

**METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF
RISPERIDONE (MARKETED) BY USING RP-HPLC**

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ABSTRACT

The method development involved optimizing the chromatographic conditions, such as mobile phase composition, column selection, and detection wavelength, to achieve good resolution, peak symmetry, and sensitivity. The developed method utilized a reversed-phase (RP) HPLC system with a C18 column and a mobile phase consisting of a mixture of acetonitrile and buffer (pH adjusted to 3.0) in a gradient elution mode. The flow rate was set at 1.0 ml/min, and the detection was performed at 280 nm. The method showed excellent linearity over the concentration range of 10-100 µg/ml, with a correlation coefficient (r^2) of 0.999. The method was validated as per International Council for Harmonisation (ICH) guidelines for specificity, accuracy, precision, robustness, and system suitability. The accuracy of the method was evaluated by performing recovery studies, which yielded results within the acceptable range of 98-102%. Precision studies demonstrated the method's repeatability (intra-day) and intermediate precision (inter-day) with relative standard deviations (RSD) below 2%. Robustness was assessed by deliberately varying the chromatographic conditions, such as flow rate, buffer pH, and column temperature, which did not significantly affect the method's performance. System suitability parameters, including retention time, resolution, and tailing factor, were within the acceptable limits, indicating the method's suitability for routine analysis.

The developed RP-HPLC method was successfully applied for the estimation of risperidone in marketed formulations. The assay results were found to be accurate and precise, demonstrating the method's reliability for routine quality control analysis. Overall, the developed method offers a simple, sensitive, and cost-effective approach for the quantitative determination of risperidone in pharmaceutical formulations.

Keywords: Risperidone, limit of detection (LOD), limit of quantification (LOQ), relative standard deviations (RSD), high-performance liquid chromatography (HPLC), reversed-phase

INTRODUCTION

Risperidone, a widely prescribed antipsychotic medication, requires accurate and reliable analytical methods for its estimation in pharmaceutical formulations. The quantitative estimation of the active constituents is an integral part of developing and manufacturing process of pharmaceutical dosage forms¹. Slight changes in the composition or in the purity of the drug itself can affect the therapeutic value². Therefore, there is a need for development of better and reliable methods for the estimation of pharmaceutical dosage form. The official methods for the analysis of active ingredients of formulations are few and the most of the methods available for the analysis of active ingredients are applicable only after prior separation that involves tedious and time-consuming procedures³. Literature survey also reveals that lesser method development of RP-HPLC method for the determination of Risperidone in bulk forms or other pharmaceutical dosage forms have been reported⁴. Therefore, in the present study an attempt will be made to develop RP-HPLC methods for the analysis and method development of the drug in pure and pharmaceutical formulations using RP-HPLC method^{5,6,7}.

The aim of this research study is to develop and validate simple, accurate, precise, sensitive and cost-effective RP-HPLC method for quantitative evaluation of Risperidone drug and to develop a validated RP-HPLC method for determination of Risperidone drug in pharmaceutical formulations which are critical for the quality control laboratories.

MATERIAL AND METHOD

HPLC grade solvents were purchased from: Acetonitrile and Methanol from merck, Water from Milli-Q, Orthophosphoric acid from Hi Media. Standard Risperidone used was 99.9% pure. Marketed formulation used was Geodon having label claim Risperidone 20mg per capsules.

Identification and Characterization of drugs: Solubility of Risperidone was observed by dissolving them in different solvents and found slightly soluble in Water, 0.1N NaOH, 0.1N HCl and Phosphate Buffer, and Soluble in Methanol, Acetonitrile and Ethanol. Melting Point of the drug for Risperidone 180-182°C found through Melting point apparatus. The pure drug solution was scanned on UV spectrophotometer, and λ_{max} was 250.0 nm determined.

