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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RISPERIDONE AND HALOPERIDOL IN TABLET DOSAGE FORMS

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ABSTRACT

A new, simple and sensitive Reverse phase performance liquid chromatography (RP-HPLC) method has been developed for the separation and quantification of Riserpidone (RIS) and Haloperidol (HPD) in tablet dosage form. The flow rate was maintained at 1.0ml/min and the eluent was monitored at 260nm. The retention time of RIS and HPD were 1.82min and 4.42 min respectively. The method was validated in terms of linearty, precision, accuracy, specificity and robustness. The method was linear and for precision studies; RSD for RIS and HPD were 0.02 and 0.04 respectively. The percentage recoveries for both drugs from their tablets were 100.80 and 99.76% respectively.

Keywords: *RP-HPLC, Risperidone, Haloperidol.*

INTRODUCTION

Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Method for analyzing drugs by HPLC demands primary knowledge about the nature of the sample, structure, polarity, volatility, stability and the solubility parameter. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be validated or revalidated1. Such as.z. Precision, Specificity, Accuracy, Linearity, Range, Limit of detection (LOD), Limit of Quantification (LOQ), Ruggedness [1-4].

MATERIALS AND METHODS [5-7]

Waters HPLC system connected with UV- Visible – SPD 10A Vpseries Detector and Empower-2 Software was used. Rheodyne 7725i injection with 20µL loop and analytical column- WATERS XTERRA RP8 4.6x150, 5microns are used. Risperidone, and Haloperidol were generously given by LARA Drugs Pvt Ltd, Hyderabad, and Telengana, India. Acetonitrile (HPLC grade) was procured from E.Merck (India) Ltd, Mumbai. Methanol and orthophosphoric acid (AR grade) were procured from Qualigens fine chemicals, Mumbai2. Water (HPLC grade) was obtained from a Milli-QRO water purification system.

Preparation of mobile phase:

Accurately measured 400 ml (40%) of above buffer and 600 ml of A triethylamine HPLC (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Preparation of Standard Solution:

Accurately weighed amount of 1 mg Risperidone and 10 mg Haloperidol were taken to a 10 ml cleaned and dried volumetric flask. This was then diluted with 7ml of diluent and was sonicated. The volume was made to 10 ml with the same solvent. This was marked and labeled as Stock solution. Further, an amount of 0.3 ml Risperidone and Haloperidol each were pipette from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluents to get 30 µg/ml of Risperidone and 15 µg/ml of Haloperidol.

Preparation of Sample Solution:

Accurately weighed amount of 1 mg risperidone and 10 mg haloperidol were taken to a 10 ml cleaned and dried volumetric flask. This was then diluted with 7ml of diluent and was sonicated3. The volume was made to10 ml with the same solvent. This was marked and labeled as Stock solution. Further, an amount of 0.3 ml Risperidone and Haloperidol each were pipette from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluents to get 30 µg/ml of Risperidone and 15 µg/ml of Haloperidol. The standard and sample solution of 30 µg/ml of Risperidone and 15 µg/ml of Haloperidol was injected for five times and the peak areas were recorded.

Validation Parameters: [8-10]

System suitability: The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can commence. Set up the chromatographic system; allow the HPLC system to stabilize for 40 min4. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like resolution, tailing factor, theoretical plate count and % RSD for peak area of six replicate injections of LMS standard (% RSD NMT 2.0). The system suitability data is reported in Table 1.

Linearity: The linearity of the method was determined at five concentration levels ranging from 10-50 µg/ml for Risperidone and 5 to 25µg/mL for Haloperidol respectively the linearity was evaluated by linear regression analysis, using least squares method. The slope and intercept value for calibration curve was y = 19288X (r2 =0.9996) for Risperidone and y=16616X (r2 =0.999) for Haloperidol. The results shows that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above. The calibration curves areshown in Table 3 and Figure 2.

Accuracy: For accuracy determination, three different concentrations were prepared separately6 i.e. 80%, 100% and 120% for the analyte and chromatograms are recorded for the same. The data was given in the Table 2 and Figure 1.

Intermediate Precision: 30 µg/ml of Risperidone and 15 µg/ml of Haloperidol of the above sample solution were injected for five times

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in five different days and peak areas were recorded. Chromatograms were recorded and results are shown in Table 6.

Limit of Detection: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response .The LOD for Risperidone and Haloperidol found to be 30µg/mL and 15 µg/mL, respectively.

Limit of Quantification: The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10)10. The LOQ was 30 µg/mL and 15 µg/mL for Risperidone and Haloperidol, respectively in Table 4.

Ruggedness: The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-2010AHT), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C18, Phenomenex Gemini C18 and Hichrom C18. Data is represented in Table 7 & 8.

