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Paliperidone Determination by Using HPLC Method in Blood Plasma Samples-Stability Indicating Method

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Abstract

Paliperidone product is used to control schizoaffective disorder in adults. In the market 3mg, 6mg, 9mg and 12mg strength tablet dosage form is available. Regular used strength tablets are 3mg and 6mg. Objective was to develop a simple HPLC method to evaluate the qualitative and quantitative quantity of the Paliperidone with known impurities separation. Blood plasma samples were prepared and injected into the HPLC. Simple HPLC method was developed and validated the method. *Buffer:* 2.1g of TBAHS in 100 mL of HPLC grade water, *Mobile phase:* Buffer, Acetonitrile 90:10 v/v, Zorbax SB C18 100 x 4.6 mm, 3.5 µm, flow rate 1.0 mL/min, 275 nm wavelength, 10 µL injection volume, column temperature 40°C were used. Run time 20 min was performed. Method validation was performed with precision, linearity, accuracy, ruggedness, robustness and specificity (interference and force degradation). Optimized method can be used to determine the Paliperidone in tablets dosage form.

Keywords

Paliperidone, Schizoaffective disorder, Dopamine antagonist, HPLC method development and validation.

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INTRODUCTION

Paliperidone is a dopamine antagonist and 5HT2A antagonist. The chemical name is (±)-3-[2-[4-(6-fluoro-1, 2benzisoxazol-3-yl)-1-piperidinyl] ethyl]-6, 7, 8, 9-tetrahydro-9-hydroxy-2-methyl-4Hpyrido [1, 2-a] pyrimidin-4-one. Molecular formula is $C_{23}H_{27}FN_4O_3$ and molecular weight is 426.49 g/mol. Paliperidone is sparingly soluble in 0.1N HCl and methylene chloride; practically insoluble in water, 0.1N NaOH, and hexane; and slightly soluble in N, N-dimethylformamide. It is an antipsychotic class drug product. Paliperidone is used for the treatment of schizophrenia and schizoaffective disorder. Paliperidone is used for mental/ mood disorders, medication can decrease hallucinations and increase the more clearly and positively thinking ness. Paliperidone side effects are tachycardia, somnolence, insomnia, headache, hyperprolactinaemia and sexual dysfunction.

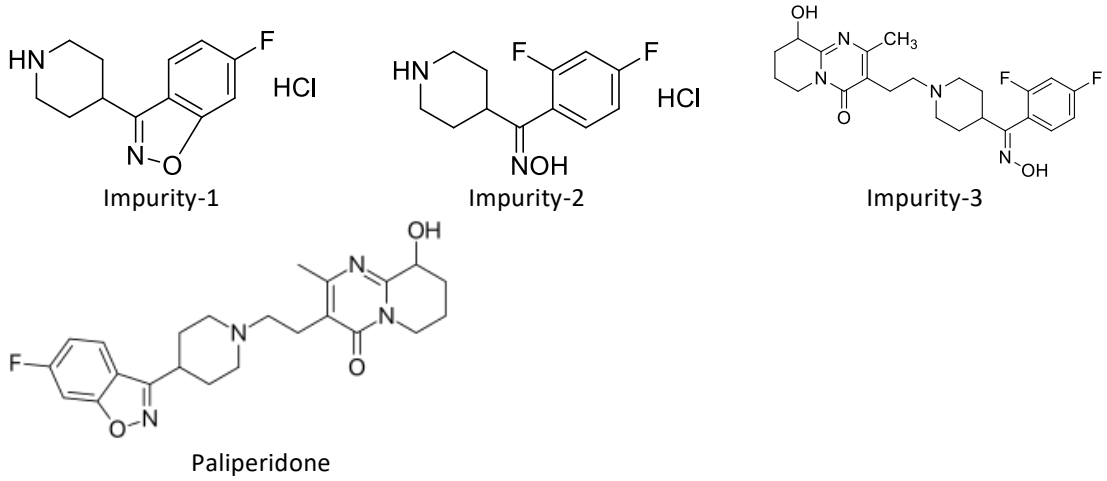

Figure-1: Chemical structure of the Paliperidone and its impurities.

Impurities Chemical names:
Impurity-1: 6-fluoro-3-(piperidin-4-yl) benzo[d]isoxazole hydrochloride
Impurity-2: (2, 4-difluorophenyl) (piperidin-4-yl) methanone oxime hydrochloride
Impurity-3: (Z)-3-(2-(4-((2,4-difluorophenyl)(hydroxyimino)methyl)piperidin-1-yl)ethyl)-9-hydroxy-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one.

A complete literature survey was conducted to understand the literature and publications. Few methods were reported on HPTLC (Patel RB, 2010) [7], extraction HPLC method (SL Mendez, 2014) [8], paliperidone and its related impurities method (Bindu KH, 2010) [9] and (Jadhav SA, 2011) [10], (Rao KN 2013) [11] and few methods reported for enantiomers (Locatelli I, 2009)[12] and (Swarnalatha G, 2014) [13]. Based on the literature understanding there is no method reported to determine the paliperidone and its impurities for the determination of the blood plasma samples. Our objective of this study was to develop a simple and stability indicating HPLC method for the quantification of the paliperidone in blood plasma samples.

MATERIAL AND METHODS

Reagents:
Analytical reagent grade Tetrabutyl ammonium hydrogen sulphate (TBAHS), HPLC grade water and methanol were used.

HPLC Instrument:
Waters Alliance 2695 separations module equipped with gradient elution capability, 2487 UV detector and an auto sampler. Empower work station data handling system.

