

# DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF PALIPERIDONE PALMITATE AS BULK DRUG

<sup>1</sup>Dr. Mrinalini C. Damle

Professor

Department of Quality Assurance

AISSMS College of Pharmacy, Savitribai Phule University, Kennedy Road, Near RTO, Pune 411001, Maharashtra, India

<sup>2</sup>Swapnil S. Waghmare

Research Scholar

Department of Quality Assurance

AISSMS College of Pharmacy, Savitribai Phule University, Kennedy Road, Near RTO, Pune 411001, Maharashtra, India

<sup>3</sup>Purushotam Sinha

Research and Development Department,

Wockhardt pharmaceuticals Pvt. Ltd., Aurangabad, Maharashtra, India.

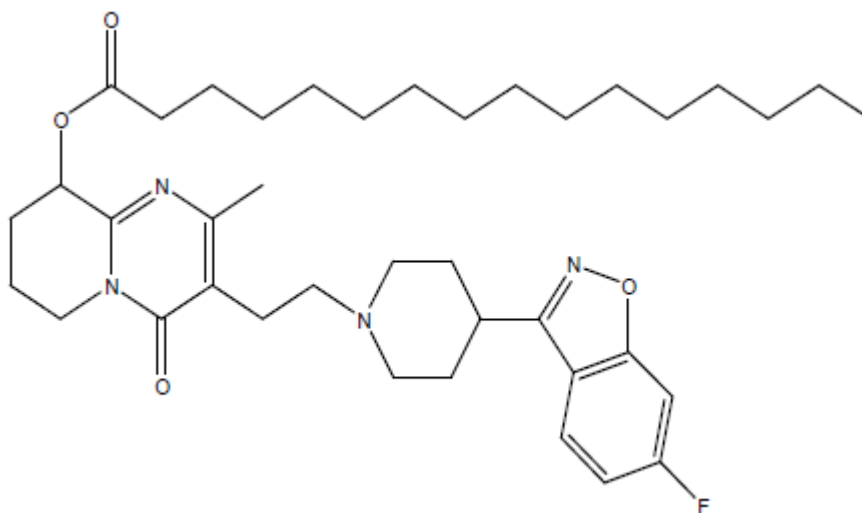
## Abstract:

A simple, economical and precise thin-layer chromatography method has been developed for determination of Paliperidone palmitate. The method was validated as per International Conference on Harmonization (ICH) guidelines. Aluminium TLC plates precoated with silica gel 60F<sub>254</sub> were used as the stationary phase and chloroform: methanol 3:7 (v/v) as mobile phase. A compact band (Rf 0.54 ± 0.03) was obtained for Paliperidone palmitate. Densitometric analysis was performed in the absorbance mode at 236 nm. The calibration curve was found to be linear in the concentration range of 500-2500 ng/band. The limits of detection and quantitation were found to be 47.70ng/band and 144.56ng/band respectively. Paliperidone palmitatae was subjected to stress conditions like hydrolysis under acidic, basic and neutral conditions, oxidation, heat and photolysis.

**Keywords:** Paliperidone Palmitate, HPTLC, Stability indicating method

## INTRODUCTION

Paliperidone is the primary active metabolite of the older antipsychotic risperidone. While its specific mechanism of action is unknown, it is believed that Paliperidone and Risperidone act via similar if not the same pathways. 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 (5HT<sub>2A</sub>) receptors and H<sub>1</sub> histaminergic receptors, which may explain some of the other effects of the drug. Paliperidone chemically described as 3-[2-[4-(6-fluoro-1,2benzisoxazol-3yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-ethyl-4H-pyrido[1,2-a]pyrimidin-4-one(Fig. 1)[1].



**Figure I. chemical structure of paliperidone palmitate**

Paliperidone was approved by the FDA for treatment of schizophrenia on December 20, 2006 [2]. According to literature survey, there are few UV[3,4], stability indicating HPLC[5-8], HPLC[9-11], MS-MS[12], UPLC[13], RP-RRLC[14], LC-MS[15], ULC[16] methods were reported as bulk or in pharmaceutical formulation.

To the best of our knowledge, no stability indicating HPTLC method has been reported for the determination of Paliperidone palmitate as bulk drug. The objective of this research work was to develop a simple, economical and stability-indicating HPTLC method for the determination of Paliperidone palmitate as bulk drug.

