Journal of Applied Pharmaceutical Science Vol. 3 (07), pp. 087-092, July, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3716
ISSN 2231-3354

A Rapid, Stability Indicating RP-UPLC Method for Determination of Paliperidone Palmitate in a Depot Injectable Formulation

Rakshit Kanubhai Trivedi*[1, 2], Prashant Jain[1], Mukesh C Patel[2], Parimal M Chatrabhuji[2], Dipika R Trivedi
[1]Analytical Research and Development, Integrated Product Development, Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad-500 072, India.
[2]P.S. Science and H.D. Patel Arts College, S.V. Campus, Kadi-382 715, Gujarat, India.

ARTICLE INFO

Article history:
Received on: 06/05/2013
Revised on: 29/05/2013
Accepted on: 25/06/2013
Available online: 30/07/2013

Key words:
Depot , Chromatography, Extended-release suspension, Assay, Method validation, Paliperidone palmitate injection, Schizophrenia.

ABSTRACT

A rapid, stability-indicating reversed phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of paliperidone palmitate (PP), in depot injectable dosage form. The chromatographic separation was achieved on an Acquity BEH C18 (50 mm × 2.1 mm, 1.7 µm) column, with a mobile phase consisting of ammonium acetate buffer, and acetonitrile at a ratio of 10:90 (v/v) and a flow rate of 0.6 mL/min. The eluted compound was monitored at a wavelength of 238 nm using a UV detector. The method described herein separated paliperidone palmitate from all other formulation components and two major known degradation products (N-Oxide and paliperidone) within a run time of 2.5 min. The method also generated linear results over a PP concentration range of 156 to 468 µg/mL. The stability indicating capability of the method was established by performing forced degradation experiments. The RP-UPLC method that was developed was validated according to the International Conference on Harmonization (ICH) guidelines. This method was successfully applied in the quantitative determination of PP in a stability study of paliperidone palmitate depot injection. The procedure described herein is simple, selective, and reliable for routine quality control analysis as well as stability testing.

INTRODUCTION

Paliperidone palmitate is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives. Paliperidone palmitate ER injection contains a racemic mixture of (+)- and (-)-paliperidone palmitate. The chemical name is (9RS)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)piperidin-1-yl]ethyl] -2- methyl4-oxo-6,7,8,9-tetrahydro- 4H -pyrido [1,2-a] pyrimadin-9-yl hexadecanoate. Its molecular formula is $C_{39}H_{57}FN_4O_4$ and its molecular weight is 664.89. The structural formula is presented in Figure 1.

Paliperidone palmitate is very slightly soluble in ethanol and methanol, practically insoluble in polyethylene glycol 400 and propylene glycol, and slightly soluble in ethyl acetate. INVEGA® SUSTENNA® is available as a white to off-white sterile aqueous extended-release suspension for intramuscular injection in dose strengths of 39 mg, 78 mg, 117 mg, 156 mg, and 234 mg paliperidone palmitate. The drug product hydrolyzes to the active moiety, paliperidone, resulting in dose strengths of 25 mg, 50 mg, 75 mg, 100 mg, and 150 mg of paliperidone, respectively.

The inactive ingredients are polysorbate 20, polyethylene glycol 4000, citric acid monohydrate, disodium hydrogen phosphate anhydrous, sodium dihydrogen phosphate monohydrate, sodium hydroxide, and water for injection (http://www.rxlist.com/invega-sustenna-drug.htm). Ultra-performance liquid chromatography (UPLC) is a new separation technique based upon the well-established principles of liquid chromatography, which utilizes sub-2 mm particles for the stationary phase. These particles operate at elevated mobile phase linear velocities to affect a dramatic increase in resolution, sensitivity, and speed of analysis. Because of its speed and sensitivity, this technique has gained considerable attention in recent years for pharmaceutical and biomedical analysis (Harshal *et al*., 2012; Rakshit *et al*., 2012 & 2011). The UPLC system will significantly decrease the time and cost per sample in the analytical process while improving the quality of the results. By outperforming traditional or optimized HPLC, the system allows chromatographers to work at higher efficiencies with a much wider range of linear velocities, flow rates, and backpressures. In this present work, the technology has been applied for the method development and method validation study for assay determination of Paliperidone palmitate in depot formulation.

