Research Article

Development and Validation of A Stability Indicating Method For Paliperidone In Paliperidone Depot Injection Using Reversed Phase High Performance Liquid Chromatography

VS. Mastiholimath[1]*, G. Gupta[1], PM. Dandagi[2], AP. Gadad[2] and VR. Pai[1]

[1]Department of Quality Assurance, KLE University's College of Pharmacy,
Near KLES Prabhakar Kore Hospital, Nehru Nagar,
Belgaum-590 010, Karnataka, India.
[2]Department of Pharmaceutics, KLE University's College of Pharmacy,
Near KLES Prabhakar Kore Hospital, Nehru Nagar,
Belgaum-590 010, Karnataka, India.

ABSTRACT
A stability-indicating RP-HPLC method was developed and validated for the determination of paliperidone in paliperidone depot injection using C18 column (250mm x 4.6mm, 5µm) with mobile phase consisting of methanol : buffer in the ratio of 35:65 respectively with a flow rate of 1.5mL/min (UV detection at 236nm). Linearity was observed over the concentration range 53.6-91.1µg/mL (r^2 = 0.9997) with regression equation y = 21026x - 69083. Paliperidone was subjected to stress conditions including acidic, alkaline, oxidation, and thermal degradation. Paliperidone is more sensitive towards alkali degradation. The method was validated as per ICH guidelines.

Keywords: Paliperidone; Reversed-phase HPLC; Stability indicating; Validation.

1. INTRODUCTION

Paliperidone (Figure 1), (*RS*)-3-[2-[4-(6-fluorobenzo[*d*]isoxazol-3-yl)-1-piperidyl]ethyl]-7-hydroxy-4-methyl-1,5-diazabicyclo [4.4.0] deca-3,5-dien-2-one, is the major active metabolite of risperidone (9-hydroxy-risperidone) and is an atypical antipsychotic that belongs to the chemical class of benzisoxazole derivatives [Richelson and Souder 2000, Cada, Baker and Levien 2007, Kane et al 2007, Fowler, Bettinger and Argo 2008, Vermeir et al 2008].

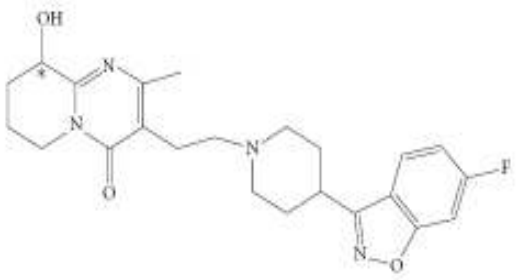

FIGURE 1: Chemical structure of paliperidone. *indicates position of chiral carbon atom.

The drug was approved on December 20, 2006 by the United States Food and Drug Administration (FDA) for the treatment of schizophrenia [Corena-McLeod et al 2008]. It binds to both D2 and serotonin-2A (5-HT2A) receptors; antagonism at these receptors is thought to account for the therapeutic activity of this drug in schizophrenia, as demonstrated in both *in vitro* and *in vivo* animal and human studies [Vermeir et al 2008, Karlsson et al 2005, Janssen-Cilag 2007, Luthringer 2006]. Paliperidone is also active as an antagonist at alpha 1 and alpha 2 adrenergic receptors and H1 histaminergic receptors, which may explain some of the other effects of the drug. A series of studies have evaluated the pharmacodynamics, efficacy, and tolerability of paliperidone. The results obtained from randomized, double-blinded, placebo-controlled studies indicate that the drug is effective and safe at all doses, resulting in significant improvements in the symptoms of schizophrenia and related disorders [Kane et al 2007, Davidson et al 2007, Marder et al 2007, Canuso et al 2008, Dunner et al 2010, Marchese et al 2010].