## MATERIALS AND METHODS

### Chemicals and reagents

Paliperidone palmitate was provided as a gift sample by Wockhardt research and development centre, Aurangabad. Methanol, Chloroform and all other chemicals used in this study were of AR grade purchased from LOBA chemie Pvt. Ltd. Mumbai, India.

### Instruments

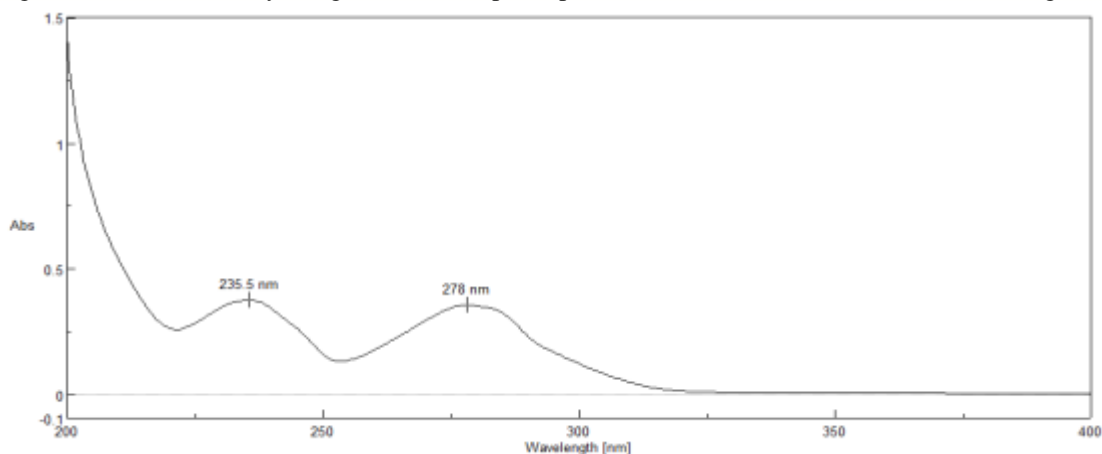
Linomat-5 sample applicator (Camag, Switzerland), twin trough chamber (10 x 10 cm; Camag, Switzerland), TLC scanner 3 (Camag, Switzerland), WinCATS version 1.4.3 software (Camag, Switzerland), Photostability chamber (Newtronics NEC103RSPI), Shimadzu balance (Model AY-120), Camag 100  $\mu$ l sample syringe (Hamilton, Switzerland) were used in the study.

### Preparation of standard solutions

Standard stock solution of Paliperidone palmitate was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000  $\mu$ g/ml. From the standard stock solution, working standard solution was prepared containing 100  $\mu$ g/ml of Paliperidone palmitate.

### Selection of analytical wavelength

The standard solution of Paliperidone palmitate of concentration 100 $\mu$ g/ml was prepared using methanol and scanned over the wavelength range 200 nm to 400 nm by using UV-Visible spectrophotometer.  $\lambda_{\max}$  was found to be 236 nm (Fig. II).



**Figure II. UV spectrum of pal. palmitate (10  $\mu$ g/ml)**

### Chromatographic conditions

Initially, mobile phase optimization trials were conducted using Toluene: ethyl acetate, chloroform: methanol in the various ratios. Optimized mobile phase was chloroform: methanol (3:7 v/v). TLC plates precoated with silica gel 60 F<sub>254</sub>, of dimension 10 cm × 10 cm with 250 μm layer thickness were used as stationary phase. TLC plates were pre-washed with methanol and dried. The standard solution of Paliperidone palmitate was spotted on the dried, pre-coated TLC plate as a band with 4 mm width. The chromatographic development was carried out by using chloroform: methanol (3:7 v/v). as mobile phase with 15 min chamber saturation time and run up to distance 90 mm. Densitometric scanning was performed at 236 nm.

## RESULTS AND DISCUSSION

### Optimization of mobile phase

Method development for Paliperidone palmitate was started with the development of densitogram using neat solvents and combinations of toluene, ethyl acetate and methanol in different ratios. Chloroform: methanol in the ratio of (3: 7 v/v) was selected as mobile phase for Paliperidone palmitate which gives accepted peak parameters. The R<sub>f</sub> was found to be 0.54 ± 0.03 for Paliperidone palmitate. The standard densitogram of Paliperidone palmitate (2500 ng/band) is shown in (Fig. III).

