Research Article **CODEX: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

METHOD DEVELOPMENT AND VALIDATION OF RISPERIDONE BY RP-HPLC

K. Manikandan*[1] and K. S. Lakshmi[1]

*[1]Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur, Kancheepuram, Tamilnadu, India.

ABSTRACT

An isocratic Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed and subsequently validated to develop new simple and rapid analytical method to estimate the Resperidone in pharmaceutical dosage form. As the drug is polar in nature, it was proposed to select isocratic RP-HPLC method. The separation was achieved with an LC-2010C_{HT} SHIMADZU C_{18} (150 x 4.6 mm), 5 µm column and Methanol: Acetonitrile: Potasium dihydrogen ortho phosphate (60:30:10v/v/v) as a eluent, at flow rate 1.0 mL/min. UV detection was performed at 234nm. The developed method was validated by accessing various parameters like specificity, linearity, LOD, LOQ, precision, robustness, ruggedness and system suitability studies. From the results it was found that all the parameters are within the acceptable range. Hence the proposed method was found to be satisfactory and would be used for the routine quality control analysis of Risperidonel bulk and Formulation.

KEY WORDS

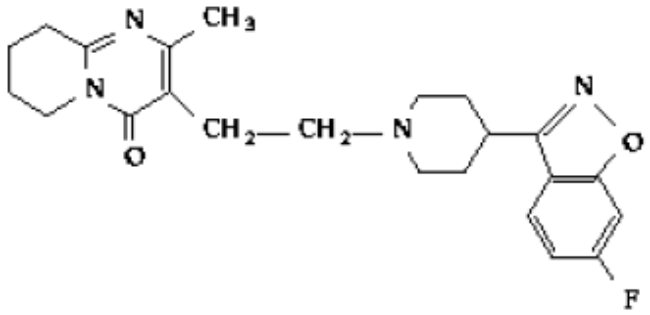
Resperidone, RP-HPLC, Method development and Validation.

Author for Correspondence:
K. Manikandan,
Department of Pharmaceutical Analysis,
SRM College of Pharmacy, SRM University,
Kattankulathur, Kancheepuram, Tamilnadu, India.

Email: gurumani12@gmail.com

INTRODUCTION

Resperidone chemically 4-[2-[4-(6-fluorobenzo [*d*] isoxazol-3-yl)-1-piper idyl] ethyl] -3- methyl-2, 6-diazabicyclo [4.4.0] deca-1, 3-dien-5-one. This drug belongs to a class of Anti-psychotic known as atypical neuroleptics. It is a strong dopamine antagonist. It has high affinity for D2 dopaminergic receptors. It has actions at several 5-HT (serotonin) receptor subtypes. The latter action may lead to an increased release of dopamine from mesocortical



neurones in the brain. Risperidone is metabolized fairly quickly, so this potential for nausea subsides usually in two to three hours. Literature survey revealed that Risperidone and 9-hydroxy Risperidone in plasma by HPLC with detection by UV. In HPLC they have used C_{18} column of diameter (150mm x 3.9mm) and the mobile phase was Methanol: Water: Dimethylamine in the ratio of (60:40:04 v/v/v) [1].

Some other methods have developed for the Determination of Risperidone in human plasma by HPLC-MS/MS and its application to a pharmacokinetic study in Chinese volunteers[2], HPLC-DAD determination of plasma levels of the antipsychotic Risperidone and its main metabolite for toxicological purposes[3], analysis of the novel antipsychotic drug Quetiapine in human plasma[4], validated LC-MS/MS methods for the determination of Risperidone[5], a method for the Determination of the novel antipsychotic drug Olanzapine in human plasma using HPLC with amperometric detection[6], development and validation Simultaneous determination of the antipsychotic drugs Levomepromazine and Clozapine and their main metabolites in human plasma by a HPLC-UV method with solid-phase extraction[7], a method for Analysis of the recent antipsychotic Aripiprazole in human plasma by capillary electrophoresis and high-performance liquid chromatography with diode array detection[8], developed a method Stability Indicating HPLC Determination of Risperidone in Bulk Drug and Pharmaceutical Formulations[9], developed a Spectrophotometric Determination of Risperidone In Tablet Formulations[10]. But none of methods were found simple, reliable, and reproducible. Hence an attempt has been made to develop new isocratic RP-HPLC methods to estimate the Resperidone in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility respectively.

MATERIAL AND METHODS

An isocratic high pressure liquid chromatography (Shimadzu with LC-2010-C-Class up software. Micro balance-Sartorius-model cp-225D, Millipore filter (0.45 µm),Millipore mill Q Water instrument and column employed C_{18} (150 x 4.6 mm) 5 µm with UV detector at 234nm.