Paliperidone has been recently investigated with regard to its determination as a main metabolite of risperidone. Previous studies have described the analysis of risperidone and 9-hydroxy-risperidone in plasma, urine and serum using LC, LC/MS-MS and MEPS-LC-UV [De Meulder et al 2008, Kirschbaum et al 2008, Mandrioli et al 2010]. Locatelli et al. proposed the determination of these substances in biological fluids using HPLC methods, allowing for the simultaneous analysis of enantiomers. The method combines reverse phase chiral separation and electrochemical detection, which guarantee sufficient sensitivity to investigate stereo selectivity. Paliperidone has also been determined in bulk using enantioselective analytical methods, liquid chromatography, and capillary electrophoresis [Danel, Chaminade et al 2007, Danel, Barth´el´emy et al 2007, Danel, Azaroual 2009]. Using a dual cyclodextrine mode in the chiral capillary electrophoretic method, the kinetics of racemisation under acidic and basic conditions (a process related to the configurational stability of the drug) have been described [Danel, Azaroual et al 2009]. Literature survey reveals that there is no stability indicating assay method reported for Paliperidone in Paliperidone Depot injection dosage form by HPLC. We developed a simple, specific, accurate, precise and robust liquid chromatographic method for the determination of Paliperidone in Paliperidone Depot injection. Stability indicating assay method (SIAM) of Paliperidone Depot injection by RP-HPLC was also developed.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Paliperidone was obtained from Ranbaxy Research Labs Ltd., Gurgaon, India. HPLC grade methanol was obtained from Loba Chemie Pvt. Ltd., India. Analytical reagent grade Ammonium Dihydrogen Orthophosphate, hydrogen peroxide, potassium di-hydrogen ortho phosphate was obtained from Fischer Scientific, India. Analytical reagent grade sodium hydroxide was obtained from Merck specialties Pvt. Ltd, India, Analytical reagent grade potassium hydroxide and hydrochloric acid was obtained from RFCL Pvt. Ltd. Highly pure water was prepared by using Millipore Milli Q plus purification system.

2.2 HPLC instrumentation and chromatographic conditions

The chromatographic apparatus consisted of an HPLC system (Waters TM 996, China) equipped with a gradient pump, vacuum degasser & mixer, and PDA detector. Data acquisition was performed with Empower software, version 2.0. Separation was performed on a Kromasil C18 column (4.6 mm × 250 mm, 5 µm) and Hypersil BDS C-18 column (4.6 mm × 250 mm, 5 µm); M/S Thermo Fisher Scientific Inc., U.S.A. The mobile phase used was in the ratio of 35:65:: methanol: buffer. 0.5M ammonium di-hydrogen orthophosphate buffer containing 0.1% triethylamine of pH 4.6 adjusted using orthophosphoric acid (40:60, v/v) was used. All analyses were carried out under isocratic conditions at a flow rate of 1.5 mL/min and at 45ºC temperature. PDA detector was set at 236nm. The mobile phase was prepared daily and degassed by sonication. All solvents were filtered through a 0.45-µm disposable membrane filter immediately before use.

2.3 Assay

2.3.1 Preparation of diluent-1

Prepare a suitable quantity of a mixture of Methanol and Acetonitrile in the ratio of 50:50 and mix.

2.3.2 Preparation of diluent-2

Prepare a suitable quantity of a mixture of Diluent-1 and Water in the ratio of 50:50 and mix.

2.3.3 Preparation of standard solution

Paliperidone working standard equivalent to about 75 mg was accurately weighed and transferred to a 100mL volumetric flask. Dissolved in about 50mL of diluent-1 by sonication and made up the volume. 5 ml of this solution was diluted to 50 mL with diluent-2. Solution was filtered through 0.45 µm nylon filter discarding first 5mL of filtrate.

2.3.4 Preparation of sample solution

Accurately weighed and transferred the sample solution equivalent to about 75 mg of Paliperidone with the help of a hypodermic needle of 20 gauge into 100 ml volumetric flask. Sample was dissolved in about 50mL of diluent-1 by sonication made up the volume with diluent-1. 5 ml of this solution was diluted to 50 mL with diluent-2. Solution was filtered through 0.45 µm nylon filter by discarding first 5mL of filtrate.

2.4 Method Validation

The chromatographic method was validated based on specificity, linearity, accuracy, precision, robustness, limit of quantitation, limit of detection and system suitability [USP 2008,US FDA Orange book 2011, Mandal et al

2010, ICH Q2 (R1) 2005]. The stability-indicating capability was determined under forced degradation conditions, including heat, oxidation, and acidic and basic degradation [ICH Q1A (R2) 2003].

2.4.1 System suitability
System suitability test was performed by preparing standard solutions of 75 ppm of Paliperidone and injected five times. System suitability parameters like theoretical plates, tailing factor and retention period were calculated.

2.4.2 Specificity
The specificity was determined by injecting Control sample of Paliperidone in triplicate and spiked sample in triplicate. The possible interferences were analyzed on the basis of RT and peak purity, which were calculated using software.