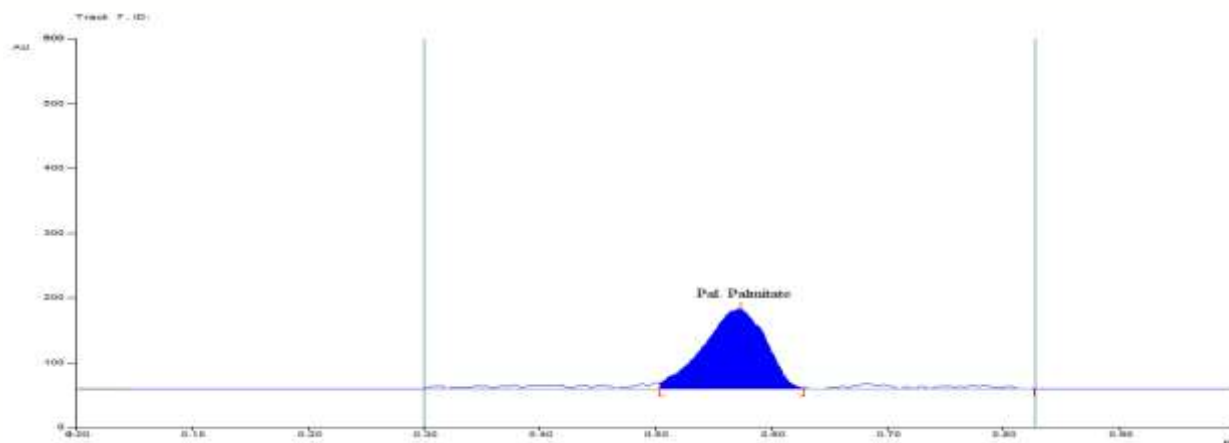


Figure III. densitogram of standard solution of pal. palmitate (2500 ng/band)

### Stress degradation studies of bulk drug

Stress testing studies were carried out as per ICH Q1A (R2) [17] on how the quality of drug varies under various stress conditions like oxidation, hydrolysis, photolysis and thermal, etc. Optimization of stress conditions was done by changing the strength of reagent and duration of exposure to get 10-30 % degradation.

### Optimization trials

Initially trials were conducted using various normalities of HCl and NaOH by keeping the sample solution overnight. For the thermal study sample was heated at 80 °C for 4 h to 8 h and for oxidation, trials were conducted using 30 % H<sub>2</sub>O<sub>2</sub> by keeping the sample solution overnight. It was observed that the drug gets degraded partially.

### Optimized stress conditions

#### Alkaline hydrolysis

1 ml working standard solution of Paliperidone palmitate (1000 μg/ml) was mixed with 1 ml of 1 N NaOH and volume was made up to 10 ml with methanol. The solution was kept for overnight at room temperature. Average 75.45 % of Paliperidone palmitate was recovered with no peak of degradation.

#### Acid hydrolysis

1 ml standard solution of Paliperidone palmitate (1000 μg/ml) was mixed with 1 ml of 0.5 N HCl and volume was made up to 10 ml using methanol. The solution was kept for overnight at room temperature. Average 78.09 % of Paliperidone palmitate was recovered with no peak of degradation.

### Oxidative degradation

1 ml standard solution of Paliperidone palmitate (1000 µg/ml) was mixed with 3 ml 30% v/v H<sub>2</sub>O<sub>2</sub>, volume was made up to 10 ml using methanol. The solution was kept for overnight at room temperature. Average 70.56 % of Paliperidone palmitate was recovered with no peak of degradation.

### Degradation under dry heat

Dry heat study was performed by keeping the drug in hot air oven at 80 °C for 8 hours. Average 81.80 % of Paliperidone palmitate was recovered with no peak of degradation.

### Degradation under neutral condition

To 1 ml of 1000 µg/ml solution of Paliperidone palmitate, 1 ml of distilled water was added. The volume was made up to 10 ml with methanol. The solution was kept for overnight at room temperature. Average 89.15% of Paliperidone palmitate was recovered with no peak of degradation.

### Photo-degradation studies

Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h/m<sup>2</sup> and subsequently to cool white fluorescent light to achieve an illumination of 1.2 million Lux hours. The sample was weighed, dissolved and diluted to get 20 µg/ml as final concentration and was applied to TLC plate. After the photo degradation study under UV light 105.68% and Fluorescence light 108.81% Paliperidone palmitate was recovered with no peak of degradation. Spotting of 10000 ng/band was done for samples at each stress condition to locate peak for a degradation product if any.