Chromatographic conditions:
Zorbax SB C18 100x4.6 mm, 3.5µm equivalent column, flow rate 1.0 mL/min, 275 nm wavelength, 10 µL injection volume, column temperature 40°C were used. Run time 20 min was performed.

Mobile phase:
Buffer: 2.1g of TBAHS weighed and transferred into a beaker containing 100 mL of HPLC grade water and mixed to dissolve. Filtered through 0.45 micron or finer porosity membrane filter.
Mobile phase: Buffer, Acetonitrile 90:10 v/v.

Diluent: Mobile Phase.

Standard solution:
Weighed accurately 50.0 mg of Paliperidone a working standard into a 100 mL clean, dry volumetric flask, added 60mL of diluent and sonicated to dissolve. Made up to volume with diluent and mixed.

Sample solution:
12.5 mg of Paliperidone into 25mL volumetric flask, 10 mL of diluent added and mixed for 20 min further diluted and mixed. Allowed settling the solution and filtered the clear supernatant solution using a 0.45µm syringe filter.

Plasma sample solution preparation:
Blood plasma samples were prepared with extraction process. Lquid-liquid extraction process was applied. 100mg was spiked in to 10ml plasma and stored for 1day (24 Hours). For processing, the stored spiked samples were withdrawn from the freezer and allowed to thaw at room temperature. An aliquot of 500µL was transferred to prelabeled 10.0 mL polypropylene centrifuge tubes. Extraction solvent, 5.0 mL of ethyl acetate, was then added to extract the drug. The samples were then kept on a vibramax unit and vortexed for 15 min. Samples were then centrifuged at 5000 rpm for 5 min in a refrigerated

centrifuge (4°C). Supernatant solution, 1 mL was then transferred into pre-labeled polypropylene tubes and was allowed to evaporate to dryness under nitrogen at constant temperature of 40°C. The dried residue was then dissolved in 200µL of mobile phase and transferred into shell vials containing vial inserts for analysis. Dilution was performed to reach 500ppm concentration.

System suitability:

1. Tailing factor should be NMT 2.0 for the Paliperidone standard peak.
2. Theoretical plates corresponding to Paliperidone peak should NLT 5000.
3. The %RSD for five replicate standard injections should be NMT 2.0%.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT:

Literature published methods were reviewed and understood the Paliperidone chemical and physical properties. Three impurities were evaluated and those three impurities are forming during synthesis and tablets formulations. All three known impurities were evaluated to understand the UV maximum absorbance. All three impurities and Paliperidone have 230nm maximum absorbance so 230nm was selected to perform the analysis. Figure-2 represented the UV spectrums for all four analytes.

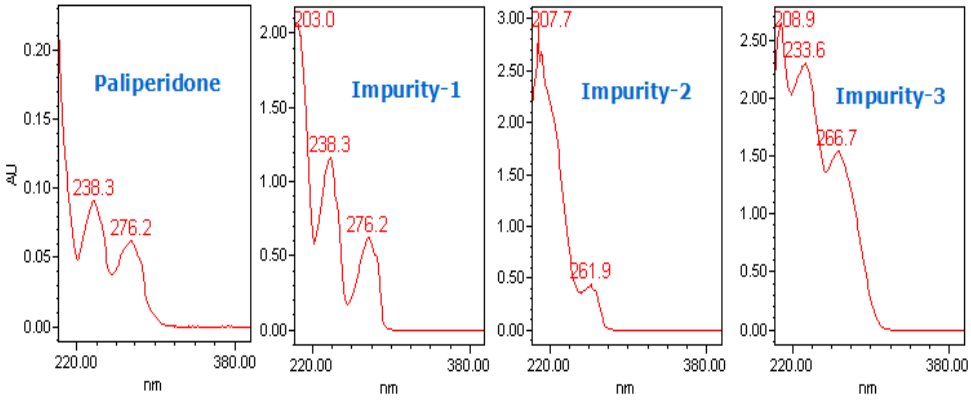

Figure-2: UV spectrums for Paliperidone and its impurities

Initial HPLC method development was performed with ammonium acetate buffer and acetonitrile as organic modifier. Experimental trials were discussed.

Experiment-1: mobile phase: 0.03M ammonium acetate in water as buffer and acetonitrile 60:40v/v; diluent: water and acetonitrile 60:40v/v; column YMC pack C18 150*4.6mm, 3µm; flow rate 1.0ml/min; 230 nm wavelength; 50µL injection volume; 40°C column oven temperature; sample solution 1.0mg/ml and standard solution 0.2% concentration with respect to sample solution.

Results: Paliperidone was eluted at 3 min and other impurities were eluted very near. Base line noise was observed. Ionic pair buffer salt may give separation

Experiment-2: mobile phase: 2g TBAHS (tetra butyl ammonium hydrogen sulphate) in 100 ml water as buffer and acetonitrile 60:40v/v; diluent: water and acetonitrile 60:40v/v; column YMC pack C18 150*4.6mm, 3µm; flow rate 1.0ml/min; 230 nm wavelength; 50µL injection volume; 40°C column oven temperature; sample solution 1.0mg/ml and standard solution 0.2% concentration with respect to sample solution.

Results: Paliperidone was eluted at 4 min. Three impurities were eluted very early about 2min with poor peak shape.