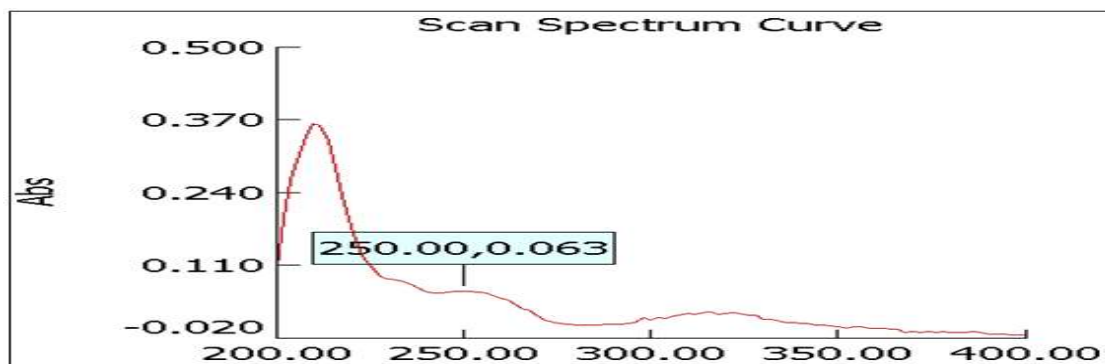


Figure 6.8: Determination of λ_{max} of Risperidone

Selection of Mobile Phase

Initially to estimate Risperidone in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No.of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: Acetonitrile in the ratio of 50:50 v/v. The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Procedure for preparation of mobile phase: 100 ml of methanol and 100ml of acetonitrile Filtered through 0.45 μ m filter paper.

Table 6.9: Mobile phase selection

Mobile Phase	Ratio	Remark
Methanol : water	50 : 50 v/v	Poor resolution
Acetonitrile : Metanol	50 : 50 v/v	Most Suitable

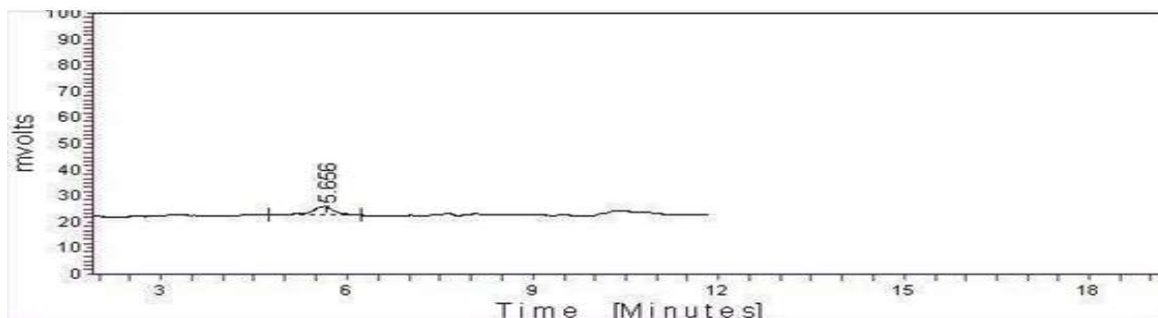


Figure 6.9: Trail graph of methanol: water

Selection of diluents: Diluent used for preparation of samples were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Linearity and Calibration Graph: To establish the linearity of analytical method, a series of dilution ranging from 5-25µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 254 nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Table 6.11: Linearity of Risperidone

Standard Concentration µg/ml	Area Under Curve (AUC)						
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Mean
0	0	0	0	0	0	0	0
5	302.965	306.700	311.762	311.462	319.481	322.465	312.472
10	612.487	622.651	631.461	632.481	639.490	643.781	630.391
15	933.489	937.660	932.551	929.483	940.739	944.485	936.401
20	1231.198	1227.591	1219.481	1222.460	1233.481	1242.486	1229.449
25	1539.511	1541.791	1541.789	1551.461	1539.788	1551.463	1544.300
r ²	0.999	0.999	0.999	0.999	0.999	0.999	0.999
Slope (m)	61.43	61.51	61.40	61.72	61.60	61.51	61.49