2.4.3 Linearity
Calibration curves were constructed by plotting peak areas vs. concentrations of Paliperidone and regression equations were calculated. The calibration curves were plotted over the concentration range 52.5-90.5 µg/mL for Paliperidone. For each drug, six injections of different concentrations (within their respective prescribed limit) were injected and area for each peak was calculated. Concentration vs. area plots were constructed in each case and correlation coefficient (r^2) were determined.

2.4.4 Accuracy
Accuracy of the method was determined by replicate analysis of three sets of samples at high, middle and low quantity control concentrations and comparing the difference between the actual value and recovered value. Accuracy was expressed as % Recovery.

2.4.5 Precision
For system precision six replicate injections of standard solution having 75 ppm Paliperidone were injected to calculate the percent relative standard deviation (%RSD) and hence the system precision.
The method precision data were obtained by repeatedly analyzing the drug samples. For this six times sample was prepared as per the procedure laid down in sample solution preparation. Each solution was injected into the HPLC system and their respective chromatograms were studied in terms of peak areas for Paliperidone. Mean, ±SD (standard deviation) and %RSD were calculated for each drug.

2.4.6 Robustness
In order to demonstrate that the analytical method is capable to yield reproducible results, a small but deliberate variation in method parameters during normal usage such as change in flow rate ,change in temperature, change in organic phase composition, change in wavelength was made and % RSD was calculated in each case.

2.5 Stability Indicating Assay Method
In order to determine the stability of sample solution of Paliperidone, sample was injected at various time intervals at room temperature. The area of peak was determined and % RSD was calculated.

2.5.1 Force Degradation Studies
Stress degradation study was carried to confirm that during stability study or through its shelf life, any degradation product if found would not interfere with the peaks of Paliperidone. Sample and placebo were separately treated under degradation studies.
All solutions for use in stress studies were prepared at the initial concentration of 1.5mg/mL of paliperidone using diluent-1. All samples were then diluted in diluent-2 to give a final concentration of 75µm/mL and filtered before injection.
Acid degradation was carried out in 5N HCl and Alkali degradation was conducted using 5N NaOH, refluxed for 2 hours at 60^0C. After cooling the solutions were neutralized and diluted with diluent-2.
Solutions for oxidative stress studies were prepared using 30% w/v H_2O_2 solution at a concentration of 1.5mg/mL of paliperidone and after refluxation for 2 hours on thermostat the sample solution was cooled and diluted accordingly with diluent-2.
For thermal degradation one complete vial of Paliperidone Depot injection was exposed at 80º C for 3 hours, cooled and used in the same way as used for other solutions.

3. Results
The representative chromatogram obtained for paliperidone showing system suitability is shown in Fig. 2. The calibration curve was linear over the concentration range 53.6-91.1µg/mL (Table II) and the regression equation was found to be y = 21026x - 69083 with correlation coefficient of 0.99962. The RSD in precision studies was found to be 0.67% (system precision) and 0.57 (method precision) (Table IV and Table V). The %RSD in accuracy studies (Table VI) and robustness studies (Table VII) was found to be less than 2.0%, indicating that the method is precise,

accurate and robust. The LOQ was found to be 2.042µg/mL and the LOD was found to be 0.67µg/mL. The proposed method was applied for the determination of paliperidone injections and the results of these assays yielded 98.4-99.6% respectively with RSD <2.0% (Table 5). The capacity factor was more than 2, theoretical plates were 6780 (not less than 4000) and tailing factor was 1.3 (not more than 1.5) for the paliperidone peak. The %RSD value of assay determined under original conditions and robust conditions was less than 2.0%, indicating that the developed method was robust. Paliperidone degraded in range of 15% in thermal, 19% in alkali and 15% in oxidation conditions (Table VIII). But Acid, Photolysis and Humidity doesn't have much influence on the product. Above data clearly shows that the product was much subjective to Alkali degradation. Also peak purity passed for every condition supporting the specificity in favor of the method in worst situations. The demonstration of the specificity (Table V) and the ability of the method to monitor changes in the chemical properties of the drug over time invariably calls for a forced degradation (stress testing) study to be performed on the drug substance and drug product [Ngwa G 2010]. According to FDA guidelines [FDA Guidance for industry 2000], stress testing of a drug substance can aid in the identification of likely degradation products, which can help to establish degradation pathways and reveal the intrinsic stability of the molecule. Stability indication refers to a process whereby it is demonstrated that, after degradation of the test article, the analytical assay method is capable of producing reliable results for its active substances and preservatives, if applicable, without interference from degradation products [Nilsen 2010]. Typical chromatograms obtained following the assay of stressed samples are shown in Fig. 3,4,5,6 respectively.