Experiment-3: mobile phase: 2g TBAHS (tetra butyl ammonium hydrogen sulphate) in 100 ml water as buffer and methanol 60:40v/v; diluent: water and acetonitrile 60:40v/v; column Zorbax SB C18 100*4.6mm, 3µm; flow rate 1.0ml/min; 230 nm wavelength; 50µL injection volume; 40°C column oven temperature; sample solution 1.0mg/ml and standard solution 0.2% concentration with respect to sample solution.

Results: Paliperidone was eluted at 6.5 min, impurity-1 eluted at 9.7min, impurity-2 eluted at 2.39min and impurity-3 eluted at 2.4min. Three impurities were well separated and have good peak shape. Each analytes Paliperidone and its impurities 1, 2 and 3 chromatograms were represented in Figure-3 to 6.

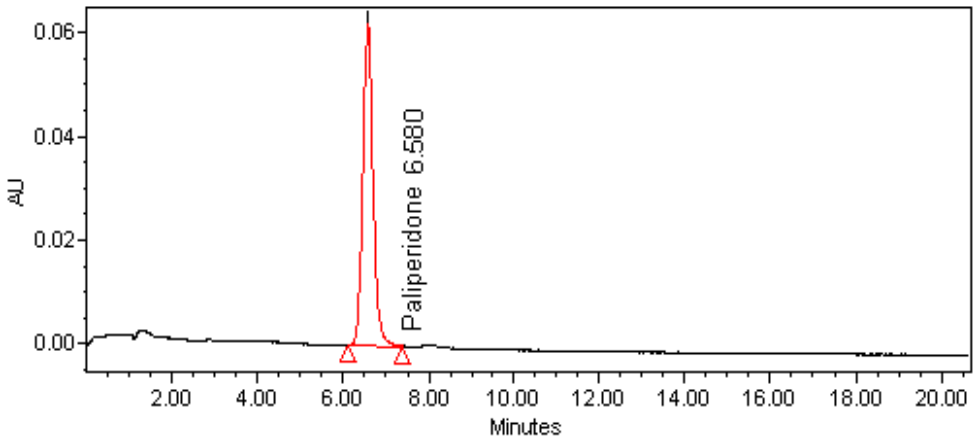

Figure-3: Paliperidone chromatogram

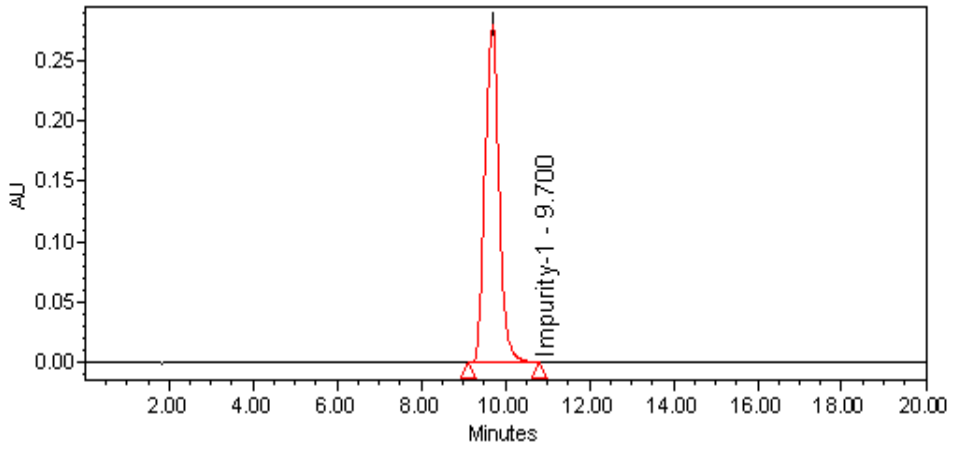

Figure-4: Impurity-1 Chromatogram

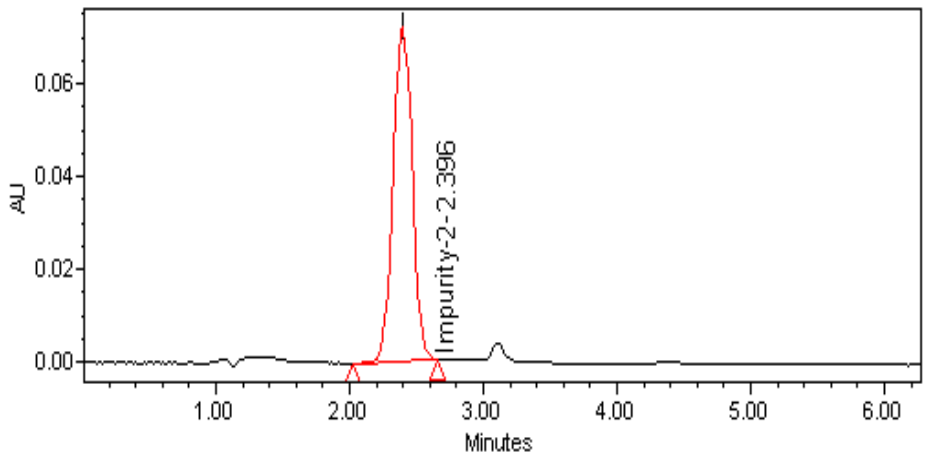

Figure-5: Impurity-2 Chromatogram

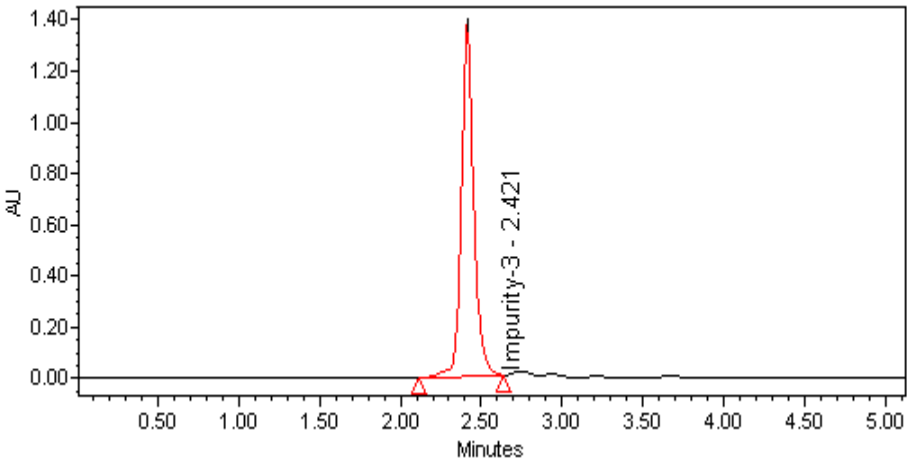

Figure-6: Impurity-3 Chromatogram

Specificity:

Specificity experiments were performed with acid, base, peroxide, thermal, UV light, humidity and water hydrolysis stress degradation studies. Interference between known and unknown impurities was evaluated and found that there is no interference. % of assay for all stress studies were reported in table-1. Stress studies chromatograms were represented in figure-7 to 14.

