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Stability Indicating HPLC method for Determination of Paliperidone in Bulk

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1. Introduction

Paliperidone (PALI) is second generation atypical antipsychotic drug used for treatment of schizophrenia in adults. It is the major active metabolite of risperidone and also known as 9-hydroxy risperidone. Its therapeutic activity is mediated through antagonism of central dopamine type D2 receptors and serotonin 5-HT2A receptors. It is practically insoluble in water, slightly soluble in dimethylformide, sparingly soluble in 0.1 N HCl[1-2]. Various analytical methods have been reported in literature for the analysis of PALIsuch as High performance liquid chromatography[3-4], Liquid chromatography-Mass Spectroscopy[5]andUltra performance liquid chromatography[6].

The current investigations was aimed to develop a simple, sensitive, precise and accurate stability indicating HPLC method for the determination of PALI in bulk.

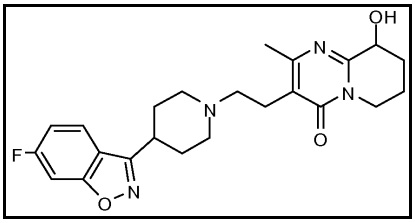

Fig. 1 Chemical structure of Paliperidone

2. Experimental

Reagents and chemicals

PALI was kindly gifted by Inventia Healthcare Pvt. Ltd., Thane, India. All other chemicals and solvents used throughout the study were HPLC/ AR grade. Acetonitrile, trimethylamine, hydrochloric acid (HCl), Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from SDFine Chemicals Lld., Mumbai, India. Water was purified with Milli-Q Millipore system.

Instrumentation and analytical conditions

The analysis of the drug was carried out on HPLC instrument (Perkin Elmer, Series 200 EP) equipped with binary pump and photodiode array (PDA) detector and TotalChrom™ Software. Chromatographic analysis

was performedin isocratic elution mode using Inertsil ODS© C18 (250 x 4.6 mm, 5µ) HPLC column and mobile phase consisting of 0.05 M KH_2PO_4 (pH adjusted to 6.0 with triethylamine) and acetonitrile in a ratio of 30:70v/v at 1 ml/min flow rate.The detection wavelength was set at 237 nm.

Preparation standard solution of PALI

Standard solution of PALI

A quantity of PALI equivalent to 25 mg was measured and transferred to 50 ml volumetric flask. 30 ml of acetonitrile (ACN) was added to it and sonicated for 10 min. Volume was made up to the mark with ACN to obtain a stock solution of the concentration 500 µg/ml. An aliquot of this stock was diluted with mobile phase to get standard working solution of the concentration 50 µg/ml.

Optimization of HPLC method

Selection of mobile phase

The mobile phase was selected on basis of good resolution, peak purity, peak symmetry, theoretical plates etc. An optimized chromatographic condition is one which meets all system suitability parameter. Different combinations of solvents in various ratios were tried to get peak of PLDN which meet all system suitability parameter. The typical chromatograms of PALI in optimized chromatographic conditions are shown in Fig. 2.

Selection of pH

The pH of mobile phase was selected on basis of good resolution, peak purity, peak symmetry, theoretical plates etc.

System suitability study[7]

The system suitability was assessed by three replicate analyses of the drug at a concentration of 50 µg/ml. System suitability of the method was evaluated for parameters viz. peaks symmetry (symmetry factor), theoretical plates of the column (N), retention time (Rt).