**Table I. summary of stress degradation study of paliperidone palmitate**

Stress degradation condition	%Recovery	%Degradation	Peak purity	
			r(s, m)	r(m, e)
Acid (0.5 N HCl) kept for overnight at RT	78.09	21.91	0.999	0.995
Base (1 N NaOH, overnight at RT)	75.45	24.55	0.999	0.997
Oxidation (30% v/v H <sub>2</sub> O <sub>2</sub> ) overnight at RT	70.56	29.44	0.999	0.999
Neutral (kept for overnight at RT)	89.15	10.85	0.999	0.998
Dry heat (80 °C 8 hr)	81.80	18.2	0.999	0.997
Photo stability UV 200 watt hrs/m <sup>2</sup>	105.68	-	0.993	0.992
Flourescence 1.2 million lux. Hrs	108.81	-	0.997	0.994

### VALIDATION PARAMETER

The developed method was successfully validated according to the ICH Q2 (R1) [18] guidelines.

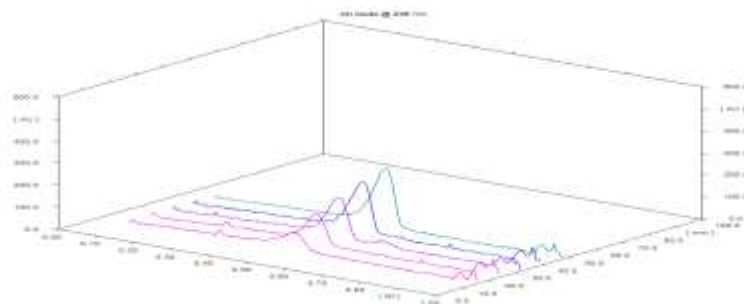
#### Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for the drug in a sample was confirmed by comparing the R<sub>f</sub> and the spectra of the spot with that of the standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at the peak end.

#### Linearity and range

The calibration curve was obtained in the range of 500-2500 ng/band by applying different volumes (5-25 µl) of stock solution of (100 µg/ml) on a TLC plate. Each standard in five replicates was analyzed and peak areas were recorded. The relationship between peak area and concentration was established by the simple regression equation method. (Fig. IV).

A)



B)

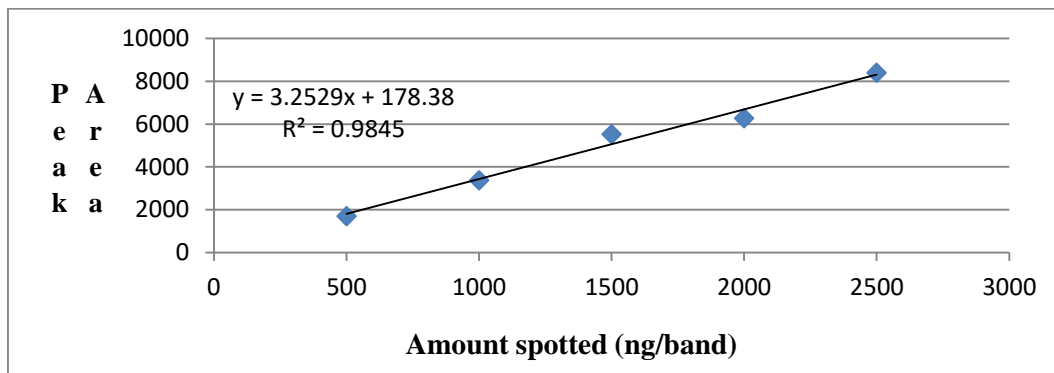


Figure IV. A - densitogram of standard solution of paliperidone palmitate (500-2500 ng/band) & B-calibration curve of paliperidone palmitate

**Assay**

20 tablets were accurately weighed and powdered. From the powder, an amount equivalent to 10 mg of Paliperidone palmitate was accurately weighed and transferred to 10 ml volumetric flask. Methanol was added, sonicated for 15 min, a solution was filtered. Dilutions were made to get the final concentration 100 µg/ml. The assay was calculated by extrapolation from standard curve which was found to be 98.67 %.