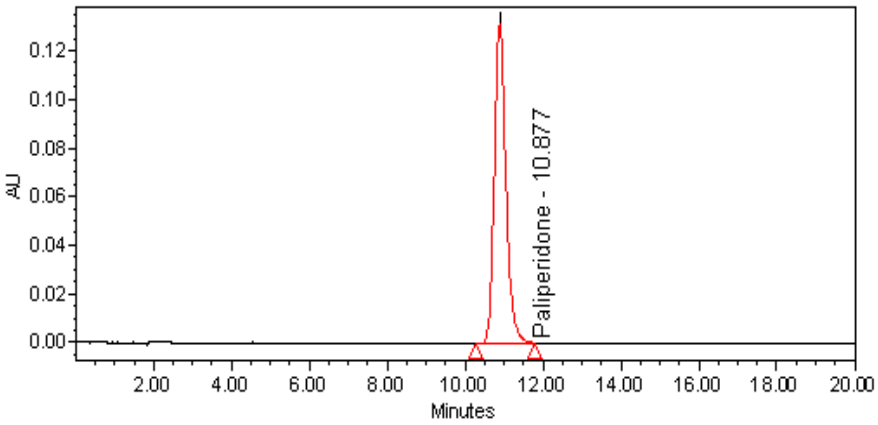

Figure-7: As such test solution as such 6 mg tablets chromatogram

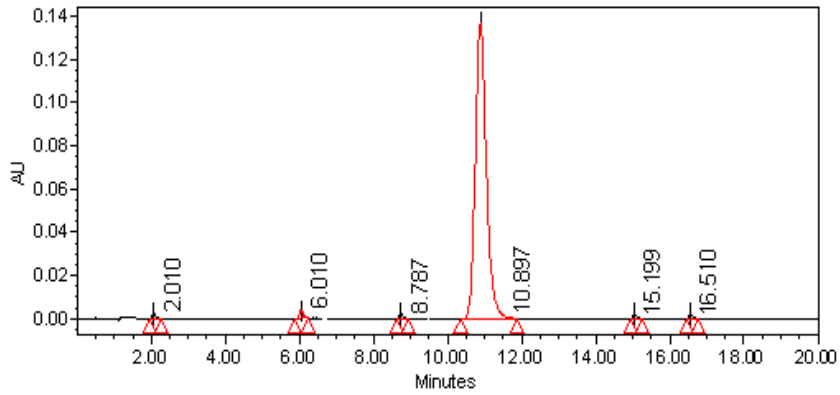

Figure-8: Acid degradation sample chromatogram

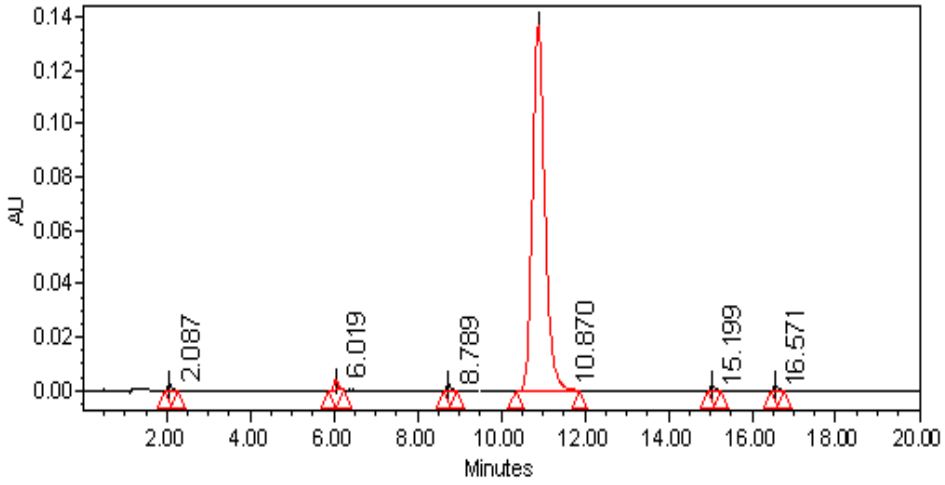

Figure-9: Base degradation sample chromatogram

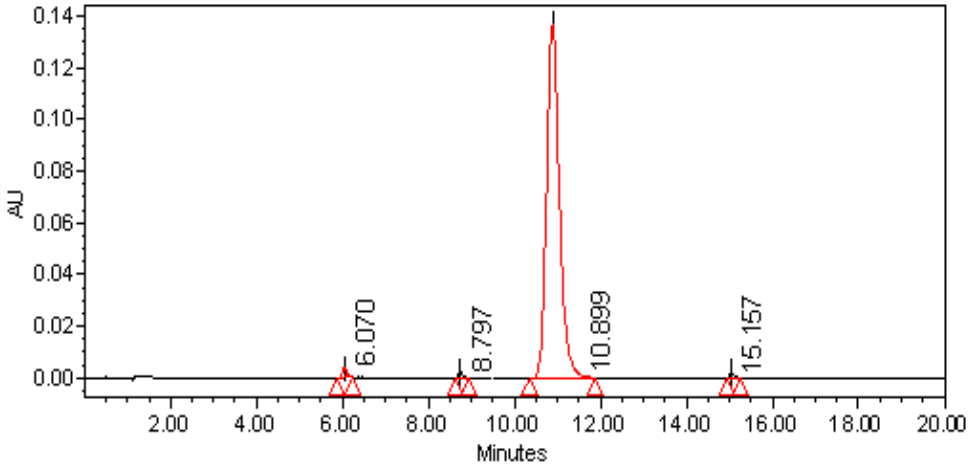

Figure-10: Peroxide degradation sample chromatogram

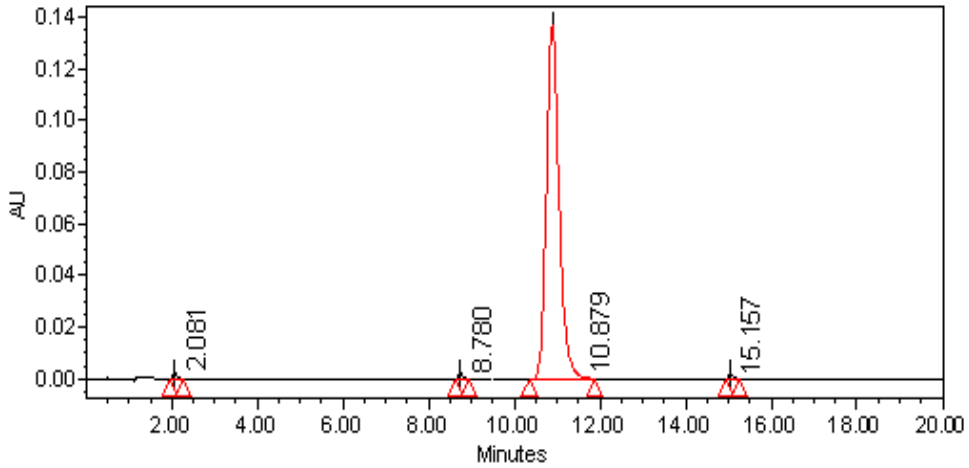

Figure-11: Thermal degradation sample chromatogram

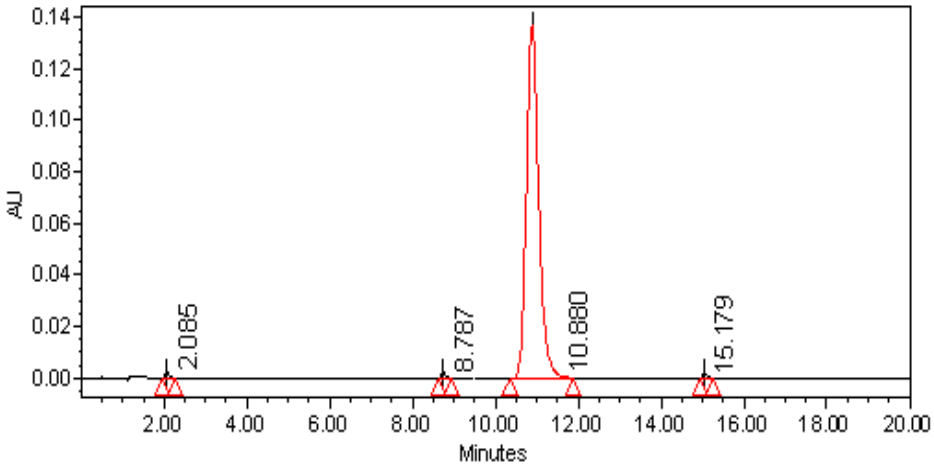

Figure-12: UV degradation sample chromatogram

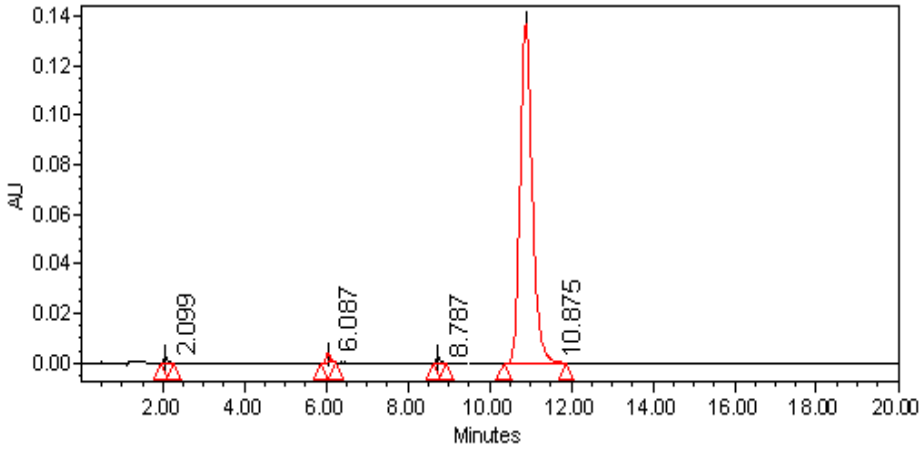

Figure-13: Water hydrolysis sample chromatogram

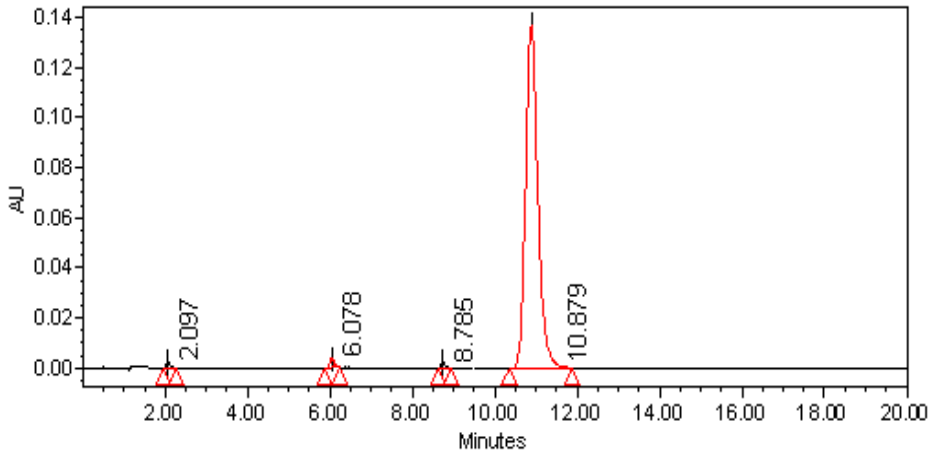

Figure-14: Humidity degradation sample chromatogram