Chemicals and reagents

All the chemicals used were of HPLC grade and AR.grade. Distilled water was used for making the solutions. The commercially available Resperidone tablets were procured from the local market.

Chromatographic Conditions

The content of the mobile phase was Methanol: Acetonitril: Potassium dihydrogen orthophosphate (60:10:30 v/v/v). The mobile phase was filtered through 0.45 µm membrane filter and sonicated for 15 min. The flow rate of the mobile phase was maintained at 1.0 ml/min. The column temperature was set ambient and the detection was carried out by UV-detector wavelength at 234 nm. The run time was set at 10 min and the volume of the injection loop was 20 µL. Priorto injection of the drug solution, the column was equilibrated for atleast 30 min with the mobile phase flowing through the system.

PROCEDURE

Preparation of Risperidone Standard Solution

25 mg of drug was accurately weighed and then it was transferred in to 25 ml of volumetric flask, dissolve in 5 ml of Methanol and it was diluted up to the mark with Methanol it was then the resultant solution was further diluted to 10 ml with Methanol to obtain a final standard solution of 100 µg/ml of Risperidone. The resultant solution was filtered through Millipore filter paper.

Preparation of Risperidone tablet solution

Twenty tablets were weighed and powdered. A quantity of powder equivalent to 25 mg of Risperidone was taken in 25 ml of volumetric flask and it was dissolved with Methanol and it was made up to the mark. The solution was further diluted to 10 ml with Methanol to obtain a final solution of 100 mcg/ml of Risperidone. The resulting solution was sonicated and filtered using millipore filter paper. 20 µl of this solution was injected and the chromatogram was recorded. The content of Risperidone present in each tablet formulation was calculated by comparing the peak area of the standard and sample reports and shown in Table No.1 and 2.

Amount of drug in each tablet of Risperidone

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{A. wt of tablet}$$

$$\% \text{ Content} = \frac{\text{Amout present}}{\text{Label Claim}} \times 100$$

METHOD VALIDATION[11-14]

Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application. Typical analytical parameters used in assay validation are,

1. Specificity
2. Linearity
3. Limit of detection
4. Limit of quantification
5. Accuracy
6. Precision -System precision
   -Method precision
7. Robustness
8. Ruggedness
9. System suitability studies
   Resolution
   Number of theoretical plates
   The tailing Factor (T)

Specificity

The specificity of the method was evaluated by analyzing the sample solution added (known amount) with excipients at appropriate levels that the assay result is unaffected by the presence of extraneous materials.

Linearity

Linearity was assessed by performing single measurement at several analyte concentrations. A minimum of five concentrations. A minimum of five concentrations were recommended for linearity studies.

The linearity of an analytical method, its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity of an analytical method is determined by mathematical treatment of test result obtained by analysis of samples with analyte concentration across claimed range. Graph of area VS CONCENTRATION IS PLOTTED and percentage curve fitting is calculated.

LIMIT OF DETECTION

The limit of detection is the lowest concentration of the analysis in a sample that can be detected but not necessarily determined in quantitatively using a specific method under the required experimental conditions. Such a limit is expressed in terms of a concentration of analyte (Example: - µg/ml) in the sample.

$$\text{LOD} = \frac{3.3 \times \text{Standard deviation of the response}}{S}$$

S = Slope of the calibration curve of the analyte

Limit of Quantification

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be qualitatively determined with suitable precision and accuracy.

$$\text{LOQ} = \frac{10 \times \text{Standard deviation of the response}}{S}$$

S = Slope of the calibration curve of the analyte.

Accuracy

The accuracy of an analytical method is the closeness of the test result obtained by that method to the true value. Accuracy is measured as the percentage of the analyte recovered by the assay spiked samples were prepared in triplicate at three intervals at a range of 80-120 % of the target

concentration, and injected in to the HPLC system. Acceptance criteria: percentage recovery should be within 98 to 102 %.

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision of analytical method is usually expressed as the standard deviation (or) relative standard deviation. There are two methods for determination of precision.

1. System precision
2. Method precision

Robustness

Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example, change in physical parameters like flow and mobile phase ratio.

Robustness

The evaluation of robustness showed the reliability of analysis with respect to deliberate variations in method parameters. The various concentrations were prepared and injected into sample injector of HPLC six times under different parameters like deliberate variations in flow rate, detection (nm).

System Suitability Studies

A Solution of 100 µg/ml of Risperidone was prepared by diluting suitably with mobile phase and same was injected.