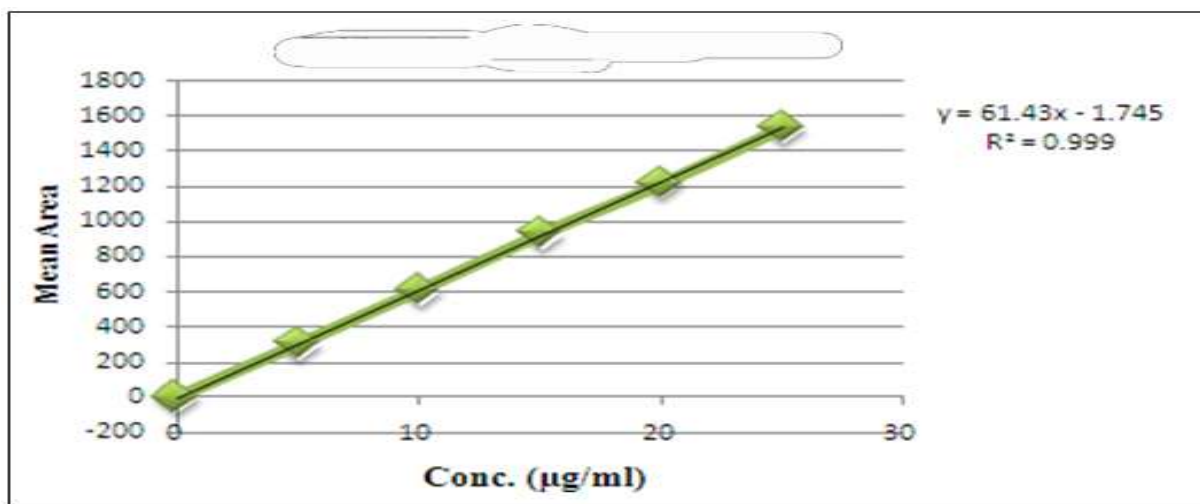


Figure 6.10: Calibration Curve of Risperidone

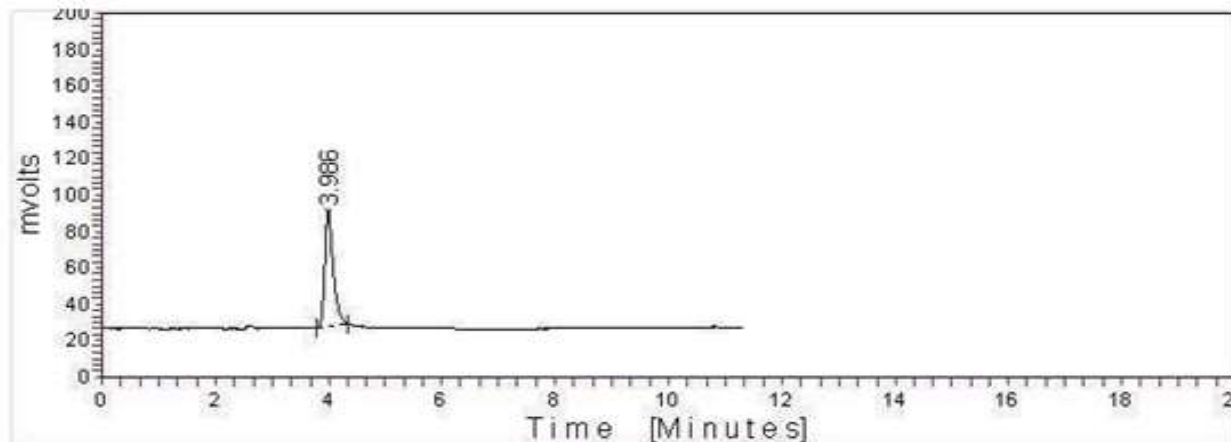


Figure 6.11: Chromatogram of Risperidone

System Suitability Parameters: Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Risperidone 10 µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram

Table 6.12: System suitability parameters of Risperidone

System suitability Parameter	RT	AUC	No. of theoretical plates	Tailing factor
Rep-1	3.990	612.559	3553	0.99
Rep-2	3.991	627.661	3539	0.96
Rep-3	3.989	629.461	3547	0.97
Rep-4	3.990	633.481	3590	0.94
Rep-5	3.989	637.493	3550	0.95
Rep-6	3.991	643.789	3561	0.96
Mean	3.990	630.740	3556.66	0.961
S.D.	0.001	10.4122	16.223	0.0162
% R.S.D.	0.025	1.65699	0.462	1.7199

Validation of Developed Method

Linearity: Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was

contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Table 6.13: Response ration data for linearity of Risperidone

Replicates	Concentration (µg/ml)	Mean AUC	Response Ratio
Rep-1	5	301.972	60.3944
Rep-2	10	610.564	61.0564
Rep-3	15	929.502	61.9668
Rep-4	20	1218.196	60.9098
Rep-5	25	1536.512	61.4604
Mean			61.1575
SD			0.591748
%RSD			0.967579