* *Corresponding Author*
E-mail: triveditr@yahoo.com (Rakshit. K. Trivedi)

A detailed literature survey for PP revealed that a gradient RP-UPLC method is available for the related substances determination of PP in bulk drug (Hima *et al.*, 2012). Reported method has long run time for the determination of PP. Moreover, PP is not officially represented in any pharmacopoeia to date, thus necessitating the development of a new stability-indicating method to assay (PP) in pharmaceutical formulation.

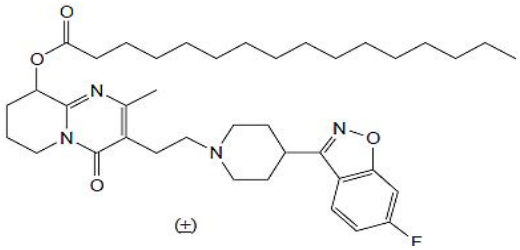

Fig. 1: Chemical structure of Paliperidone palmitate.

The purpose of this study was to develop a rapid, stability-indicating method for the determination of PP in injection formulation. The method developed was able to separate PP from its known degradation products [N-Oxide, ((9RS)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl) piperidin-1-yl]ethyl]-2-methyl-4-oxo-6,7,8,9-tetrahydro-4H-pyrido[1,2-a] pyrimadin-9-yl-1-N-oxide hexadecanoate) and Paliperidone, (3-(2-(4-(6-fluorobenzo[d] isoxazol-3-yl)piperidin-1-yl) ethyl)- 2-methyl -7,8- dihydro-4H-pyrido[1,2-a]pyrimidine-4,9(6H)-dione)] and other excipients of a drug product within 2.5 min. Upon successful separation, this method was validated as per ICH guidelines (ICH, Geneva, Switzerland, 2005) and successfully applied in the separation and quantification of PP in paliperidone palmitate extended release injectable suspension.

EXPERIMENTAL

Materials and Reagents

Depot injection and placebo solution, working standard and impurities standard were provided by Dr Reddy's Laboratories Ltd. HPLC grade acetonitrile was obtained from J.T. Baker (NJ., USA) and HPLC grade tetrahydrofuran was obtained from Merck Ltd. GR grade ammonium acetate, triethylamine and glacial acetic acid were obtained from Merck Ltd. (Mumbai, India). Nylon membrane filters (0.22 µm) were purchased from Pall Life Science Limited (India). High purity water was generated with Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

Buffer preparation

A solution of ammonium acetate buffer (0.05 M) and 3.0 mL of glacial acetic acid were prepared using Milli-Q water. The pH was adjusted to 4.5 with glacial acetic acid. The buffer preparation was stable with respect to pH and maintained visual clarity for 48 h.

Diluent preparation

Mixture of buffer and tetrahydrofuran in the ratio of 40:60 v/v.

Chromatographic conditions

Analysis was performed on an Alliance Waters UPLC system consisting of a quaternary solvent manager, sample manager, and PDA (photo diode array) detector. System control, data collection, and data processing were accomplished using Waters Empower chromatography data software. The chromatographic conditions were optimized on an Acquity BEH C18 (50 mm × 2.1 mm, 1.7 µm) column. The mobile phase was a mixture of buffer and acetonitrile at a ratio of 10:90 (v/v). The mobile phase was filtered through 0.22 µm nylon membrane filter and degassed under vacuum prior to use. Purified water was used as a diluent. The optimized conditions were as follows: an injection volume of 1 µL, isocratic elution at a flow rate of 0.6 mL/min, 50°C (column oven) temperature, and 238 nm detection wavelength. The stress degraded samples were analyzed using a PDA detector over a range of 200 - 400 nm.

Standard solution preparation

The standard solution was prepared by dissolving the standard in diluent to obtain a solution containing 312 µg/mL of PP.

Sample solution preparation

For the preparation, 0.5 gm of sample solution was accurately transferred into a 250 mL volumetric flask. Approximately 200 mL of diluent was added to the volumetric flask, which was then sonicated in an ultrasonic bath for 5 min. The resulting solution was then diluted up to the mark with diluent and mixed well.

Placebo solution preparation

In preparing the placebo solution, 0.5 gm of placebo solution was accurately transferred into a 250 mL volumetric flask. Approximately 200 mL of diluent was added to the volumetric flask, which was then sonicated in an ultrasonic bath for 5 min. The resulting solution was then diluted up to the mark with diluent and mixed well.

Method validation

The method described herein has been validated for assay determination by UPLC.