**Accuracy**

To check accuracy of the method, recovery studies were carried out by adding a standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of the sample chosen was 1000 ng/band. The drug concentrations were calculated by using the regression equation of Paliperidone Palmitate (Table-II).

Table II. result of accuracy (recovery) study of paliperidone palmitate

Concentration (ng/band)		Amount Recovered	%Recovery	%RSD
Sample conc	Amount added			
1000	800	1769.70	98.31	0.86
1000	1000	1982.01	99.10	1.95
1000	1200	2207.62	100.34	0.93

**Precision**

The precision of the method was demonstrated by intra-day and inter-day studies. In the intra-day studies, 3 replicates of 3 standard solutions were analyzed in a same day and percentage RSD was calculated. For the inter-day, 3 standard solutions were analyzed on three consecutive days and percentage RSD was calculated (Table III & IV).

Table III. intra-day precision (n=3)

Concentration (ng/band)	Area	%RSD
500	1703.33	1.12
1000	3372.67	1.96

1500	5512.5	1.17
------	--------	------

n = number of determinations, %RSD = %Relative Standard Deviation

**Table IV. inter-day precision (n=3)**

Concentration (ng/band)	Area	%RSD
500	1683.53	1.61
1000	3424.17	1.27
1500	5547.9	1.42

n = number of determinations, %RSD = %Relative Standard Deviation

### Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated as  $3.3 \sigma/S$  and  $10 \sigma/S$  respectively. Where  $\sigma$  is the standard deviation of the lowest concentration response and  $S$  is the slope of the calibration plot. The LOD and LOQ were found to be 47.70ng/band and 144.56ng/band respectively.

### Robustness

The robustness of the method were studied during method development, by small but deliberate variations in chamber saturation time (13, 17 min), change in mobile phase composition, Time was changed from spotting to development and development to scanning and the effect on the peak area was noted (Table V).

**Table V. robustness study of paliperidone palmitate (n=3)**

Parameters	Robust Condition	Area	% RSD
Chamber saturation time (15 min) $\pm$ 2 min	13 min	3586.5	0.72
	17 min	3124.37	1.85
Mobile phase composition chloroform: methanol(3 :7 v/v) $\pm$ 0.2 methanol	Chloroform: methanol(3.2: 6.8 )	3039.27	1.51
	Chloroform: methanol(2.8: 7.2)	3641.03	1.20
Time from application to development (immediate)	After 30 min	3108.2	1.66
	After 1 h	3214.07	1.59
Time from development to scanning (immediate)	After 30 min	3292.33	0.91
	After 1 h	3111.47	1.56

n = number of determinations, %RSD = %Relative Standard Deviation

The method validation results were satisfactory as per ICH Q2 R1 guidelines. The peak area was found to be linear over the concentration range of 500-2500 ng/band with a correlation coefficient of 0.984. Method specificity can be proved using peak purity parameter in WinCATS software of HPTLC. There is a provision to compare the UV spectrum at the start, middle and end of any peak. Inter and Intra-day precision was less than 2%. Percent recovery in an accuracy study was within the limit of 98 to 102 %. The results of validation are summarized in table 6.

**Table VI. summary of validation parameter**

Validation parameter	Results
Linearity	$Y = 3.25x + 178.3$ $R^2 = 0.984$
Range	500-2500 ng/band
Precision	(%RSD)
A) Intra-day	1.41
B) Inter-day	1.43

Assay	98.67%
Accuracy	(%Recovery)
80%	100.42
100%	100.73
120%	99.51
LOD	47.70 ng/band
LOQ	144.56 ng/band
Specificity	Specific
Robustness	Robust

## DISCUSSION

A thorough study of literature revealed few stability indicating methods by chromatographic techniques. The drug has been reported to be sensitive to hydrolytic conditions. A variation in observations was seen with respect to oxidative degradation conditions. Study by Divya I. et al indicates that the drug is stable to oxidation where as studies by Trivedi R. et al, Boga H. et al and Sherje A. et al reflect oxidative degradation to different extent. Studies by Trivedi R. et al and Sherje A. et al indicates the drug is stable to thermal and photolytic conditions. We have observed that Paliperidone is photo-stable but prone to other condition of degradation.

## CONCLUSION

The developed method is simple and stability indicating. It can be used for estimation of Paliperidone palmitate. The method has inherent advantage of high throughput.