4. DISCUSSION

No stability indicating method is available in the official compendia using HPLC for analyzing Paliperidone in Paliperidone Depot injection till now. The complete separation of the analytes was accomplished in 12 min and the method can be successfully applicable to perform long-term and accelerated stability studies of Paliperidone Depot injection. According to ICH [ICH Q2 (R1) 2005], the analytical procedure refers to the method of performing the analysis, describing in detail the steps necessary to perform each analytical test. The goal of any analytical method is to produce analytical results that reflect the content of the samples with an acceptable standard of accuracy [Feinberg 2007].

5. ACKNOWLEDGMENT

The authors are thankful to Principal, KLE University's College of Pharmacy; Belgaum, Karnataka and Ranbaxy Laboratories Pvt. Ltd Gurgaon - Haryana for providing necessary facilities for research work in their esteemed organization.

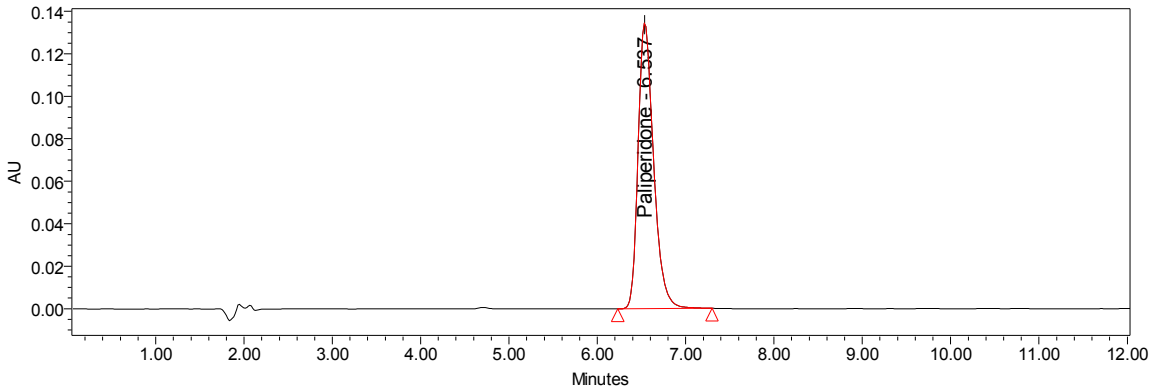