System suitability

System suitability parameters were performed to verify the system performance. System precision was determined on six replicate injections of standard preparations. All the important characteristics, including the relative standard deviation, peak tailing, and theoretical plate number, were measured.

Specificity

Forced degradation studies were performed to demonstrate selectivity and stability-indicating the capability of the proposed method. The sample was exposed to acidic (0.5 N HCl, 60 °C, 1 h), alkaline (0.5 N NaOH, 60 °C, 1 h), strong oxidizing (3 % H_2O_2, 30 min), thermal (60 °C, 6 h) and photolytic

(1.2 million Lux hours) degradation conditions. All exposed samples and standards were then analyzed by the proposed method.

Linearity

Linearity was demonstrated from 50 to 150 % of standard concentration using a minimum of five calibration levels (50 %, 75 %, 100 %, 125 % and 150 %) for PP. The method of linear regression was used for data evaluation. The peak area of the standard compound was plotted against the respective PP concentrations. Linearity was described by the linearity equation and the correlation coefficient was also determined.

Precision

The precision of the system was determined using the sample preparation procedure described above for six real samples of depot injection and analysis using the same proposed method. Intermediate precision was studied using different columns and was performed on different days.

Accuracy

To confirm the accuracy of the proposed method, recovery experiments were carried out by the standard addition technique. Three levels (50 %, 100 % and 150 %) of standards were added to pre-analyzed samples in triplicate. The percentage recoveries of PP at each level and each replicate were determined. The mean of percentage recoveries (n = 9) and the relative standard deviation were also calculated.

Robustness

The robustness is a measure of the capacity of a method to remain unaffected by small but deliberate changes in flow rate (± 0.05 mL/min), change in column oven temperature (± 5 °C), change in pH of buffer (± 0.1) and change in wavelength nm (± 2 nm).

Stability of sample preparation

The stability of the sample solution was established by storage of the sample solution at ambient temperature for 24 h. The sample solution was re-analyzed after 24 h, and the results of the analysis were compared with the results of the fresh sample.

RESULTS AND DISCUSSION

Method development and optimization

The main criterion for developing an RP-UPLC method for the determination of PP using a UV detector was to estimate the amount of PP in a single run, with emphasis on the method being accurate, reproducible, robust, stability indicating, linear, free of interference from other formulation excipients, major degradation product (N-Oxide and paliperidone) and convenient enough for routine use in quality control laboratories.

Table 1: Label claim of PP with its working concentration (specification limit).

Compound	Label claim mg/mL	Working concentration	
		(mg/mL)	(µg/mL)
PP	156 mg	0.312	312

A spiked solution of PP (312 µg/mL) with its major degradation product (paliperidone and N-Oxide) and placebo peaks were subjected to separation by RP-UPLC. Initially, the separation of all peaks was studied using water as mobile phase A and acetonitrile as mobile phase B on an UPLC column (Acquity BEH C8, 50 mm × 2.1 mm, 1.7 µm) and Waters (UPLC) system with a linear gradient program. The 0.5 mL/min flow rate was selected to achieve the separation of peaks. The column oven temperature was maintained at 25°C. These conditions resulted in merging of the PP peak with the impurity peaks, and high tailing factor for PP peak. Based on this result, the C8 column was replaced with a C18 column in an effort to achieve high resolution between the two known (major degradation product) impurities peak and the PP peak. With the Acquity BEH C18 (50 mm × 2.1 mm, 1.7 µm) column, different combinations of mobile phase A and B were studied to optimize the method, and the results of the optimization are summarized in Table 2, including any observations noted. From the mobile phase selection study, the optimized HPLC parameters were as follows: flow rate, 0.6 mL/min; column oven temperature, 50°C; injection volume, 1 µL; and an isocratic program with a mixture of buffers (3.0 mL triethylamine was added in 0.05 M ammonium acetate buffer, adjusted to pH 4.5 with glacial acetic acid) and acetonitrile in the ratio of 10:90 (v/v) as the mobile phase. The column oven temperature was also studied; it was found that 50°C was a more appropriate temperature with respect to peak separation and shape. Based on the UV spectrum of the compound, 238 nm was found to be appropriate for the determination of PP in pharmaceutical formulations. No chromatographic interference due to the blank (diluent), other excipients (placebo) and two major degradation product (N-Oxide and Paliperidone) at the retention time of PP was observed, as shown in Figure 2 and Figure 4.