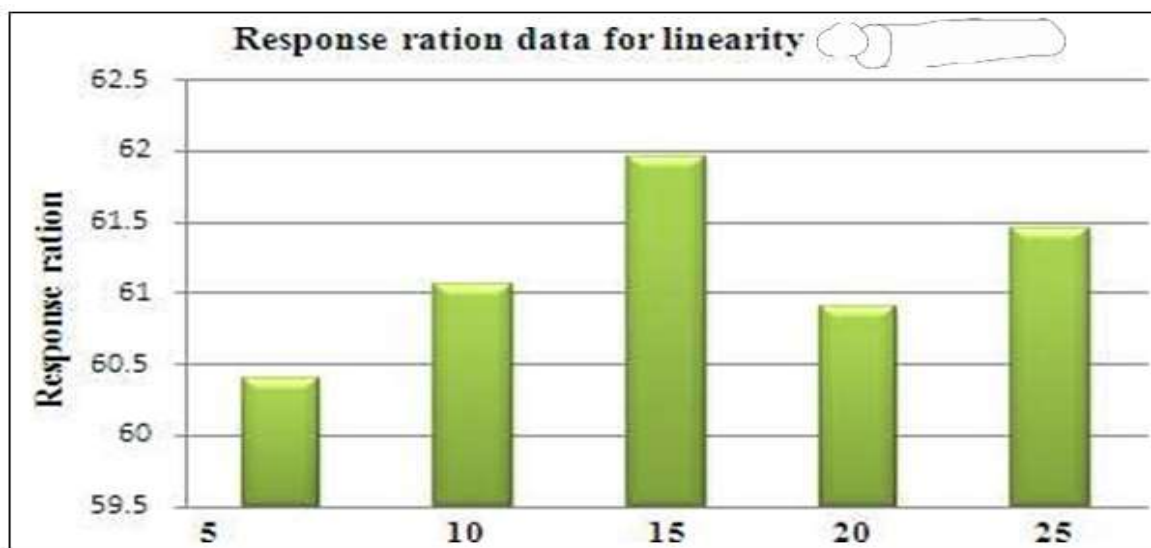


Figure 6.12: Response Ratio Curve of Risperidone

Specificity: Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

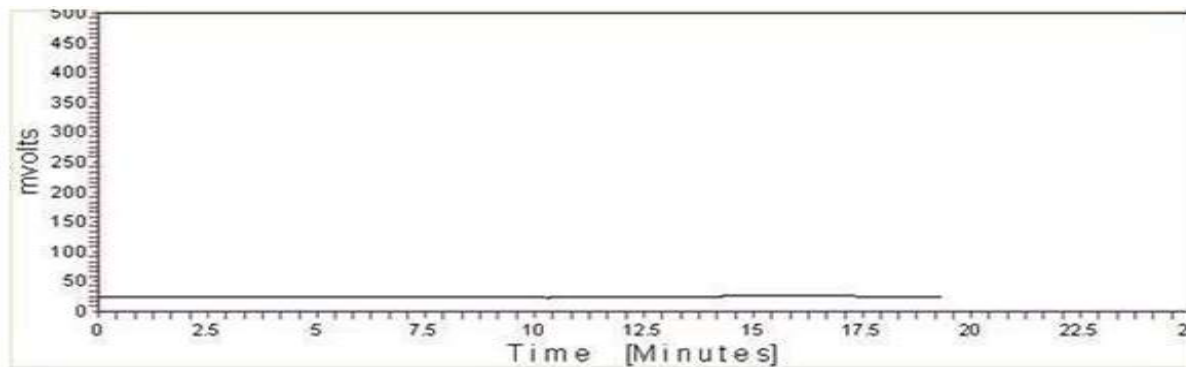


Figure 6.13: Chromatogram of blank diluent

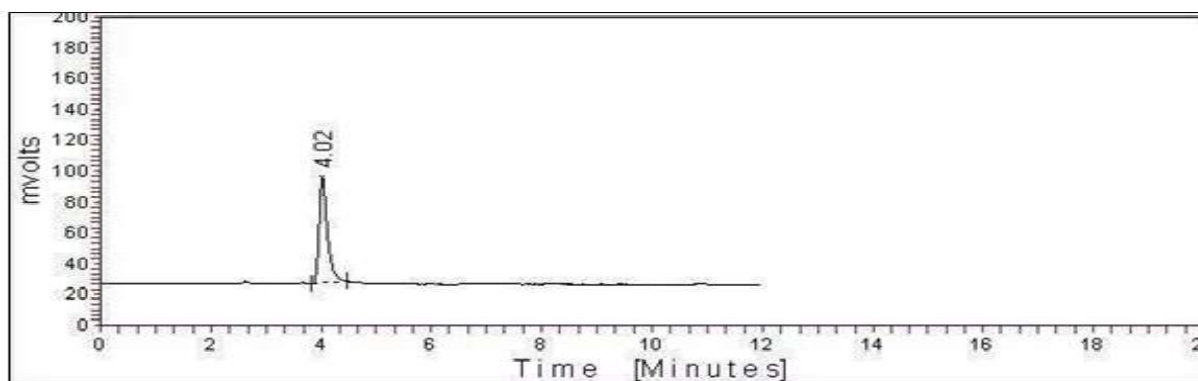


Figure 6.14: Chromatogram of pure drug

Accuracy: Recovery studies were performed to validate the accuracy of developed method. To pre analyzed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Table 6.14: Recovery study of Risperidone (80% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found (µg/ml)			% Conc. Found			Mean % conc
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	4	3.96	4.00	3.99	99.81	100.33	99.67	99.93