Fig. 2: Chromatogram of System Suitability of Paliperidone Standard

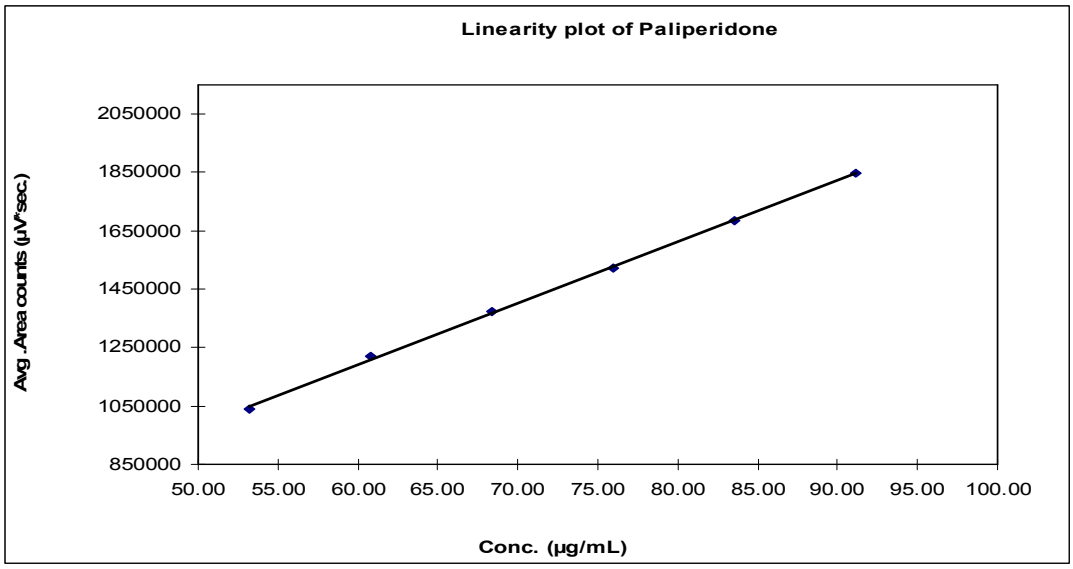

Fig.3: Linearity plot of paliperidone

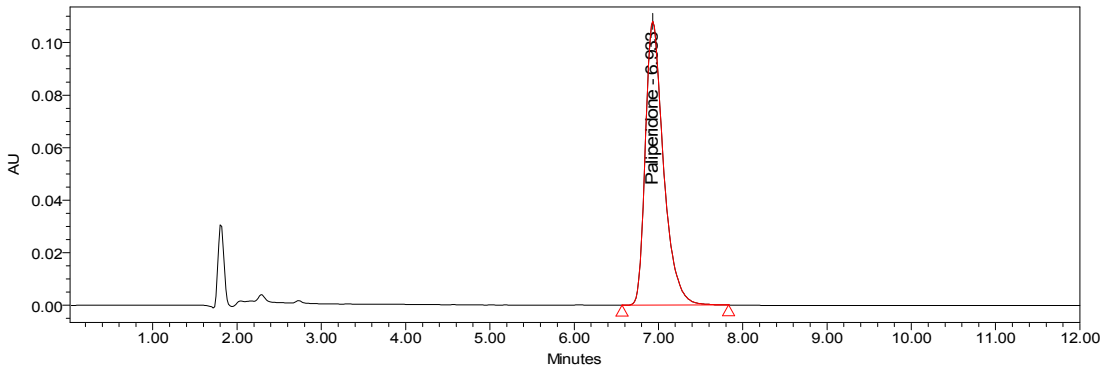

Fig. 4: Chromatogram of Acid Degradation Sample

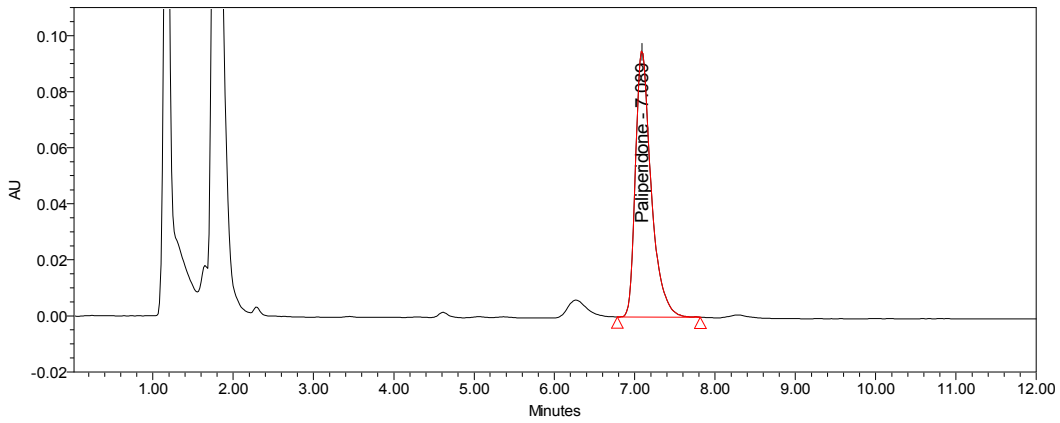

Fig. 5: Chromatogram of Alkali Degradation Sample

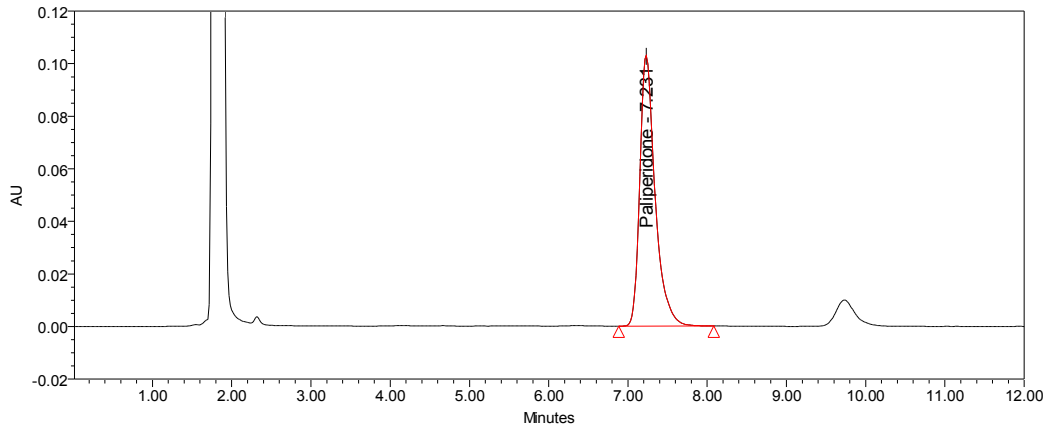

Fig. 6: Chromatogram of Peroxide Degradation Sample

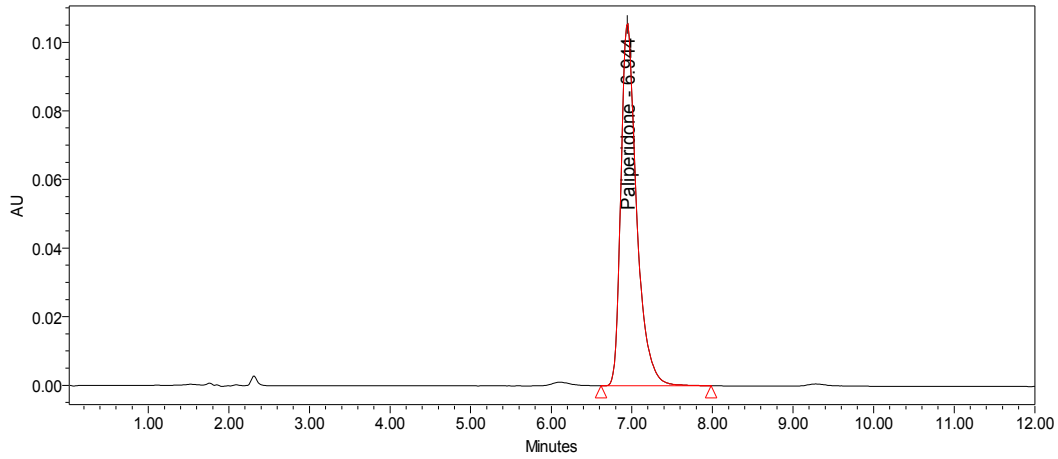

Fig. 7: Chromatogram of Thermal Degradation Sample

Table I: Data sheet for system suitability

	Paliperidone			
Inj. No	**RT**	**Area**	**USP Tailing**	**USP Plate count**
1	6.520	1630018	1.33	6759
2	6.522	1631330	1.33	6654
3	6.524	1633401	1.34	6780
4	6.529	1632478	1.33	6595
5	6.537	1631756	1.32	6747
6	6.537	1635205	1.33	6801
Mean		**1632364**		
SD		**1794.59**		
% RSD		**0.11**		

Table II: Linearity response for Paliperidone

S.No	Concentration (µg/ml)	Area Counts (µV sec.) Inj#1	Area Counts (µV sec.) Inj#2	Mean Area Counts (µV sec)