Table. 2: Summary of method optimization .

Experimental condition	Observation
Water(MP-A) and acetonitrile (MP-B), linear gradient; Acquity BEH-C8 (50 mm × 2.1 mm, 1.7 µm); 25°C	PP peak was merged with impurity peak
0.01 M KH_2PO_4 (MP-A) and acetonitrile (MP-B), linear gradient; Acquity BEH-C18 (50 mm × 2.1 mm, 1.7 µm); 25°C	PP peak was merged with impurity peak
3.854 gm Ammonium acetate + 1.5 mL of triethylamine, pH 6.0 with Glacial acetic acid (buffer) and acetonitrile, (10:90) v/v; Acquity BEH-C18 (50 mm × 2.1 mm, 1.7 µm); 40°C	PP peak was separated from impurities but found tailing [Figure 3]
3.854 gm Ammonium acetate + 3 mL of triethylamine, pH 4.5 with Glacial acetic acid (buffer) and acetonitrile, (10:90) v/v; Acquity BEH-C18 (50 mm × 2.1 mm, 1.7 µm); 50°C	Satisfactory peak separation and peak shape

Analytical parameters and validation

After development, this method was subjected to validation according to ICH guidelines [6]. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, accuracy, precision, linearity, robustness, solution stability and stability-indicating capability).

System suitability

The percentage relative standard deviation (RSD) of area from six replicate injections was below 2.0 %. Low values of RSD for replicate injections indicate that the system is precise. The results of other system suitability parameters such as peak tailing and theoretical plates are presented in Table 3. As seen from this data, the acceptable system suitability parameters would be as follows: the relative standard deviation of replicate injections is not more than 2.0 %, the tailing factor for the peak of PP is not more than 1.5 and the theoretical plates are not less than 3000.

Table 3: System suitability results (precision, intermediate precision and robustness).

Parameter	Theoretical plates*	Tailing factor*	% RSD* of standard
Precision	3950	1.1	1.00
Intermediate Precision	3522	1.0	0.72
At 0.55 mL/min flow rate	3604	1.2	0.54
At 0.65 mL/min flow rate	3734	1.1	0.82
At 45°C column temp.	3678	1.1	0.67
At 55°C column temp.	3879	1.0	0.78
At buffer pH 4.4	3750	1.1	1.10
At buffer pH 4.6	3938	1.1	0.71
At 236 nm	3812	1.1	0.53
At 240 nm	3910	1.1	0.58

*… Determined on six values

Specificity

Forced degradation studies were performed to demonstrate the selectivity and stability-indicating capability of the proposed RP-UPLC method. Figure 2 shows that there is no interference at the RT (retention time) of PP from the blank and other excipients.

Significant degradation was not observed when PP was subjected to acid, base, thermal, hydrolytic and UV conditions, whereas significant degradation was observed when the PP was subjected to oxidative hydrolysis (3 % H_2O_2, 30 minutes), leading to the formation of N-Oxide and paliperidone. The oxidative product (N-Oxide and paliperidone) and PP are well separated from each other, as seen in Figure 4.

The peak attributed to PP was investigated for spectral purity in the chromatogram of all exposed samples and was found to be spectrally pure. The purity and assay of PP was unaffected by the presence of other excipients and thus confirms the stability-indicating power of this method. The results of the forced degradation study are presented in Table 4.

Table. 4: Summary of forced degradation results .

Degradation condition	Assay (% w/w)	Purity Flag	Observation
Control sample	99.8	No	Not applicable
Acid hydrolysis (0.5 N HCl, 60°C, 1 h)	98.5	No	PP found stable
Alkaline hydrolysis (0.5 N NaOH, 60°C, 1 h)	98.4	No	PP found stable
Oxidation (3 % H_2O_2, 30 min)	78.3	No	PP found sensitive
Thermal (60°C, 6 h)	100.3	No	PP found stable
Exposed to UV (1.2 million Lux hours)	100.1	No	PP found stable

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in that sample within a given range.

The response was found to be linear from 50 % to 150 % of standard concentration. The regression statistics are shown in Table 5, with the linearity curve for PP represented in Figure 5.

Table. 5: Regression statistics.