10	8	7.89	7.99	7.98	99.91	99.82	99.44	99.72
15	10	9.60	9.61	9.63	99.93	99.61	99.68	99.74
MEAN								99.79
SD								0.086
% RSD								0.087

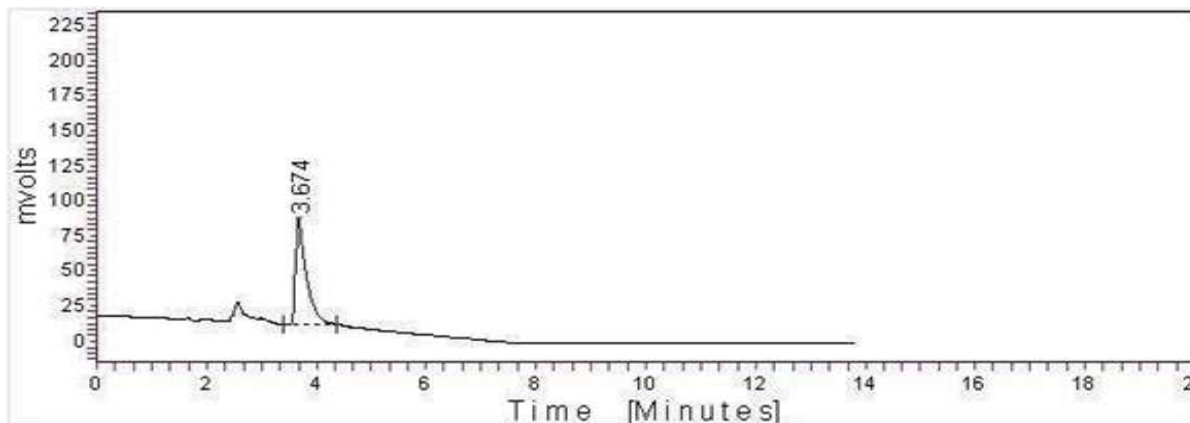


Figure 6.15: Graph of Recovery study of Risperidone (80% Level)

Table 6.15: Recovery study of Risperidone (100% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found (µg/ml)			% Conc. Found			Mean % conc
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	5	4.99	4.98	5.00	99.65	99.09	100.02	99.58
10	10	9.99	9.98	9.96	99.83	99.92	99.61	99.78
15	15	14.98	14.99	15.00	99.71	99.91	99.95	99.85
MEAN								99.79
SD								0.091
% RSD								0.092