Lin-1	53.16	1037517	1038345	1037931
Lin-2	60.76	1219748	1223756	1221752
Lin-3	68.35	1377831	1365949	1371890
Lin-4	75.95	1519195	1529586	1524391
Lin-5	83.545	1681053	1685027	1683040
Lin-6	91.14	1853254	1843970	1848612
Slope			**21027**	
Intercept			**-69153**	
Correlation coefficient			**0.99962**	

Table III: Data for System precision

Inj. #	Standard Area Counts (µV sec.)
1	1571189
2	1547399
3	1548183
4	1545798
5	1552591
6	1542147
Mean	1551218
SD	10355
%RSD	0.67

Table IV: % RSD of Method Precision

Inj. #	Standard Area Counts (µV sec.)
1	1571189
2	1547399
3	1548183
4	1545798
5	1552591
6	1542147
Mean	**1551218**
SD	**10355**
%RSD	**0.67**

Table V: Data sheet for assay of Control samples (Specificity)

Sample	wt. of sample	Inj# 1	Inj#2	Mean	Assay (mg/tab)	Assay (% Claim)
Control-samp-1	375.71	1545440	1535710	1540575	149.4	99.6
Control samp-2	387.04	1579933	1576019	1577976	148.5	99.0
Control samp-3	381.31	1555713	1558536	1557125	148.8	99.2
				Mean	148.9	99.3
				SD	0.46	0.31
				%RSD	0.31	0.31