RESULTS AND DISCUSSION

System suitability:

Table No. 1: Results of system suitability parameters for risperidone and haloperidol

S.No.	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Risperidone	1.826	379477	56515	--	1.15	2685
2	Haloperidol	4.443	287871	15973	5.91	1.44	2269

Accuracy:

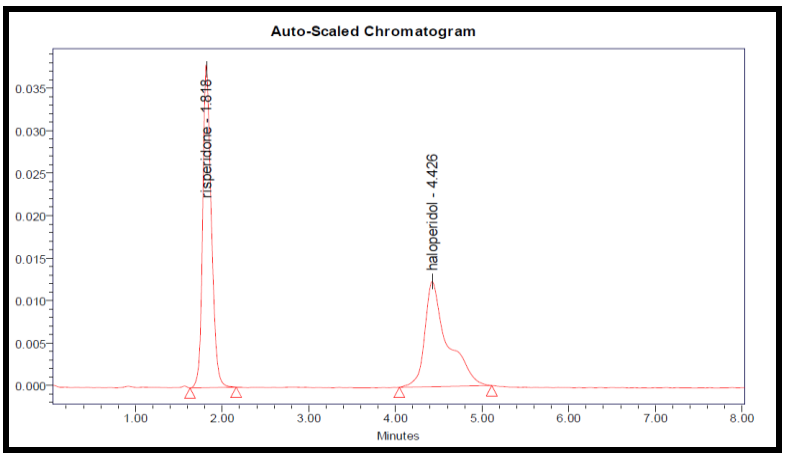

Fig. 1: Chromatogram for sample concentration-80%

Table No. 2: Results of Accuracy for sample concentration-80%

S.No.	Name	RT (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Risperidone	1.822	264326	38605	--	1.19	2672
2	Haloperidol	4.432	222044	12431	8.25	1.59	2201
3	Risperidone	1.824	257566	37548	--	1.17	2583
4	Haloperidol	4.436	222571	11819	7.58	1.62	2910
5	Risperidone	1.818	262184	38105	--	1.21	2515
6	Haloperidol	4.426	224674	12405	8.61	1.60	2192

Linearity:

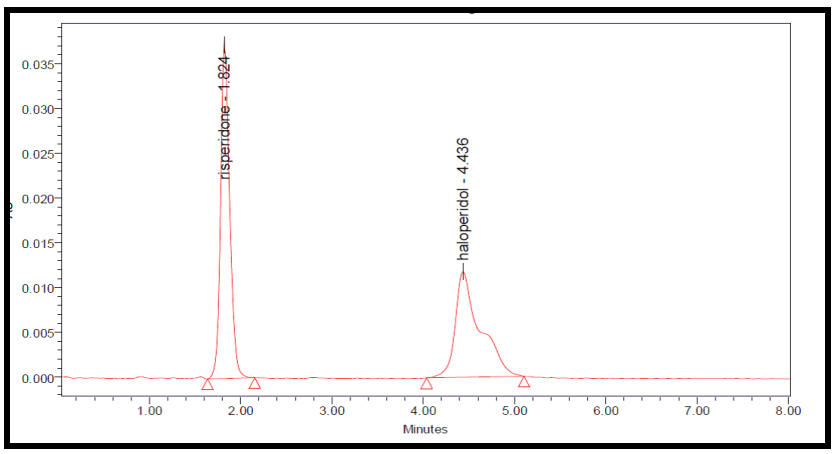

Fig. 2: Calibration curve of haloperidol and risperidone

Table No. 3: Results of method Linearity for Haloperidol

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	Haloperidol	4.444	41922	3260	3045.7	1.2	9.8
2	Haloperidol	4.430	141840	8019	2262.7	1.6	8.7
3	Haloperidol	4.432	275422	14434	2617.2	1.6	6.8
4	Haloperidol	4.435	307888	15858	2311.3	1.5	6.3
5	Haloperidol	4.435	384272	20111	2592.7	1.6	6.7
Mean			230268.8		2965.9	1.5	7.6
Std. Dev.			136998.7				
% RSD			59.5				

Table No. 4: Results of LOQ

S. No.	Name	RT (min)	Area	Height	USPResolution	USPTailing	USPPlateCount	Injection
1	Risperidone	1.826	364591	52312	--	1.13	2596	3
2	Haloperidol	4.465	258472	14772	6.46	1.67	2299	3

Table No. 5: Results of method linearity for Risperidone

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Risperidone	1.819	107480	14701	2440.0	1.2
2	Risperidone	1.820	212590	31050	2615.4	1.2
3	Risperidone	1.822	385507	54259	2582.4	1.2
4	Risperidone	1.822	417339	56591	2406.0	1.1
5	Risperidone	1.821	518455	75642	2617.1	1.2
Mean			328274.1		2532.2	1.2
Std. Dev.			165461.6			
% RSD			50.4			

Intermediate Precision:

Table No. 6: Results of method precession for Risperidone

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Risperidone	1.825	377285	55373	2569.8	1.2
2	Risperidone	1.824	379768	56287	2691.8	1.2
3	Risperidone	1.826	380712	56033	2632.8	1.2
4	Risperidone	1.822	382966	55330	2592.1	1.2
5	Risperidone	1.822	388290	57003	2668.9	1.2
Mean			381804.2		2631.1	1.2
Std. Dev.			4158.5			
% RSD			1.1			

Ruggedness:

Table No. 7: Results of method Ruggedness for Risperidone

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Risperidone	1.827	380388	55093	2653.9	1.2
2	Risperidone	1.819	384418	53697	2397.9	1.2
3	Risperidone	1.820	388201	53851	2442.2	1.2
Mean			384335.6		2498.0	1.2
Std. Dev.			3907.5			
% RSD			1.0			

Table No. 8: Results of method Ruggedness for Haloperidol

S. No.	Peak Name	RT (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Haloperidol	4.418	286892	15040	2833.4	1.5
2	Haloperidol	4.433	288548	15833	2184.3	1.6
Mean			287831.6		2498.0	1.2
Std. Dev.			850.3			
% RSD			0.3			

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of risperidone and haloperidol. in Tablet Dosage Form 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, approved testing laboratories, biopharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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