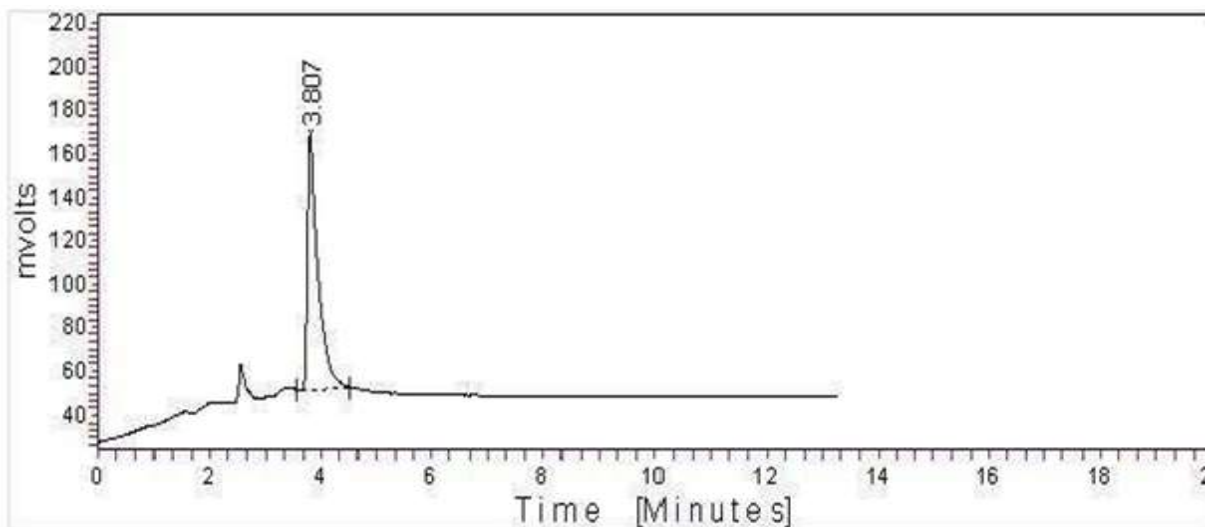
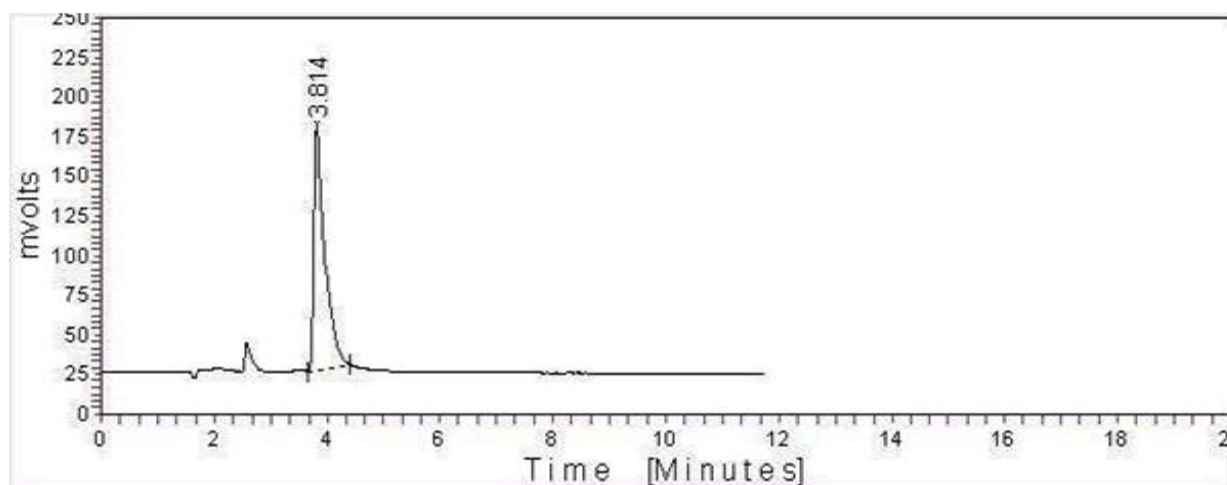


Figure 6.16: Graph of Recovery study of Risperidone (100% Level)

Table 6.16: Recovery study of Risperidone (120% Level)

Conc. of sample ($\mu\text{g/ml}$)	Amt. Added ($\mu\text{g/ml}$)	Conc. Found ($\mu\text{g/ml}$)			% Conc. Found			Mean % conc
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	6	5.95	5.98	5.99	99.17	99.67	99.83	99.56
10	12	11.95	11.98	12.01	99.58	99.83	100.8	99.83
15	18	17.98	18.01	17.99	99.89	100.6	99.94	99.96
MEAN								99.784
SD								0.170
% RSD								0.170

**Figure 6.17: Graph of Recovery study of Risperidone (120% Level)**

Precision: The stock solution was prepared as stated in 5.4.6. The precision are established in three differences:

- Repeatability
- Intermediate precision: (a) Day to Day (b) Analyst to Analyst
- Reproducibility

Repeatability: The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 $\mu\text{g/ml}$ for Risperidone indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively.