Forced Degradation Studies[8]

Forced degradation of PALI was performed to provide information about the degradation behaviour of drug during stability studies and specificity of the method. PALI was subjected to stress conditions including acid hydrolysis, base hydrolysis, oxidative, thermal and photo degradation. PALI was subjected to acid degradation (0.1 M HCl, reflux at 80ºC for 3 h), alkaline degradation (0.1 M NaOH at room temperature for 8 h), oxidative degradation (1% H_2O_2 at room temperature for 7 h), thermal degradation (80°C for 72 h) and photo degradation (UV light for 24 h). The sample solution of PALI under each stress condition was analysed by HPLC. The peak area of samples under each condition was compared with that of standard PALI area to calculate the % degradation using the following formula-

$$\% \text{ Degradation} = \frac{\text{Peak area of sample of stress condition}}{\text{Peak area of standard PALI}} \times 100$$

Method validation[9]

Linearity and range

To study the linearity of method, the aliquots of the standard stock of PALI were diluted with mobile phase to obtain concentrations in the range 40- 200 µg/ml. The resulting solutions were subjected to HPLC analysis. The peak areas were calculated and plotted against their respective concentrations. A linearity curve was constructed and the linearity range was determined from the regression coefficient value (r^2). The above experiment was performed in triplicate (n=3).

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were computed from the calibration curve data using ANOVA analysis. LOD and LOQ were calculated using 3.3 σ/S and 10σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of Y-intercept.

Precision

a) Repeatability

The six replicates (n=6) of test solution of PALI were subjected to HPLC analysis and their peak responses were monitored. The percent relative standard deviation (% RSD) of the peak areas of six replicate injections was calculated.

b) Intermediate precision

The intermediate precision study was carried out asinterday study. For interday study, six test solutions of PALI (50 µg/ml) were analysed on two consecutive days and their peak responses were monitored. The % RSD for 6 replicates was calculated for each day and cumulative for two days.

Accuracy

The accuracy of the method was determined by recovery study. Three levels i.e. 80, 100 and 120% of test concentration were chosen for the experimental purpose. The placebo blend of the marketed formulation were prepared and then spiked with known amount of PALI. Each concentration level was performed in triplicate. The percent recovery and % RSD were calculated.

Robustness

The effect of certain analytical parameters such as flow rate and pH was studied. The effect of change in flow rate was studied by varying the flow rate by 0.2 units i.e. 0.8 and 1.2 ml/ min, whereas, pH was varied by ±0.2 (pH 5.8 and 6.2), respectively keeping other analytical parameters constant.

3. Results and Discussion

Selection of mobile phase

A mobile phase consisting of 0.05 M KH_2PO_4 buffer (pH adjusted to 6.0 with triethylamine) and acetonitrile in a ratio of 30:70 %v/v, respectively, was selected as optimized mobile phase.

Selection of pH

A chromatogram with mobile phase of pH 6.0 was found to exhibit system suitability parameters within limit. Hence, a mobile phase (acetonitrile: 0.05 M KH_2PO_4 buffer 30:70 %v/v) of pH 6.0 was selected for analysis.

Table 1. Optimized Chromatographic conditions

Parameters	Specifications
Stationary phase	Inertsil ODS© _C18_250 * 4.6 mm_5µ
Mobile phase	KH_2PO_4 0.05 M: Acetonitrile, 70:30 v/v
pH of mobile phase	Adjusted to 6.0 using triethylamine
Flow rate (ml/min)	1.0 ml/min
Detection wavelength	237 nm

