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VALIDATION OF PALIPERIDONE IN PHARMACEUTICAL DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Paliperidone is Chemically, (±)-3-[2-[4-(6-fluoro-1,2benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4*H*-pyrido[1,2- a]pyrimidin-4-one It is a psychotropic agent belongs to the chemical class of benzisoxazole derivatives. A validation method was developed for paliperidone by using reverse phase liquid chromatography. For separation column was used as Water symmetry RP 8 The mobile phase made of buffer and acetonitrile (65:35 % v/v). A 238 nm wavelength was selected for chromatographic study. The ICH guidelines were used for robustness and stability study. The method has been successfully used to assay of pharmaceutical dosage form i.e. tablets with good recoveries.

KEY WORDS: ***Paliperidone, Tri-ethylamine, acetonitrile, orthophosphoric acid, HPLC.***

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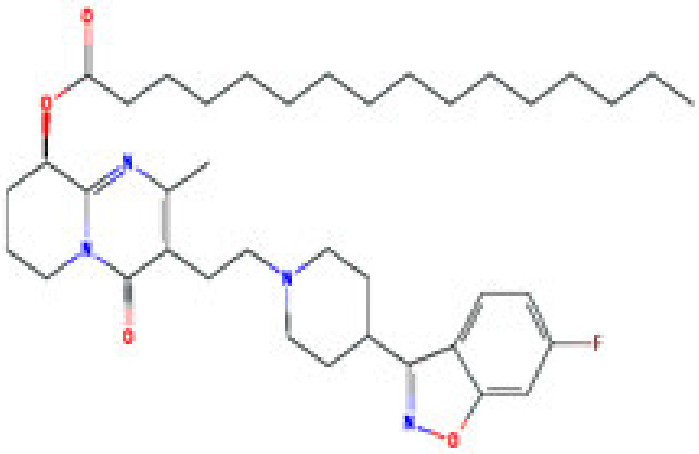
INTRODUCTION

Paliperidone is Chemically, (±)-3-[2-[4-(6-fluoro-1,2benzisoxazol-3-yl)-1-piperidinyl]ethyl] -6,7,8,9-tetra hydro-9-hydroxy-2-methyl-4*H*-pyrido[1,2-a] pyrimidin-4-one (Fig. 1). It is a psychotropic agent belongs to the chemical class of benzisoxazole derivatives, indicated for the treatment of schizophrenia1.Paliperidone is the major active metabolite of risperidone. The mechanism of action of Paliperidone, as with other drugs having efficacy in schizophrenia, is unknown, but it has been proposed that the drug's therapeutic activity in schizophrenia is mediated through a combination of central dopamine Type 2 (D2) and serotonin Type 2 (5HT2A) receptor antagonism. A literature survey reveals a spectrophotometric[1-7], HPLC[6-14], UPLC[15], HPTLC[16] methods. The proposed method is much simple and has better sensitivity than methods proposed in literature.

Figure 1
Structure of Paliperidone



MATERIAL AND METHODS

Chemical and reagents
Reference standard of paliperidone was obtained from reputed firm with certificate of analysis. Tri-ethyl amine , acetonitrile and ortho-phosphoric acid were used of analytical grade and HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer and acetonitrile (65:35 % v/v)].

Instrumentation
The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software. A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation
Standard solution
About 2.0 mg of standard paliperidone was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 2 minutes. The volume was adjusted up to the mark with diluent to give concentration as 200 µg /ml.

Sample preparation
Twenty tablets were weighed accurately and average weight of each tablet was determined. About 2 mg of paliperidone sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 200 µg /ml.

Chromatographic condition
Chromatographic separation was performed at ambient temperature on a reverse phase Water symmetry RP 8 (150 x 3.9 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile (65:35 % v/v). The buffer was mixtures of 0.1 % tri-ethyl amine adjusted the pH 3.5 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 238 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 20 µl.

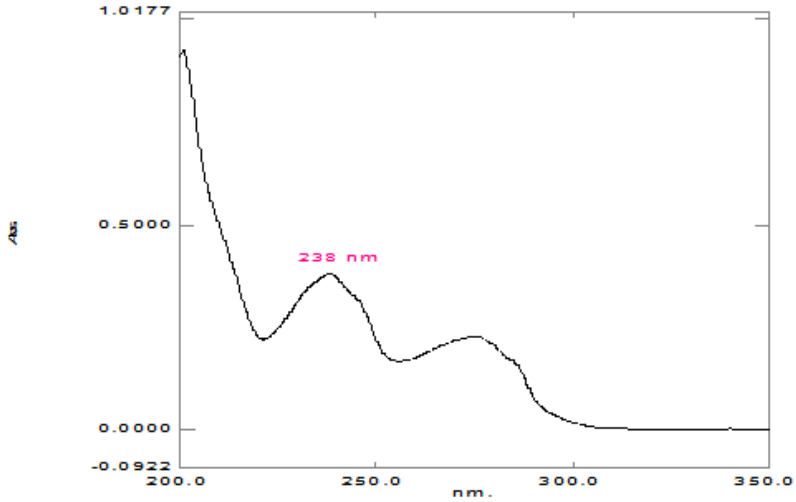


Figure 1
UV spectra of paliperidone

Method validation
System suitability
System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, area and % area were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1
System suitability parameters evaluated on standard solution of paliperidone