RESULTS AND DISCUSSION

As there is official method for the estimation of Risperidone in the British pharmacopoeia, it was necessary to develop a new sensitive method for the estimation of the parameters used for the developed method is given below:

Fixed Chromatocraphic Condition

Instrument	:	LC-2010 Shimadzu
Column	:	C_{18} (150x4.6mm) 5 µm
Wavelength	:	234 nm
Flowrate	:	1 ml/min
Injection Volume	:	20 µ l
Mobilephase	:	Methanol: Acetonitrile: Potassium dihydrogen orthophosphate (60:10:30 v/v/v)
Retention Time	:	Risperidone–3.850 minutes

The amount of drug present in the tablet formulation was determined and the results were obtained in the following Table No.4.

Specificity of the method was found out through non-interference of the placebo identical conditions of assay. This uniform the specificity of the proposed method.

Linearity of the drug was obtained in the range of 20 to 100 mg/ml for Risperidone. The linearity correlation, Co efficient and percentage curve fitting was found to be 0.9971 for Risperidone 99.71. The limit of detection was found to be 0.17 mg/ml for Risperidone. The limit of quantification was found to be 0.5mg/ml for Risperidone.

Accuracy of the method was determined through recovery studies of the drug. Recovery of the drug was well within acceptance limit (97% to 102 %).

Precision of the method was determined by analyzing the drug formulation by replicate injection and system precision was determined by standard solution %RSD the result was found to be within the limits of 2%. Thus developed method was found to provide high degree of precision and reproducibility.

Ruggedness of the method was determined by performing the assay with different analyst in different days perform the assay to check the reproducibility. The test results were found within limit 97.2% to 102%. The results were found to be reproducible. Inspite of variation in condition with could be normally expected from analyst to analyst.

Robustness was determined by carrying out the assay during change in the mobile phase ration and

flow rate. The results obtained with the change in mobile phase ratio makes it possible to carry out the method for Risperidone with small variations in mobile phase ratio. System suitability was determined by performed the assay with the same sample repeatedly. The number of the theoretical plates was found to be 24.18 for Risperidone.

Table No.1: Results showing quantitative estimation of Risperidone by using the developed method

S.No	Tablet	Peak Area (Sample)	Peak Area (standard)	% Recovery
1.	Risperidone	1096.491	1094.812	99.8%

Table No.2: % Content of Risperidone present in each tablet formulation

S.No	Sample	Area Obtained	% Content of drug (% w/w)
1.	Standard	1094.812	99.95%
2.	Standard + Placebo	1084.812	100.87%
3.	Placebo	0	0

Table No.3: Linearity data for Risperidone

S.No	CONCENTRATION (µg/ml)	PEAK AREA
1	20	150.797
2	40	333.449
3	60	518.531
4	80	696.185
5	100	884.399

Table No.4: Determination of the amount of drug present in the tablet formulation

S.No	Tablet	Peak Area (sample)	Peak Area (standard)	Percentage Content
1	Risperidone	1096.505	1094.812	99.8%

Table No.5: Validation Result for Risperidone in developed method

S.No	Parameters	Results obtained	Acceptance criteria
1.	Specificity	100.41%	99 -101%
2.	**LINEARITY RANGE**		
	Correlation Coefficient	0.9971	NLT – 0.997%
	Percentage curve fitting	99.71%	NLT – 99.7%
	Slope	8.7091	-
3.	LOD	0.17 µg/ml	-
4.	LOQ	0.5 µg/ml	-
5.	Accuracy	100.25	99-101%
6.	**PRECISION**		
	System Precision	0.378	2% (RSD)
	Method Precision	0.474	2% (RSD
7.	**RUGGEDNESS**		
	Different Analyst	99.94	99-101%
	Different Instrument	99.78	99-101%
8.	**ROBUSTNESS**		
	Robustness change in flow rate 0.8 ml	99.16%	99-101%
	1.2ml	99.05%	
	Change in mobile phase ratio 59:11:30	98.88%	99-101%
	Change in mobile phase ratio 61:10:29	99.65%	
9.	**SYSTEM SUITABILITY PARAMETER**		
	Theoretical Plates	2418	-
	Tailing factor	1.8	Not more than 2
	Resolution	2.3	Not less than 2