Table VI: Data sheet for Recovery of Paliperidone

Sample Name	Wt of Std Paliperidone taken (mg)	Amount Added (mg)	Area Counts of Paliperidone			Amount Recovered (mg)	% Recovery
			Injection-1	Injection-2	Mean		
Recovery-80-1	**59.98**	**59.98**	**1259788**	**1250713**	**1255251**	**59.24**	**98.77**
Recovery-80-2	60.10	60.10	1257800	1259192	1258496	59.40	98.84
Recovery-80-3	59.92	59.92	1260222	1260462	1260342	59.48	99.27
Recovery-100-1	75.01	75.01	1581938	1576605	1579272	74.53	99.36
Recovery-100-2	75.21	75.21	1578936	1578851	1578894	74.52	99.08
Recovery100-3	75.34	75.34	1587016	1584122	1585569	74.83	99.32
Recovery-120-1	90.38	90.38	1906457	1905594	1906026	89.96	99.54
Recovery-120-2	90.22	90.22	1903201	1904044	1903623	89.84	99.58
Recovery-120-3	90.48	90.48	1906992	1908617	1907805	90.04	99.51
						Mean	99.25
						SD	0.297
						%RSD	0.30

Table VII: Comparison of assay of Paliperidone in Paliperidone depot injection in robust conditions

Sample	Method Precision	Flow Minus	Flow Plus	Temp. Minus	Temp. Plus	Wavelength Minus	Wavelength Plus
1	99.6	98.6	99.1	96.9	96.2	99.8	100.1
2	99.3	99.3	99.1	97.8	98.1	99.1	99.5
3	98.4	97.2	99.0	96.3	96.5	98.3	98.6
4	99.5						
5	98.6						
6	98.4						
MEAN	99.0	98.4	99.1	97.0	96.9	99.1	99.4
SD	0.56	1.07	0.06	0.75	1.02	0.75	0.75
RSD (%)	0.57	1.09	0.06	0.77	1.05	0.76	0.75
Overall Mean		**98.8**	**99.0**	**98.3**	**98.3**	**99.0**	**99.1**
Overall SD		**0.76**	**0.45**	**1.14**	**1.22**	**0.58**	**0.62**
Overall RSD (%)		**0.77**	**0.45**	**1.16**	**1.24**	**0.59**	**0.63**

Table VIII: Data sheet for Degradation study

Sample	Sample wt.(mg)	Area counts of Paliperidone	Assay (mg/tab)	% Degradation	Purity Angle	Purity Threshold
Acid Degradation (5 ml 5N HCl, 2 hours @60°C)	780.68	1617545	159.3	-6%	0.107	1.112
Alkali Degradation (5ml 5N NaOH, 90min. @RT)	771.85	1289723	123.5	19%	0.189	1.181
Peroxide Degradation (5ml 30%H_2O_2, Initial)	783.78	1377956	128.5	15%	0.124	1.138
Thermal Degradation (80°C,3 hours)	764.45	1324628	128.2	15%	0.102	1.099

5. REFERENCES

1. Cada DJ, Baker DE and Levien T. Paliperidone. Hosp Pharm. 2007;42(7):637-47.
2. Canuso C, Carothers J, Dirks B, Zhu Y, Schreiner A and Kosik-Gonzalez C. A double-blind placebo-controlled trial comparing paliperidone ER and quetiapine in patients with a recent acute exacerbation of schizophrenia. Schizophr Res. 2008;102(2):252.
3. Corena-McLeod MDP, Oliveros A and Charlesworth C. Paliperidone as a mood stabilizer: a pre-frontal cortex synaptoneurosomal proteomics comparison with lithium and valproic acid after chronic treatment reveals similarities in protein expression. Brain Res. 2008;1233:8-19.
4. Danel C, Azaroual N and Brunel A. Configurational stability of 9-hydroxyrisperidone. Kinetics and mechanism of racemization.

