mg Risperidone were accurately weighed and powdered. A quantity of the powder equivalent to 10 mg was taken into a 50 mL volumetric flask and 25 mL methanol was added. Then solution was sonicated for 15 mins, dissolved and then made upto the volume with the methanol and filtered through a 0.45 µ membrane filter .From the filtrate, 5 ml of this solution was then diluted to 10 ml with methanol. Then 20 µL of the solutions were injected each time into the column at a flow rate of 1.2 mL/min. The retention time of Risperidone samples were found to be 4.55 and 4.49 minutes as shown in Fig.5 and Fig.6.

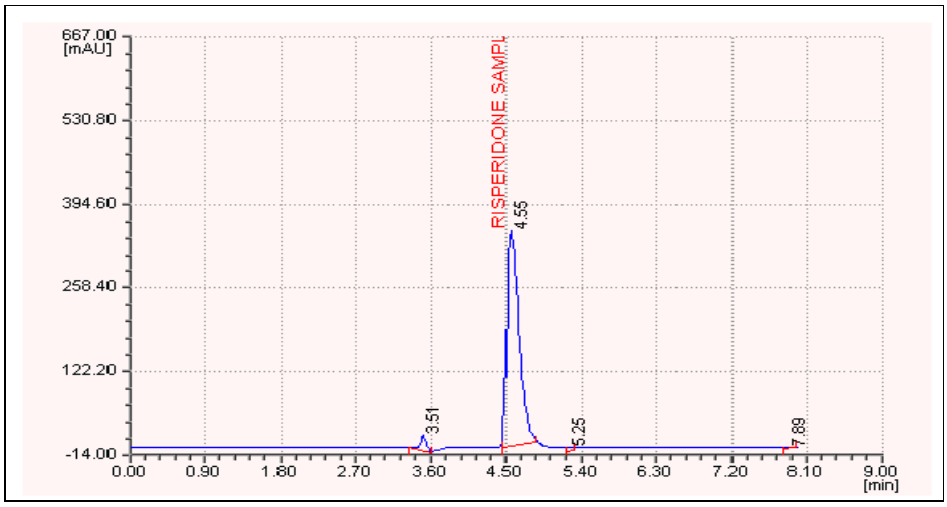

Fig.5.Chromatogram of Risperidone sample 1 (in tablet)

ISSN: 0975-8585

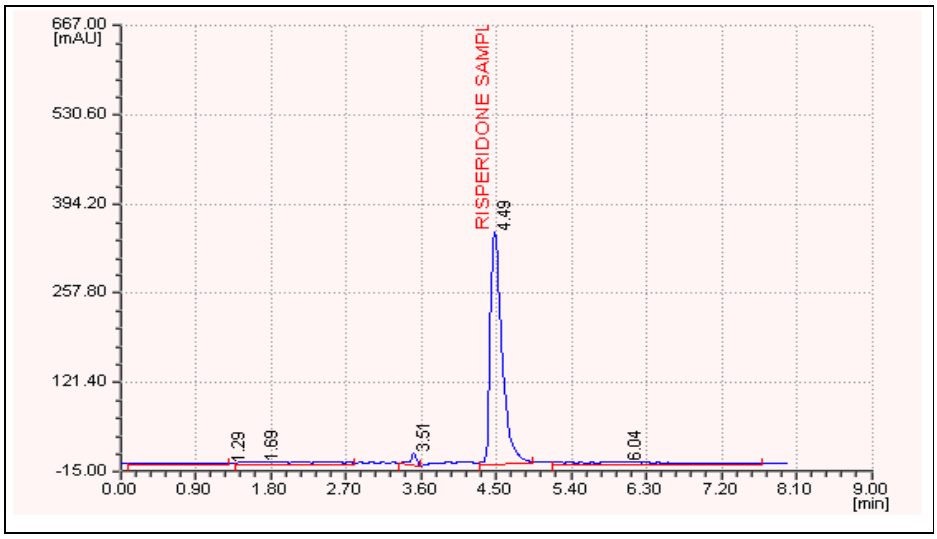

Fig.6.Chromatogram of Risperidone sample 2 (in tablet)

RESULTS AND DISCUSSION

Method Development

The present study was carried out to develop a simple, fast, accurate and precise RP-HPLC method for the analysis of Risperidone in bulk and in tablet dosage forms. For the determination of Risperidone, different compositions of mobile phases were employed. Initially, a mobile phase consisting of Methanol and Water in the ratio of 50:50 %v/v was tried. Then the composition of mobile phase was changed to Acetonitrile and Water in the ratio of 70:30 %v/v but in these conditions broad peak shape and tailing were observed. Finally the ratio was changed to 80:20 %v/v (Methanol: Acetonitrile), where Risperidone was eluted at around 4.51 min with symmetric peak shape and shorter retention time. The results of system suitability parameters were given in the Table-1.