Retention Time	Area	Area %	USP Plate Count	Asymmetry
3.050	2268116	100	3049	1.23

Specificity
Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard paliperidone was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

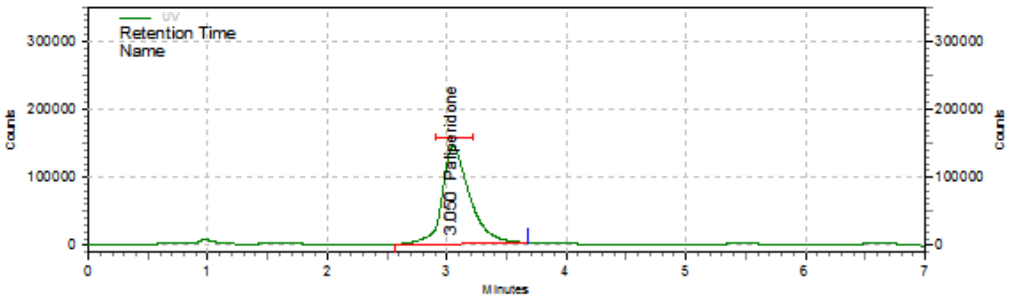


Figure 2
Typical chromatogram of paliperidone (standard)

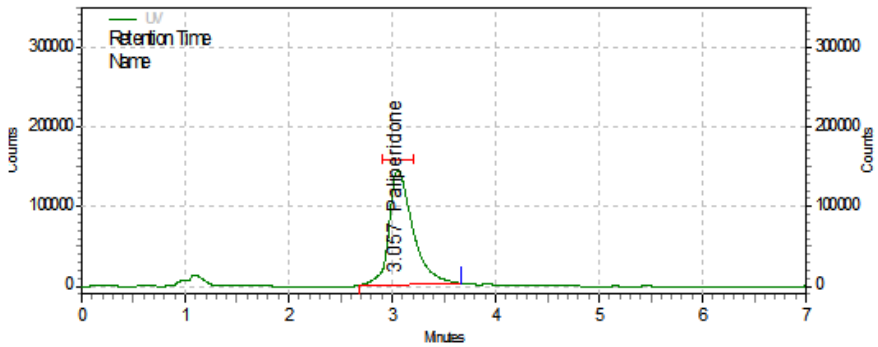


Figure 3
Typical chromatogram of paliperidone (sample)

Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2
Statistical evaluation of the data subjected to regression analysis

Parameters	paliperidone
Correlation Coefficient (r)	0.9999
% Intercept (y)	-37546
Slope (m)	11267

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 120 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Table 3
Statistical evaluation of the data subjected to accuracy of paliperidone

level	test	weight in mg	Area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	2.08	1848042	16.64	16.83	101.12	**100.97**
	2	2.07	1848482	16.64	16.83	101.14	
	3	2.06	1839303	16.64	16.75	100.64	
100%	1	2.09	2283876	20.8	20.79	99.97	**100.08**
	2	2.11	2285241	20.8	20.81	100.03	
	3	2.08	2289769	20.8	20.85	100.23	
150%	1	2.07	2784176	24.96	25.35	101.56	**101.23**
	2	2.09	2779602	24.96	25.31	101.39	
	3	2.10	2761816	24.96	25.15	100.74	

Precision

The method precision was established by carrying out the analysis of paliperidone. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table 4.

Table 4
Statistical evaluation of the data subjected to method precision of paliperidone

Test	wt of test	Area	% assay
Test solution -1	2.09	2268116	100.24
Test solution -2	2.07	2269786	98.88
Test solution -3	2.1	2266280	100.16
Test solution -4	2.08	2277563	99.70
Test solution -5	2.09	2282321	100.38
Test solution -6	2.06	2276754	98.70
	Mean Assay		**99.68**
	SD		**0.726**
	RSD		**0.729**

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in the flow rate by $\pm$ 0.2 ml /min

Variation in mobile phase composition by $\pm$ 2 %

Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application

A sample equivalent to 2 mg of paliperidone sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 minutes to dissolve it. Further volume was made up to the mark with the diluent to give 200 µg /ml. From this solution 20 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of paliperidone in the product meets the requirement.