Tetrahedron Asymmetr. 2009;20(10):1125-31.
5. Danel C, Barthelemy C and Azarzar D. Analytical and semipreparative enantioseparation of 9-hydroxyrisperidone, the main metabolite of risperidone, using high-performance liquid chromatography and capillary electrophoresis. Validation and determination of enantiomeric purity. J Chromatogra A. 2007;1163(1-2):228-36.
6. Danel C, Chaminade P and Odou P. Enantioselective analysis of the antipsychotic 9-hydroxyrisperidone, main metabolite of risperidone, by chiral capillary EKC using dual CDs. Electrophoresis. 2007;28(15):2683-92.
7. Davidson M, Emsley R and Kramer M. Efficacy, safety and early response of paliperidone extended-release tablets (paliperidone ER): results of a 6-week, randomized, placebo controlled study. Schizophr Res. 2007;93(1–3):117–130.
8. De Meulder M, Remmerie BMM and de Vries R. Validated LC-MS/MS methods for the determination of risperidone and the enantiomers of 9-hydroxyrisperidone in human plasma and urine. J Chromatogra B. 2008;870(1):8-16.
9. Dunner DL, Bossie CA, Turkoz I and Canuso CM. Paliperidone extended-release (ER) in patients with schizoaffective disorder presenting with manic or depressive symptomatology. J Affect Disorders. 2010;122(1):S61.
10. FDA, Food and Drug Administration, Guidance for industry-Analytical procedures and methods validation (draft guidance), 2000.
11. FDA, US Food and Drug Administration, Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations, 2011.
12. Feinberg M. Validation of analytical methods based on accuracy profiles. J Chromatogra A. 2007;1158(1-2):174-83.
13. Fowler JA, Bettinger TL and Argo TR. Paliperidone extended-release tablets for the acute and maintenance treatment of schizophrenia. Clin Ther. 2008;30(2):231-48.
14. ICH Q1A (R2). Stability Testing of New Drug Substance and Products: text and Methodology, in Proceedings of the International Conference on Harmonization, 2003.
15. ICH Q2 (R1).Validation of Analytical Procedure: text and Methodology, in Proceedings of the International Conference on Harmonization, 2005.
16. Janssen-Cilag, Invega Paliperidona and Sao Paulo. 2007.
17. Kane J, Canas F and Kramer M. Treatment of schizophrenia with paliperidone extended-release tablets: A 6-week placebo-controlled trial. Schizophr Res. 2007;90(1-3):147-61.
18. Karlsson R, Dencker E and Nyber S. Pharmacokinetics and dopamine D2 and serotonin 5-HT2A receptor occupancy of paliperidone in healthy subjects. Basic Neurosci. 2005;1:S386.
19. Locatelli I, Mrhar A and Grabnar I. Simultaneous determination of risperidone and 9-hydroxyrisperidone enantiomers in human blood plasma by liquid chromatography with electrochemical detection. J Pharmaceut Biomed. 2009;50(5):905-10.
20. Kirschbaum KM, Finger S and Vogel F. LC with column switching and spectrophotometric detection for determination of risperidone and 9-hydroxyrisperidone in human serum. Chromatogra. 2008;67(3-4):321-4.
21. Luthringer R. Sleep assessments in patients with schizophrenia following treatment with paliperidone extended-release tablets. Eur Neuropsychopharmacol. 2006;16(4):S224.
22. Mandal AS, Biswas N and Karim KM. Drug delivery system based on chronobiology-A review. J Control Release. 2010;147(3):314-25.
23. Mandrioli R, Mercolini L, Lateana D, Boncompagni G and Raggi MA. Analysis of risperidone and 9-hydroxyrisperidone in human plasma, urine and saliva by MEPS-LC-UV. J Chromatogra B. 2010;879(2):167-73.
24. Marchese G, Pittau B and Casu G. A comparison of continuous subcutaneous paliperidone infusion and repeated subcutaneous injection of risperidone free-base in rats. Eur Psychiat. 2010;25(2):92-100.
25. Marder SR, Kramer M and Ford L. Efficacy and safety of paliperidone extended-release tablets: results of a 6-week, randomized, placebo-

controlled study. Biol Psychiat. 2007;62(12):1363-70.
26. Ngwa G. Forced degradation as an integral part of HPLC stability-indicating method development. Drug Deliv Technol. 2010;10(5):56-9.
27. Nilsen C. Analytical methods validation, design and execution. Pharm Formulation Qual. 2010:29–32.
28. Richelson E and Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci. 2000;68(1):29-39.
29. USP, United States Pharmacopoeia. 31 ed., US Pharmacopoeial Convention. 2008.
30. Vermeir M, Naessens I and Remmerie B. Absorption, metabolism, and excretion of paliperidone, a new monoaminergic antagonist, in humans. Drug Metab Dispos. 2008;36(4):769-79.