Table-1: Stress study results

S. No.	Degradation condition	Paliperidone % of assay	Purity Angle	Purity Threshold	Peak purity
1.	Test as such	99.6%	0.073	0.255	Pass
2.	Acid (5N HCl, 1hr, 60°C)	94.2%	0.071	0.309	Pass
3.	Base (5N NaOH, 1hr, 60°C)	93.4%	0.146	0.283	Pass
4.	Peroxide (3% H_2O_2, 1hr, RT)	94.8%	0.084	0.243	Pass
5.	Water (Water, 6hr, 60°C)	94.6%	0.094	0.255	Pass
6.	Thermal (24hr, 80°C)	95.6%	0.073	0.255	Pass
7.	UV/ visible light (UV light 200 watt hr/sq. meter) (Visible light 1.2 million lux hrs)	93.9%	0.089	0.289	Pass
8.	Humidity (90%RH, 25°C, 7hr)	94.8%	0.068	0.216	Pass

Precision:

Method precision was performed for both the tablets strength 3mg, 6mg, 9mg and 12 mg. Six replicate test samples were performed freshly, analysed on HPLC and calculated the assay values and % RSD for six replicate preparations. Precision results confirmed the method repeatability and reproducibility. System suitability was evaluated with five replicate solutions. Blank, placebo and standard solutions chromatograms were represented in figure-15 to 17. Figure-18 represented the five replicate standard injections. Intermediate precision was performed with different HPLC instrument; different analyst and different lot column. Intermediate precision results were satisfactory. Precision and intermediate precision results were compared and found to be acceptable. Table-2 represented the precision and intermediate precision results.