Substance	Linearity range (µg/mL)	Correlation Coefficient (R^2)	Linearity (Equation)	Y-intercept bias in %
PP	156 to 468	0.9999	y = 1504.8429x – 69.7980	-0.01493

Precision

The purpose of this study was to demonstrate the reliability of the test results with variations. The average % assay (n = 6) of PP was 99.7 % with RSD of 1.0 %. The results are shown in Table 6, along with intermediate precision data. Low RSD values indicate that this method is precise.

Table. 6: Precision (312 µg/mL) and Intermediate precision (312 µg/mL) results.

Substance	Precision		Intermediate precision	
	% Assay #	% RSD*	% Assay #	% RSD*
PP	99.7	1.0	100.3	0.7

#Average of six determinations; *****Determined on six values

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. The amount recovered (for 50, 100 and 150 % level) was within ± 2 % of amount added, indicating that the method is accurate and that there is no interference due to other excipients presents in the injection. The results of the recovery assay are shown in Table 7.

Table. 7: Accuracy results of PP.

	At 50 % 156 µg/mL	At 100 % 312 µg/mL	At 150 % 468 µg/mL
% Recovery#	99.7	98.5	98.6
% RSD*	0.91	0.85	0.60

*****…Determined on three values; #... Mean of three determinations

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. No significant effect was observed on system suitability parameters such as RSD, tailing factor, or the theoretical plates of PP when small but deliberate changes were made to chromatographic conditions. The results are presented in Table 3, along with the system suitability parameters of normal conditions.

Thus, the method was found to be robust with respect to variability in applied conditions.

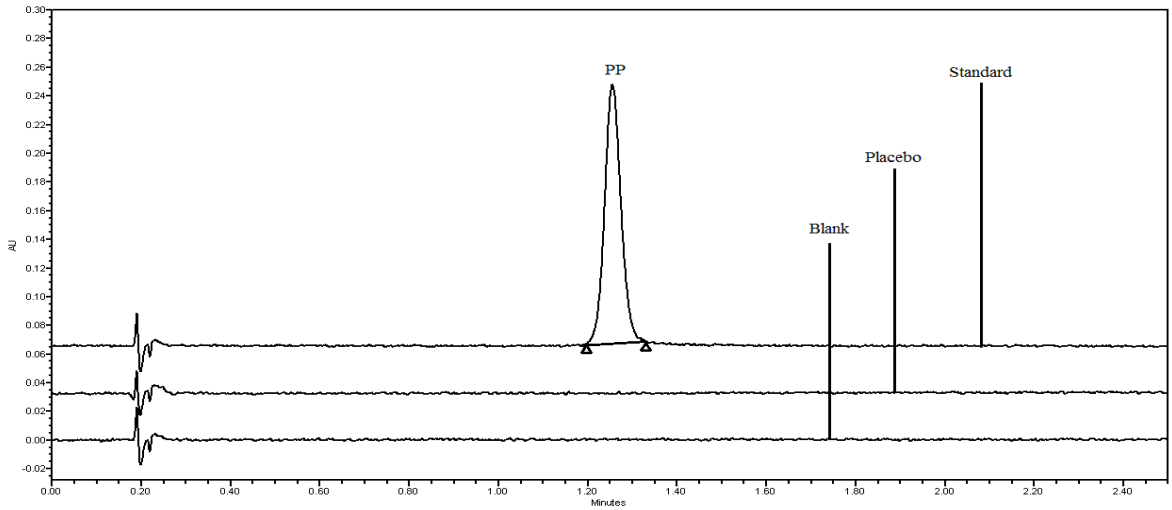

Fig. 2 Overlaid chromatograms of blank, placebo and standard (PP).