Table 6.17: Repeatability of Risperidone

REP.	CONCENTRATION FOUND ($\mu\text{g/ml}$)					MEAN
	5	10	15	20	25	
Replicate-1	4.98	10.01	14.95	19.97	24.91	
Replicate-2	4.97	9.98	14.88	19.96	24.95	
Replicate-3	5.01	10.02	14.98	19.95	24.89	
Replicate-4	4.98	10.01	14.87	19.96	24.96	
Replicate-5	4.99	10.01	14.92	19.89	24.94	
MEAN	4.986	10.006	14.920	19.946	24.930	
% MEAN	99.720	100.060	99.467	99.730	99.720	99.739
SD	0.015	0.015	0.046	0.032	0.029	0.028
% RSD	0.015	0.015	0.047	0.032	0.029	0.028

Intermediate Precision:

Day To Day Precision: Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations. Results of day-to-day intermediate precision for Risperidone reported in table respectively.

Table 6.18: Day-to-Day variation of Risperidone

REP.	CONCENTRATION FOUND ($\mu\text{g/ml}$)					MEAN
	5	10	15	20	25	
Replicate-1	4.94	9.96	14.92	19.98	24.93	
Replicate-2	4.99	9.92	14.98	19.97	24.94	
Replicate-3	4.89	9.89	14.93	19.96	24.98	
Replicate-4	4.91	9.96	14.94	19.88	24.91	
Replicate-5	4.97	9.96	14.97	19.87	24.93	
MEAN	4.931	9.911	14.951	19.919	24.919	
% MEAN	99.5002	99.031	99.678	99.598	99.679	99.276
SD	0.045	0.036	0.037	0.057	0.029	0.043
% RSD	0.901	0.369	0.241	0.278	0.119	0.384

Robustness: As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, methanol: acetonitrile (50:50 % v/v), to (45:55 % v/v). Results of robustness are reported in table 6.19.

Table 6.19: Robustness of Risperidone

REP.	CONCENTRATION FOUND ($\mu\text{g/ml}$)					MEAN
	5	10	15	20	25	
Replicate-1	4.98	9.95	14.95	19.92	24.88	
Replicate-2	4.95	9.95	14.93	19.95	24.95	
Replicate-3	4.95	9.93	14.73	19.98	24.88	
Replicate-4	4.9	9.89	14.89	19.95	24.58	
Replicate-5	4.82	9.96	14.95	19.92	24.95	
MEAN	4.928	9.914	14.91	19.936	24.916	
% MEAN	98.56	99.14	99.4	99.68	99.664	99.458
SD	0.046	0.085	0.08	0.038	0.052	0.065
% RSD	0.934	0.861	0.534	0.19	0.21	0.546

Detection Limit and Quantitation Limit: The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Table 6.20: LOD and LOQ

Name	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
Risperidone	0.095	0.271

Analysis of Tablet Sample: Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equal to 10 mg of Risperidone was taken in 100-ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration. Results of tablet analysis are reported in table.