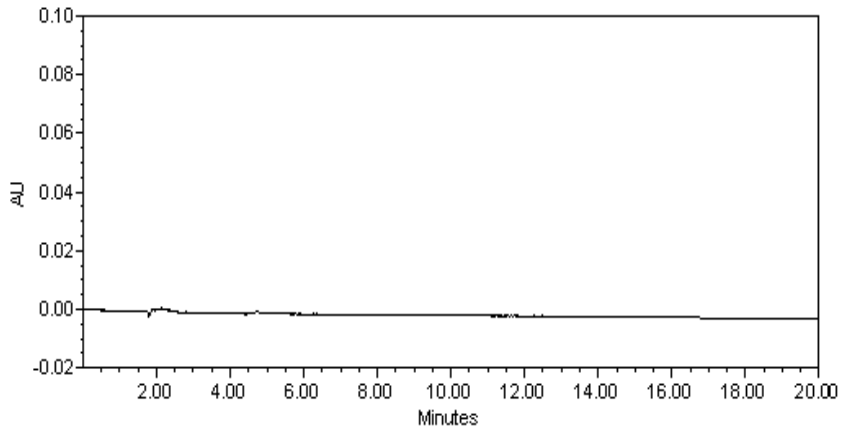

Figure-15: Blank Chromatogram

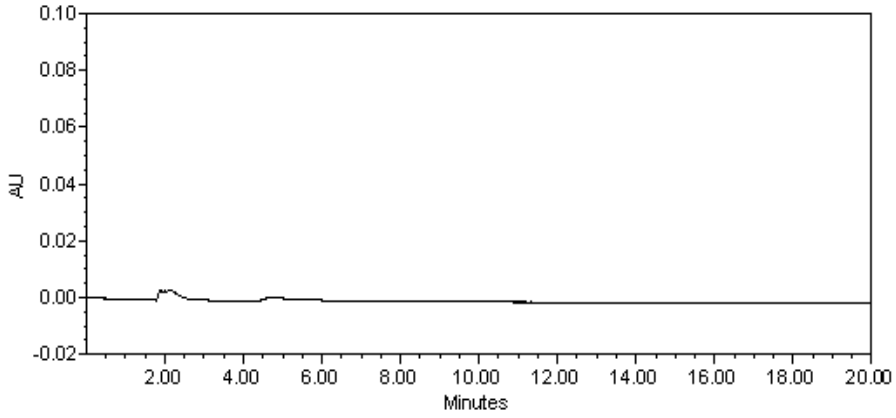

Figure-16: Placebo Chromatogram

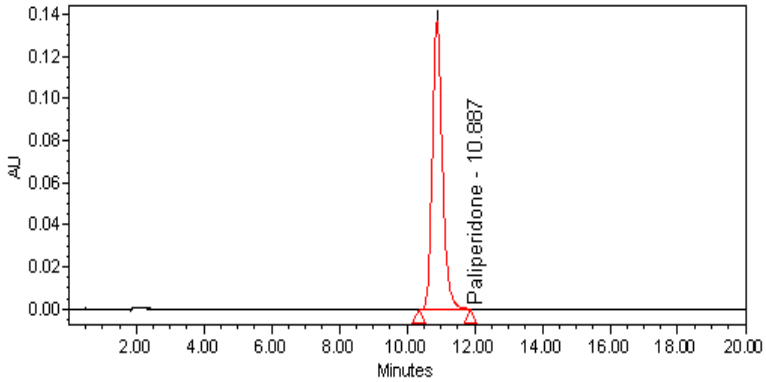

Figure-17: Standard chromatogram

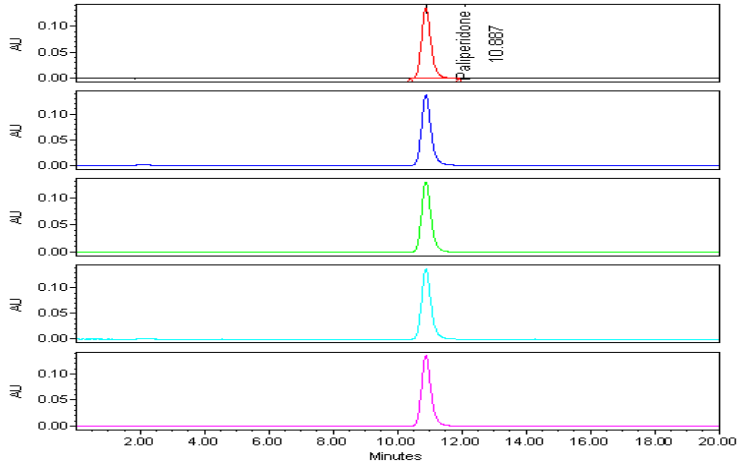

Figure-18: Five replicate standard solution overlay chromatogram

Table-2: Precision and intermediate precision results

	Precision results							
Paliperidone	**1**	**2**	**3**	**4**	**5**	**6**	**Average**	**%RSD**
Assay (%)	101.25	100.12	99.79	101.21	99.68	100.58	100.43	0.68
	Intermediate precision							
Assay (%)	100.16	100.25	99.87	100.15	99.88	100.21	100.08	0.16

Linearity:

Method linearity was performed to confirm the method linearity range. Five different concentration linearity levels were prepared with the standard material. 50% to 150% of standard and test solution concentrations were coved and confirmed the linearity correlation coefficient value. Correlation coefficient value 0.9993 was observed and linearity plot was drawn. Table-3 represented the Paliperidone linearity results. Overlay chromatograms were represented in figure-19 and linearity plot was shown in figure-20.

Table-3 Paliperidone Linearity results

Linearity level	**1**	**2**	**3**	**4**	**5**	**Corr. of coeffi.**
Conc. (µg/ml)	244	311	490	620	763	
Area	1254080	1669805	2561244	3356413	4191864	0.9993