## ACKNOWLEDGEMENT

Authors are thankful to the principal and management of the AISSMS College of pharmacy, Pune for providing required facilities for research work. Authors are also thankful to the Wockhardt pharmaceuticals Pvt. Ltd. Aurangabad for providing an active pharmaceutical ingredient (API) as gift sample.

## REFERENCES

1. <https://www.drugbank.ca>
2. <https://www.fda.gov>
3. Divya I, Panigrahy U. 2014. New method development and validation of UV spectrophotometer for the estimation of Paliperidone in bulk and pharmaceutical dosage form. *International Journal of Pharmaceutical Research and Bio-science*, 3(2): 106-125.
4. Trivedi R, Jupudi R, Patel M, Trivedi D, Ioqia H. 2018. A validated spectrophotometric method for determination of Paliperidone Palmitate in bulk drug and its pharmaceutical dosage form. *Journal of Pharmacy and Pharmaceutical Sciences*, 2(3): 37-41.
5. Boga H, Patnik A. 2014. A novel method for chromatographic determination of Paliperidone in pure form and formulation by using RP-HPLC and its stability studies. *International Journal of Innovative Pharmaceutical Sciences and Research*, 2(11): 2709-2718.
6. Sherje A, Londhe V. 2015. Stability indicating HPLC method for determination of Paliperidone in bulk, *International Journal PharmTech Research*, 8(8): 157-163.
7. Mastiholimath V, Gupta G, Dandagi P, Gadad A, Pai V. 2014. Development and validation of a stability indicating method for Paliperidone in Paliperidone depot injection using Reversed Phase High Performance Liquid Chromatography. *International Journal of Pharmaceutical and Chemical Sciences*, 3(4): 905-914.
8. Jadhav S, Landge S, Choudhari P, Solanki P, Bembalkar S, Mathad V. 2011. Stress degradation behavior of Paliperidone, an antipsychotic drug, and development of suitable stability indicating RP-LC method. *Chromatography Research International*.

9. Swarnalatha G, Vijaykumar B, Jothieswari D, Reddy J, Mohankumar M. 2014. Method development and validation of RP-HPLC method for determination of new antipsychotic agent in Paliperidone Palmitate bulk drug. *International Journal of Novel Trends in Pharmaceutical Sciences*, 4(4): 101-110.
10. Gadekar V, Rokde M. 2017. Isocratic Reversed-Phase HPLC method with PDA detector for the assay and purity evaluation of Olanzapine & Paliperidone in bulk drug. *International Conference On Emanations in Modern Technology and Engineering*, 5(3): 38-39.
11. Batthula S, Yandamuri N, Narla D, Bandam P, Allada P. 2015. Development and validation of RP-HPLC method for simultaneous estimation of Lansoprazole and Paliperidone Palmitate in bulk drugs. *An International Journal of Advance in Pharmaceutical Sciences*, 6(1); 2704-2708.
12. Farooqui M, Ahmed R, Sangshetti J, Zaheer Z, Wahab A, Deshpande M, Bhojane S, Baig S. 2013. Identification and determination of related substances of Paliperidone in bulk drug and pharmaceutical formulations by HPLC and HPLC-MS-MS. *Indo American Journal of Pharmaceutical Research*, 3(12): 1375-1383.
13. Trivedi R, Jain P, Patel M, Chatrabhuji P, Trivedi D. 2013. A rapid stability indicating RP-UPLC method for determination of Paliperidone Palmitate in depot injectable formulation. *Journal of Applied Pharmaceutical Science*, 3(7): 87-92.
14. Dadare K, Khatri N, Pant M. 2012. Application of RP-RRLC methods for estimation of Paliperidone in tablet dosage forms. *Journal of Chemical and Pharmaceutical Research*, 4(6): 3154-3157.
15. Satyanarayana P, Alavala S. 2012. LCMS method development and validation of Paliperidone in formulation dosage. *Research Desk*, 1(1): 1-9.
16. Panda S, Beg S, Bera V, Kumar R, Singh P. 2015. Analytical quality-by-design compliant Ultrafast Liquid Chromatographic method for determination of Paliperidone in extended release tablet dosage form. *Journal of Bioanalysis and Biomedicine*, 7(4): 116-123.
17. ICH guidelines, for stability testing of new drug substances and products Q1A(R2).2004.Pg. No 1-24.
18. ICH guidelines, for validation of analytical procedures: text and methodology Q2(R1 ).2005.Pg. No 1-17.