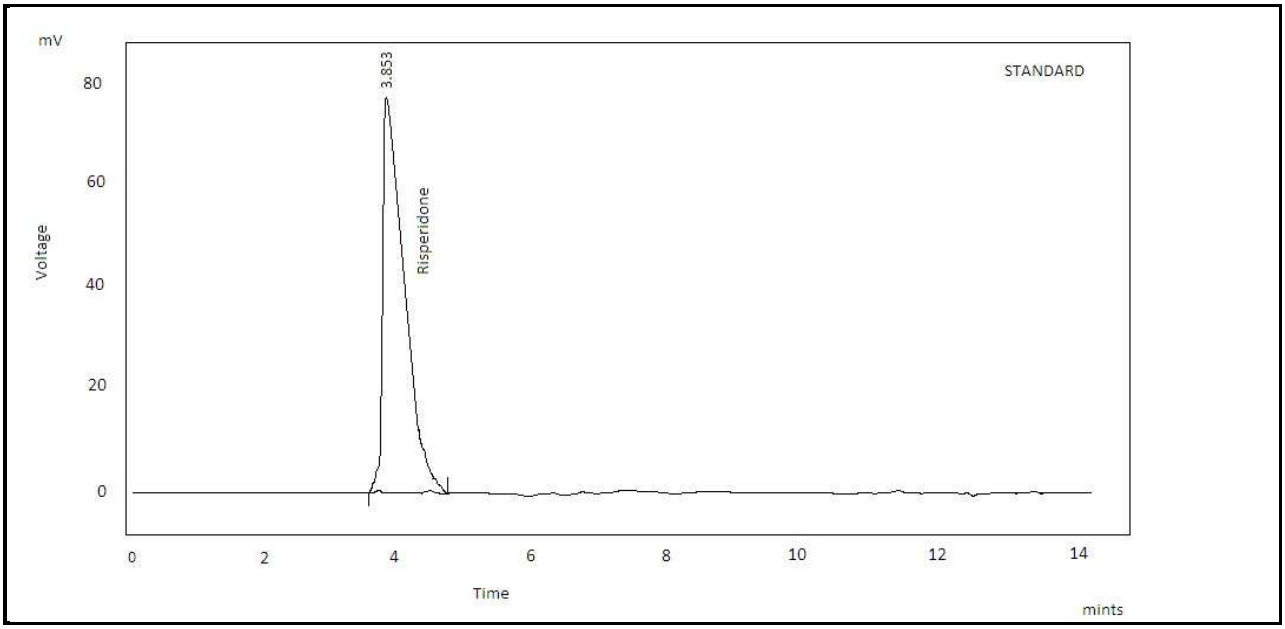


Figure No.1: Standard of Risperidone

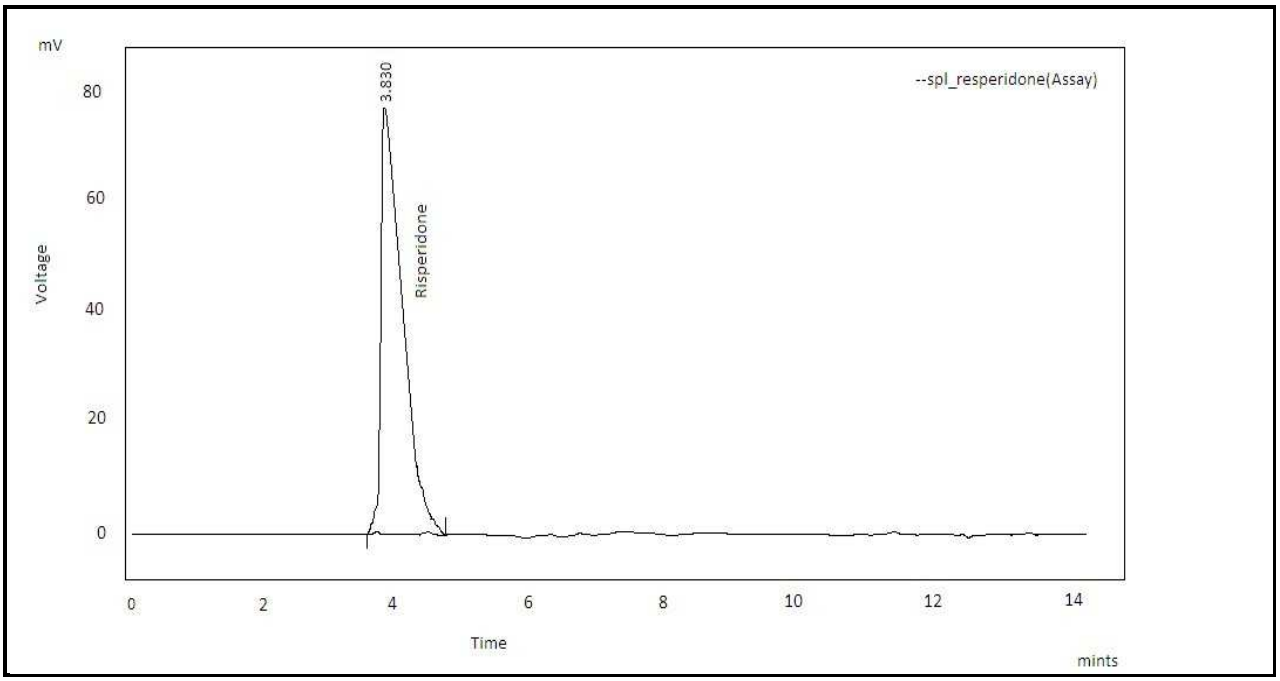


Figure No.2: Chromatograph of Spl - Risperidone

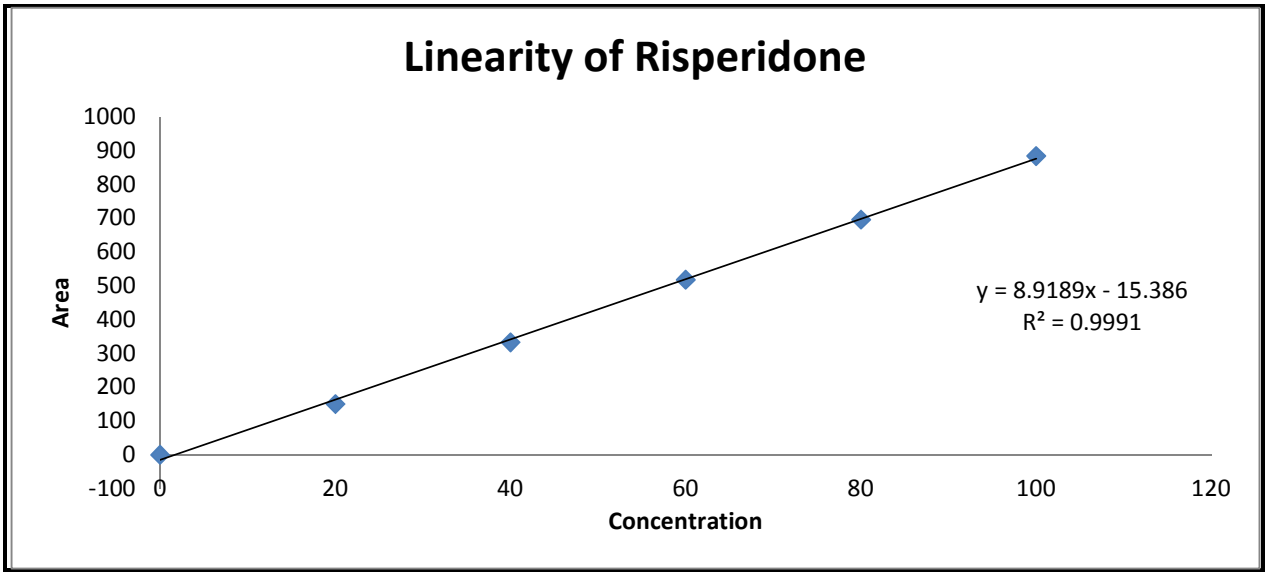


Figure No.3: Linearity of Risperidone

CONCLUSION

A HPLC-method was developed for the estimation of Risperidone in tablet dosage from using RP-HPLC. LC-2010 with UV detector 234 and C_{18} (150 x 4.6mm) 5 µl of standard was injected and evaluated with mobile phase of Methanol: Acetonitrile: Potassium dihydrogen orthophosphate (60:10:30v/v/v) which was pumped at flow rate 1ml/min and detected by UV detector at 234 nm. The peak of Risperidone was found well observed at 3.850 minutes respectively. The developed method was applied for the determination of Risperidone in tablet dosage form. The assay results are within the label claim of formulation. The developed method was validated with various parameters as per ICH guidelines like accuracy, precision, Linearity, specificity, ruggedness and robustness, system suitability. The results are within the acceptance criteria. Hence the proposed method was found to be satisfactory and would be used for the routine analysis of Risperidone in bulk and formulation.

ACKNOWLEDGEMENT

The authors are sincerely thanks to the SRM College of Pharmacy, SRM University, Kattankulathur, Kancheepuram, Tamilnadu, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Manikandan K and Lakshmi K S. Method development and validation of risperidone by RP-HPLC, *International Journal of Research in Pharmaceutical and Nano Sciences,* 1(1), 2012, 87-95.