Table-1. Results of System Suitability Parameters of Risperidone in standard and in tablet formulations:

S.No.	Parameter	Standard	Sample 1	Sample 2
1	Retention Time (Min)	4.51	4.55	4.49
2	Peak area response	351801.2	349746.6	350918.9
3	Theoretical plates(n)	4061.9	4182.63	4134.3
4	Tailing factor(t)	1.94	1.97	1.63

Method Validation

Linearity

Linearity was determined from calibration graph plotted using peak area response versus concentration of the standard solutions and it was found to be obeyed in the concentration range of 20-100 µg/ml with a good linear relationship (r=0.9997) as shown in Fig.7.The regression curve was constructed by linear regression fitting and its mathematical expression was y=3467.905x-964.9 (where y is the peak area and x is the concentration of Risperidone).

ISSN: 0975-8585

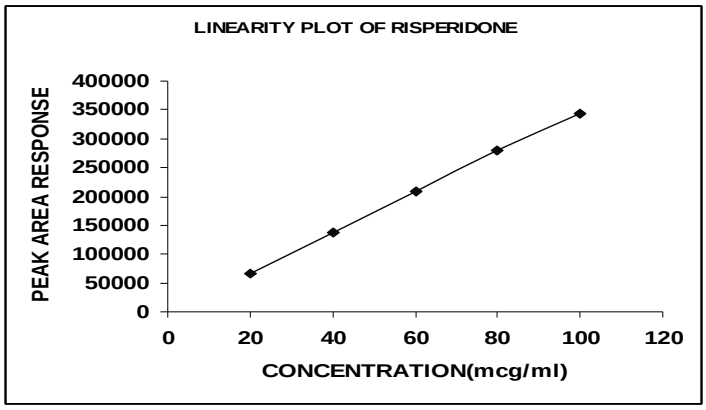

Fig.7. Linearity Graph of Risperidone.

Precision

Precision of the developed method was studied by repeatedly injecting Risperidone standard and sample solutions for six times (n=6). The % RSD was found to be 0.776 and 0.643 respectively.

Assay

The drug content (Assay) in the tablets was quantified using the proposed RP-HPLC method. The mean amount of Risperidone in two different brands of tablet dosage forms is shown in Table-2.The tablets was found to contain 99.41 % and 99.75 %of the drug.

Table-2.Results of Assay in Marketed formulation

S.No.	Brand	Standard Peak Area	Sample Peak Area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay
1	RISDONE	351801.2	349746.6	2	1.988	99.41
2	RISCLAM	351801.2	350918.9	2	1.995	99.75
				Mean	99.58	
				% RSD	0.24	

Accuracy

The accuracy of the method was evaluated by performing recovery studies by analyzing three different concentration levels ranging from 50-150% of the test concentrations. To ensure the reliability of the method, mixing a known quantity of standard drug with the pre analyzed sample formulation and the contents were reanalyzed by the proposed method. The percentage recovery was calculated and results are presented in Table-3.

Table-3.Results of Accuracy (Recovery studies, n=3)

S.no.	Test Concentration	Amount added (µg/ml)	Amount Recovered (µg/ml)	Average % Recovery	% RSD
1	50%	5	104.94	99.94	0.33
2	100%	10	110.02	100.01	0.49
3	150%	15	114.95	99.95	0.21

The developed method was validated according to the standard procedure and the summary of results obtained are presented in Table-4.

ISSN: 0975-8585

Table-4.Summary of Validation Parameters

S.no.	Parameters		Result
1	Linearity	Range (µg/ml)	20-100 µg/ml
		Correlation Coefficient (r)	0.9997
		Slope (b)	3467.905
		Intercept (a)	-964.9
		Regression Equation (y=a+bc)	3467.905 x -964.9
2	System Precision (n=6)	% RSD	0.776
3	Method Precision (n=6)	% RSD	0.643
4	Accuracy	Mean % Average Recovery	99.95
5	Assay	Mean % Assay	99.58
6	Specificity	Specific	No interference of other peak

CONCLUSION

In this present study an attempt has been made to develop RP-HPLC method for the determination of Risperidone in tablet dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. From the forgoing it is concluded that the method developed is simple, rapid, selective and precise and hence suitable for application in routine quality control analysis of pharmaceutical preparations.

ACKNOWLEDGEMENTS

The authors are thankful to Orchid Chemicals and Pharmaceuticals Ltd., Chennai, India for providing the gift sample of Risperidone and also thankful to Nimra College of Pharmacy, Ibrahimpatnam, Vijayawada for providing the necessary facilities to carry out the research work.

REFERENCES

[1] Stahl SM.Essential psychopharmacology, 2 nd ed.cambridge: Cambridge University Press; 2000, pp.425-34.
[2] Huang MZ, Shentu JZ, Chen JC, Liu J, Zhou H, J Zhejiang, Univ Sci B 2008;9(2): 114–120.
[3] Zhou Z, L Xin, Kunyan L, Zhihong X, Zeneng C, Wenxin P, Wang F, Zhu R, Huande L. J chromatogr B 2004;802 (2): 257-262.
[4] Singhvi I, Goyal A, "Visible Spectrophotometric determination of risperidone in tablet formulations" accessed on www.pharmainfo.net on 25/04/08.
[5] Baldaniya S L, Bhatt K K , Mehta R S, Shah D A, Ind J Pharm Sci 2008;70 (4): 494-497.
[6] Danel C, Barthelemy C, Azarzar D, Robert H, Bonte J P, Odou P, Vaccher C, J chromatogr A., 2007, 1163 (1-2), 228-36.