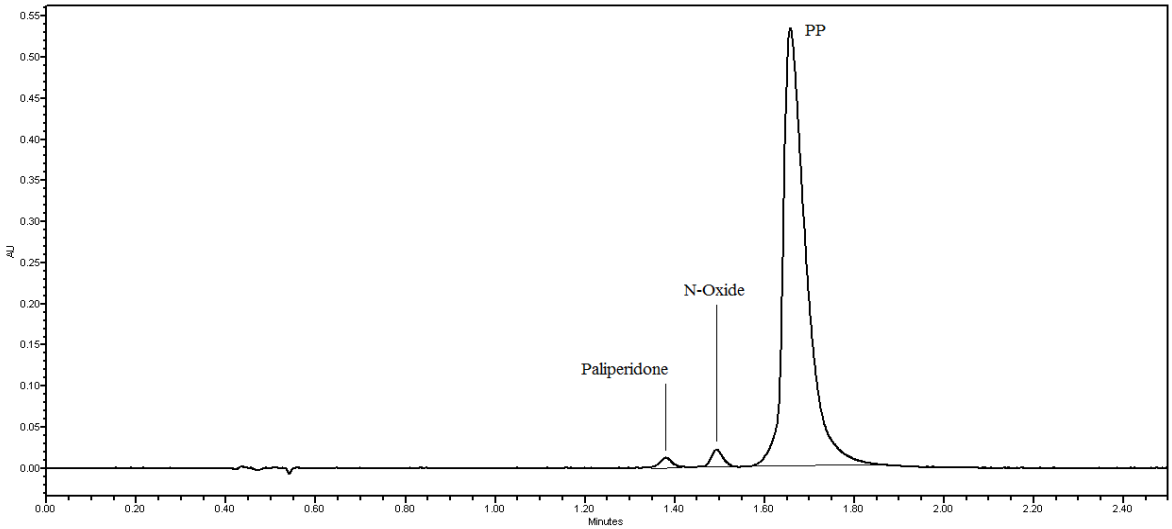

Fig. 3: Unsuccessful condition chromatogram [3.854 gm Ammonium acetate + 1.5 mL of triethylamine, pH 6.0 with Glacial acetic acid (buffer) and acetonitrile, (10:90) v/v; Acquity BEH-C18 (50 mm × 2.1 mm, 1.7 µm); 40°C], of impurities spiked solution.

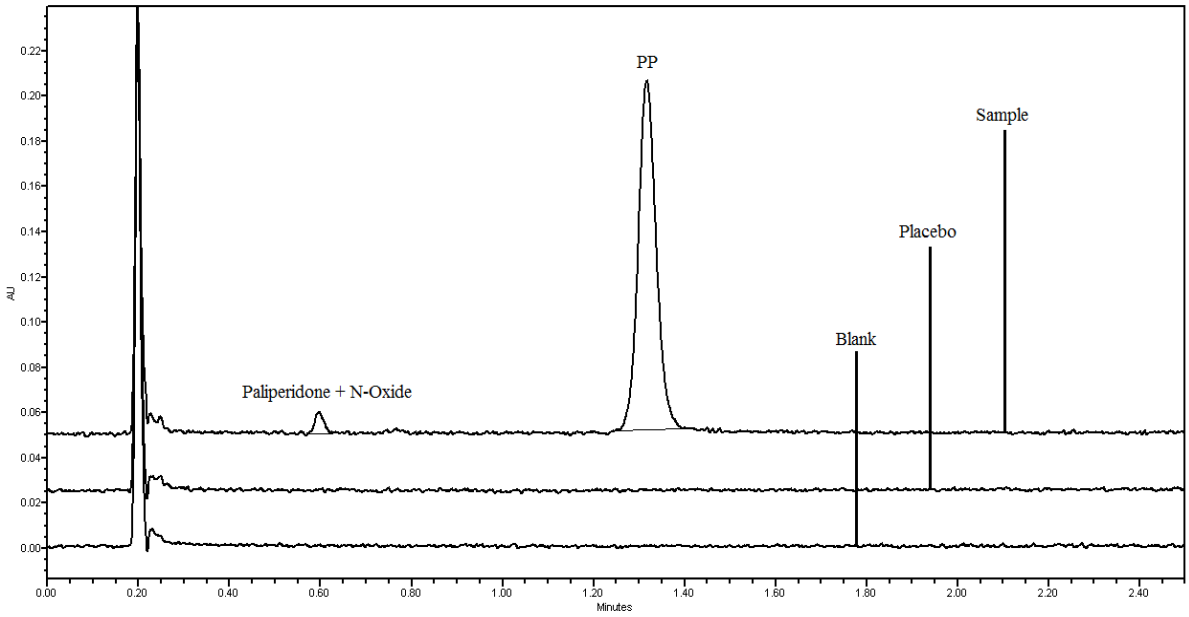

Fig. 4: Overlaid chromatograms of blank, placebo and sample (peroxide study).