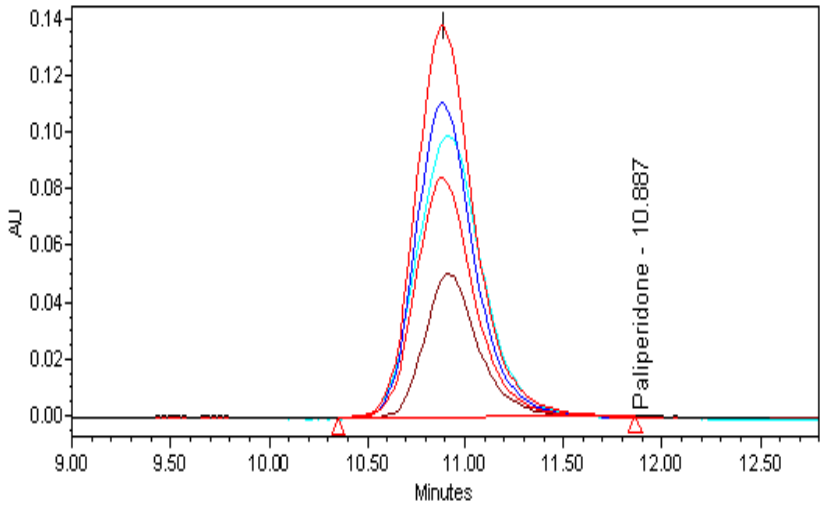

Figure-19: Linearity over lay chromatogram

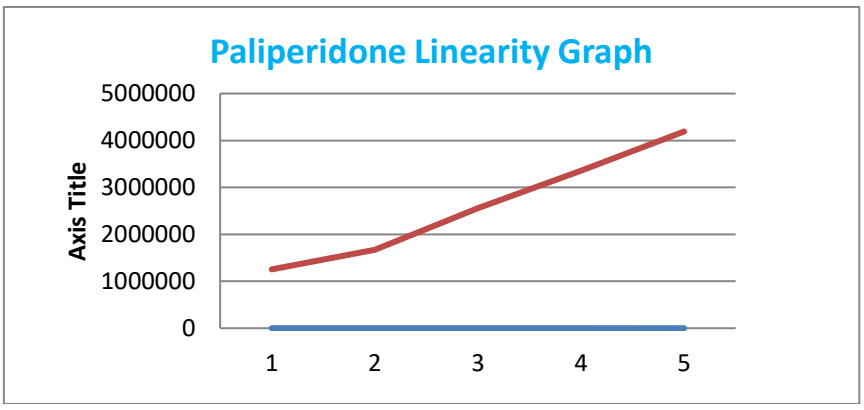

Figure-20: Linearity Graph

Accuracy:

Accuracy of the method was evaluated with three different concentration levels 50%, 100% and 150% of the test concentration. Higher concentration 150% level was prepared for six replicates and other accuracy levels were three replicates to confirm the method accuracy. % recovery was calculated and found to be within the acceptable limits (limit: 98% to 103%). Accuracy results were tabulated in 4.

Table-4: Accuracy results

	Paliperidone accuracy results											
µg	Added	248			506			750				
/mL	Recov.	243	246	251	509	510	516	742	749	751	748	743	758
% recov. (%)		97.98	99.19	101.21	100.59	100.79	101.98	98.93	99.87	100.13	99.73	99.07	101.07
Mean (%)		99.46			101.12			99.80					

Ruggedness:

Ruggedness was performed to confirm the test solutions and standard solution stability in room temperature and refrigerator storage conditions. Two precision test samples were used to study the storage stability. Day-0, 1, 3 were evaluated at room temperature storage samples and day-0, 3 and were evaluated at refrigerator storage conditions. Standard solutions were injected and systems suitability parameters tailing factor and %RSD were

evaluated. Table-5 represented the ruggedness results.

Table-5: Ruggedness results

Time in day	Bench top stability test solution				Tailing factor	%RSD	Bench top stability standard solution
			Difference				Similarity factor
	Test-1	Test-2	Test-1	Test-2			
Initial	101.25	100.12	NA	NA	1.3	1.3	NA
Day-1	100.16	100.92	1.09	0.8	1.4	1.5	0.99
Day-3	99.69	101.10	1.56	0.98	1.1	1.4	1.0
	Refrigerator stability test solution						Refrigerator stability standard solution
Initial	101.25	100.12	NA	NA	1.4	1.3	NA
Day-3	101.65	100.31	0.4	0.19	1.2	1.5	0.99
Day-5	100.89	100.96	0.36	0.84	1.1	1.2	1.01

Robustness:

Robustness was evaluated with analytical method chromatographic conditions. Mobile phase flow rate, column oven temperature and mobile phase organic solvent ratio were studied and reported the system suitability results. Table-6 represented the robustness results. Filter variations were analysed with PVDF and NYLON types. Filter variation results confirmed that there is no difference in the filter change. Filter validation results were tabulated in table-7.

Table-6: Results of Effect of variations

Condition	Flow rate			Column temperature		
	0.8 & 1.2 ml/min	0.6 & 1.0 ml/min	1.0 & 1.4 ml/min	30°C	25°C	35°C
Tailing factor	1.3	1.6	1.3	1.3	1.5	1.2
% RSD	0.52	0.58	0.49	0.39	0.42	0.49
	Mobile phase organic solvent ratio					
	pH 6.0	pH 5.8	pH 6.2			
Tailing factor	1.2	1.6	1.3			
% RSD	0.46	0.52	0.58			

Table-7: Filter Variability results

Centrifuged		Nylon filter				PVDF filter			
% assay		% assay		% Difference		% assay		% Difference	
Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2
100.64	101.01	100.31	100.69	0.33	0.32	100.63	100.89	0.01	0.12

CONCLUSION

Paliperidone medicinal product is used to treat schizophrenia and schizoaffective disorder. It is more effective than Haloperidol, Quetiapine and aripiprazole. Simple HPLC method was developed and validated to evaluate the qualitative and quantitative of Paliperidone in tablets dosage forms 3mg, 6mg, 9mg and 12 mg strengths. *Buffer:* 2.1g of TBAHS 100 mL of HPLC grade water; *Mobile phase:* Buffer, Acetonitrile 90:10 v/v, Zorbax SB C18 100x4.6 mm, 3.5µm column, flow rate 1.0 mL/min, 275 nm wavelength, 10 µL injection volume, column temperature 40°C were used. Run time 20 min. method validation was performed to confirm the each parameter such as precision, linearity, accuracy, ruggedness, robustness, specificity. Method validation results precision assay values (98% to 102%), %RSD for six replicates, linearity correlation co-efficient value not less than 0.999 were confirmed the method repeatability and reproducibility. Specificity (blank, placebo and impurities interference and force degradation) confirmed the stability indicating nature. Method can be used to study the Paliperidone in tablets dosage form.

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