RESULT

The development of an analytical method for the validation of drug by RP-HPLC has received considerable importance in quality control of drugs and

drug products in recent years. In the proposed method, the retention time of paliperidone was 3.05 min. The linearity was in the range of 10-30 µg/ml. The regression equation of the linearity was given as Y= 11267x - 37546 where X is concentration of paliperidone in µg /ml. and Y is corresponding peak area. The coefficient of co-relation was 0.9999 (Table no. 2). There is no interference peaks to diluent and at the retention time of paliperidone , the results suggest analytical procedure for the assay of paliperidone is specific. The method was found to be linear. The result shows that an excellent correlation between peak area and concentration of Paliperidone in the range indicated. The relative standard deviation for method precision was 0.729 (limit %Relative Standard deviation is 0.729 which is less than 2.0%). The mean recovery of the paliperidone was 99.68%. The high percentage recovery indicates that the proposed method is highly accurate. The use of 0.1% orthophosphoric acid in water and acetonitrile (65:35 % (v/v) gave peak with good resolution. The robustness studies indicated that there was no effect on the drug study. As per ICH harmonization drug is subjected to change in flow rate ± 0.2 ml /min, change in mobile phase composition by ± 0.2% and change in wavelength by ± 5 nm. The results were given table no 5,6 and 7. From the results obtained in table no. 5.6 and 7 the method have no significant effect on slight in change in parameters like wavelength, flow rate and mobile phase compositions.

Table 5
Robustness study of Change in wavelength

233 nm			**243 nm**		
std solution	**area**		**std solution**	**area**	
	2153153			2165158	
	2158640			2167493	
	2163888			2169827	
	2156844			2167997	
	2159764			2170705	
	2150617			2163094	
Mean	2157151		Mean	2167379	
SD	4758.224		SD	2854.609	
RSD	0.22		RSD	0.13	
test solution	Mean area	**% assay**	**test solution**	Mean area	**% assay**
2152354	2154061		2164422	2165813	
2155767		**99.95**	2167204		**100.12**

Table 6
Robustness study of flow rate

flow rate: 0.8 ml/min			**flow rate: 1.2 ml/min**		
std solution	**area**		**std solution**	**area**	
	2720283			1928394	
	2723223			1930756	
	2728327			1923415	
	2730738			1922704	
	2722898			1925849	
	2729492			1928994	
Mean	2725827		Mean	1926685	
SD	4240.187		SD	3226.929	
RSD	0.16		RSD	0.17	
test solution	Mean area	**% assay**	**test solution**	Mean area	**% assay**
2725578	2723419		1927949	1927029	
2721260		**100.11**	1926108		**100.21**

Table 7
Robustness study of mobile phase composition

mobile phase composition					
63_37			**67_33**		
std solution	**area**		**std solution**	**area**	
	2406017			2208924	
	2402258			2205060	
	2409646			2201075	
	2400149			2209426	
	2401633			2204643	
	2404738			2200098	
Mean	2404074		mean	2204871	
SD	3461.944		SD	3858.852	
RSD	0.14		RSD	0.18	
test solution	Mean area	**% assay**	**test solution**	Mean area	**% assay**
2402606	2404189		2201743	2203473	
2405772		**100.20**	2205202		**100.13**

DISCUSSION

Average recovery of Paliperidone was found to be within the acceptable limit. The method is accurate. The % RSD of system precision was found to be 0.729% .The relative standard deviation was found to be within the acceptable limit. The system is precise. The robustness of an analytical method was found to be within the acceptable limit. The relative standard deviation for the assay values of all six standard preparation were less than 2%. The % RSD of robustness was found to be satisfactory. The robustness of an analytical method was found to be within the acceptable limit. The relative standard deviation at each level was less than 2%. It can be concluded that the analytical method is robust towards the above designed changes. The % RSD, Tailing factor, Theoretical plates was found to be satisfactory. System suitability parameters were found to be within acceptable limits. The RP- HPLC method developed and validated for Paliperidone was sensitive and reproducible with consistency and could be used for routine sample. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation.(Table no.4) The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. (table no.3)

CONCLUSION

A UV Absorption maximum of paliperidone was determined and it was found to be 238 nm. Further it is employed as a UV detection wavelength in HPLC method. The HPLC method developed and validated for paliperidone in bulk as per ICH guidelines was accurate and precise; hence it can be used for assay of drug. The proposed method was found to be specific, accurate. Results showed good linearity, system and , method precision. The method showed repeatability of results with respect to robustness and system suitability conditions. In all the cases RSD was found to be less than 2% and standard deviation is within the limits. From the above studies the Paliperidone drug was estimated accurately by using the proposed RP-HPLC method in bulk samples. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation and degradation of drug in various conditions as per ICH guidelines.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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