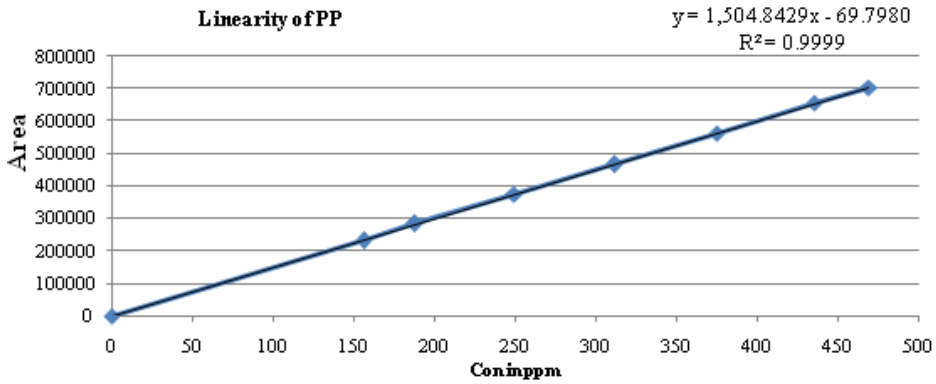

Fig. 5: Linearity of pp.

Stability of the sample solution

Drug stability in pharmaceutical formulations is a function of storage conditions and chemical properties of the PP. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data are required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling. A sample solution did not show any appreciable change in assay value when stored at ambient temperature up to 24 h (Table 8). The results from solution stability experiments confirmed that the sample solution was stable for up to 24 h during the assay procedure.

Table. 8: Solution stability results.

	Initial	After 24 hrs.
% Assay	100.2	100.4

Application of the method to stability study

The present method was applied for the estimation of PP during a stability study. The results obtained are presented in Table 9.

Table 9: Results of stability study (Paliperidone palmitate extended release injection).

Sample ID	% Assay of PP
Initial	99.8 %
1 M 40 °C /25 % RH	99.3 %
2 M 40 °C /25 % RH	99.8 %
3 M 40 °C /25 % RH	100.1 %

CONCLUSION

A new RP-UPLC method was successfully developed for the estimation of paliperidone palmitate in depot Injection. The method validation results have verified that the method is selective, precise, accurate, linear, robust and stability indicating. The run time (2.5 min) enables rapid determination of PP. This stability-indicating method can be applied for the determination of paliperidone palmitate in release testing and in stability studies of extended release Injectable suspension. Moreover, it may be applied for the determination of PP in bulk drugs, content uiformity of pre filled syringes or dissolution profile, where sample load is higher and high throughput is essential for faster delivery of results.

ACKNOWLEDGEMENT

The authors would like to thank M/s Dr. Reddy's Laboratories Ltd. for supporting this work. One of us Mr Parimal M Chatrabhuji is thankful to UGC for providing a research project [(47-2052)/2011(WRO)].

The authors' Intellectual Property Management department (IPM) has given this manuscript the internal publication number **PUB00242-13.**

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

REFERENCES

Harshal KT and Mukesh CP. Development and validation of a stability indicating RP-UPLC method for determination of rosuvastatin and related substances in pharmaceutical dosage form. Sci Pharma, 2012; 80: 393-406.

Hima BK, Nitin HD, Suryanarayana MV and Anjaneyulu AY. A validated stability indicating PLC method for simultaneous determination of assay, related substances and degradation products of paliperidone palmitate active pharmaceutical ingredient and it pharmaceutical injection forms. J Liq Chrom Rel Tech, 2012; 35(4): 533-546.

Rakshit KT and Mukesh CP. Development of a stability indicating RP-UPLC method for rapid determination of Mataxalone and its degradation products in solid oral dosage form. Sci Pharma, 2012; 80: 353-366.

RxList (The internet drug index). Available at: http://www.rxlist.com/invega-sustenna-drug.htm [Accessed 10 Jun 2011.

Trivedi RK, Patel MC and Kharkar AR. Determination of Mesalamine related impurities from drug product by reversed phase validated UPLC method. E J Chemistry, 2011; 8(1): 131-148.

ICH, 2005. Validation of Analytical Procedure, Text and Methodology Q2(R1), International conference on Harmonization, IFPMA, Geneva, Switzerland.

How to cite this article:

Rakshit Kanubhai Trivedi, Prashant Jain , Mukesh C Patel , Parimal M Chatrabhuji , Dipika R Trivedi., A Rapid, Stability Indicating RP-UPLC Method for Determination of Paliperidone Palmitate in a Depot Injectable Formulation. J App Pharm Sci, 2013; 3 (07): 087-092.