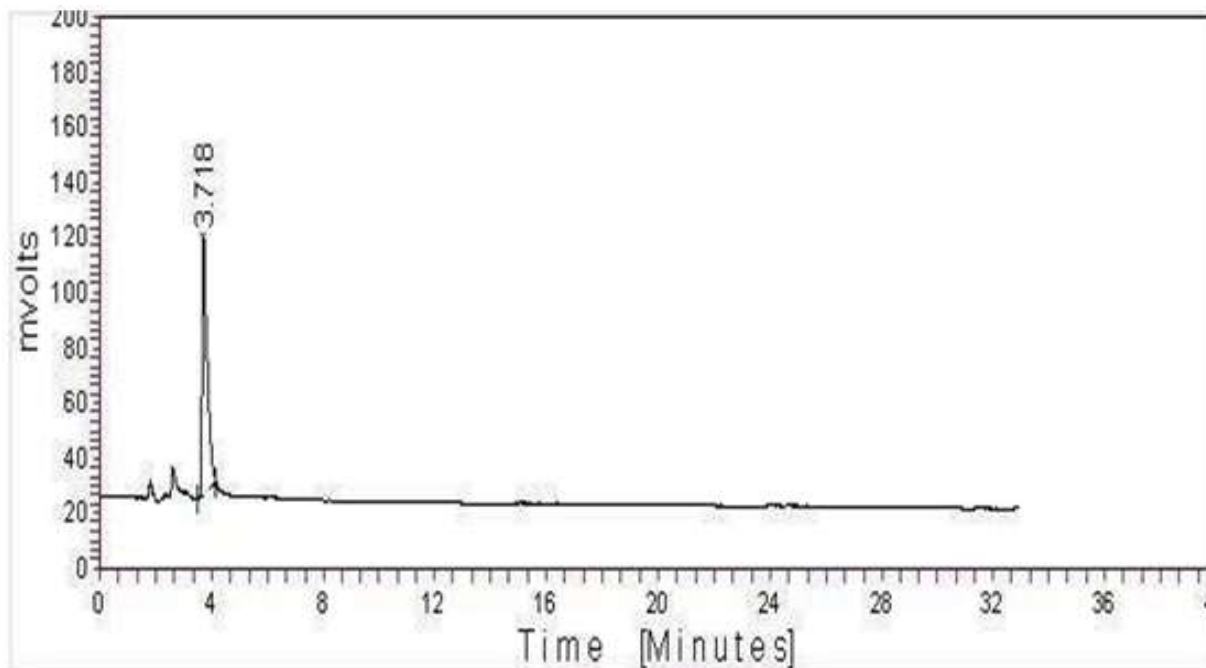


Figure 6.18: Graph of Analysis of Tablet Sample

Table 6.21: Analysis of tablet sample

S. No.	Parameter	Risperidone
1.	Mean	99.98
2.	S. D.	0.125
3.	% RSD	0.145

RESULT AND DISCUSSION

The RP-HPLC method was developed for estimation of Risperidone in bulk and capsule dosage form by isocratically using Methanol: Acetonitrile in the ratio of 50:50 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5 μ particle size) column as stationary phase and chromatogram was recorded at 250 nm. Then developed method was validated by using various parameters.

System suitability: The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 μ g/ml of Risperidone were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in table.

Table 7.1: Results of system suitability parameters

Parameters	Risperidone
No. of Theoretical Plates	3555.29
Tailing Factor	0.957
Retention time	3.991 ± 0.001

Linearity: The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 7.2.

Table 7.2: Results of linearity of Risperidone

Parameters	Risperidone
Concentration (µg/ml)	5-25
Correlation Coefficient (r^2)*	0.999
Slope (m)*	61.43
Intercept (c)*	-1.745

Specificity: Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of tablets did not interfere with standard peaks of Risperidone.

Accuracy: The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 7.3.

Table 7.3: Results of recovery study

% LEVEL	% MEAN ± SD*
80%	99.62 ± 0.084
100%	99.71 ± 0.091
120%	99.78 ± 0.170

Precision: Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and % RSD are less than 2 indicate the precision of method. Result of precision shown in table.

Table 7.4: Results of Precision

PARAMETER	% MEAN \pm SD*
Repeatability	99.739 \pm 0.028
Intermediate precision	
Day to day precision	99.276 \pm 0.041

Robustness: The robustness of developed method was checked by changing in the deliberate variation in solvent. Result of robustness shown in table 7.5.

Table 7.5: Results of robustness

PARAMETER	% MEAN \pm SD*
Robustness	99.458 \pm 0.065

LOD AND LOQ: Detection limit and quantitation limit of described method were observed as 0.095 μ g/ml, and 0.271 μ g/ml respectively based on the SD of response and slope, which meet the requirement of new method.

Assay of tablet formulation: The results of the analysis of tablet formulation were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs.

Table 7.6: Assay of tablet formulation

S. No.	Parameter	Risperidone
1.	Mean	99.98
2.	S. D.	0.125
3.	% RSD	0.145

CONCLUSION

In the present research work, a successful attempt was made for “Method Development and Validation for Estimation of Risperidone in marketed formulation by using RP- HPLC” which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling.

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation.

Proposed method was found to be linear in the range of 5-25 $\mu\text{g/ml}$ Risperidone with the correlation coefficient near to one respectively. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (80%, 100% & 120%) was ranging from 99.62 to 99.784 %, for Risperidone.

Liquid chromatographic system from waters comprising of manual injector, Waters 515 binary pump for constant flow and constant pressure delivery and U.V. detector connected to data ace software controlling the instrumentation as well as processing methanol: Acetonitrile in the ratio of 50:50 v/v at a flow rate of 1.0 ml min^{-1} . A thermo C-18 column (4.6 x 250mm, 5 μ particle size) was used as the stationary phase,

250.0 nm was selected as the detection wavelength for UV-vis. detector.

The proposed methods were found to be linear in the range of 5-25 $\mu\text{g/ml}$ with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent.

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and % RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drugas well as in tablet dosage form.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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