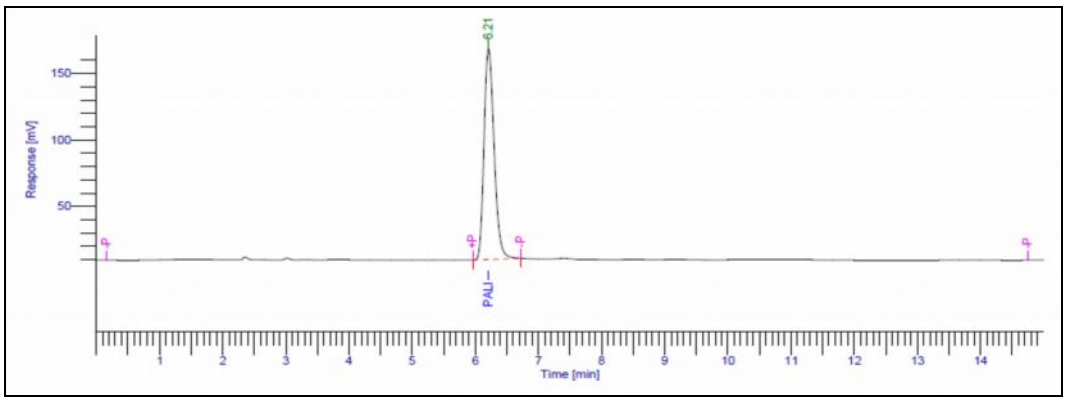

Fig. 2. A typical chromatogram of PALI (Rt = 6.21)

System suitability study

The system suitability parameters were in compliance to the USP guidelines. The theoretical plates (N) were found to be more than 2000, tailing factor was 1.14 and the retention time (Rt) was found to be 6.21.

Forced Degradation Studies

During acid hydrolysis, PALI refluxed for 3 h with 0.1 M HCl showedappearance of degradation peaks at 4.97 and 7.32 retention times. About 13-15 % degradation of PALI was observed in acidic condition.PALI solution treated with 0.1 M NaOH at room temperature showed 31-33 % degradation. The degradation peaks in alkaline stress condition were observed at 3.82, 4.60, 4.90 and 7.27 min. The PALI was observed to be liable to oxidation (more than 30 % degradation) at 1% H_2O_2 at room temperature for 7 h. The degradants were eluted at 4.92 and 7.40 min. During thermal degradation (treatment to dry heat at 80°C for 72 h) and photo degradation (exposed to UV light for 24 h), the chromatogram of PALI did not show appearance of any degradation peak. From the forced degradation study, it can be concluded that PALI is prone to degradation in acidic, alkaline and oxidative conditions. However, it was observed to be stable to thermal condition and photo stress degradation condition. The degradation peaks were well separated from the PALI peak. The method is able to determine PALI in presence of its degradation products and hence can be said to be specific for determination of PALI.

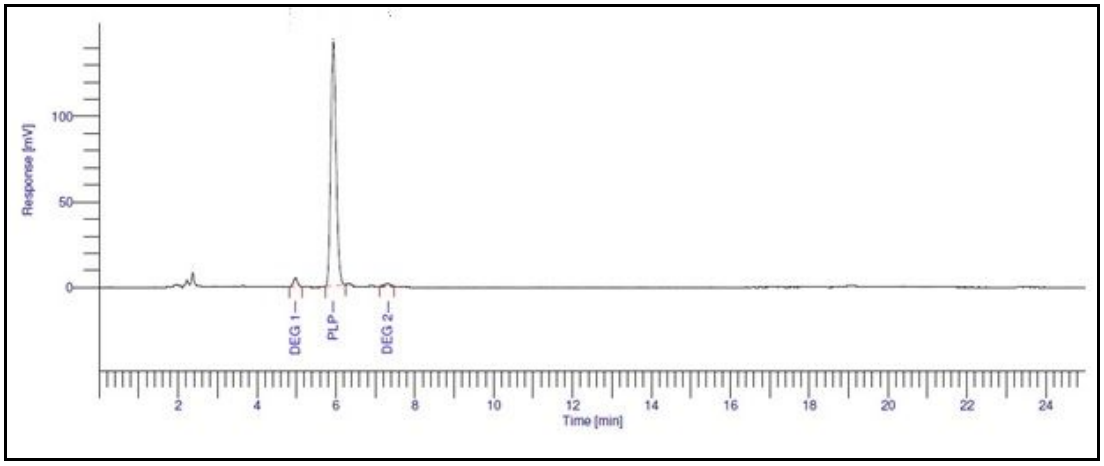

Fig. 3 Acidic degradation of PALI (0.1 M HCl, 3h reflux at 80ºC)

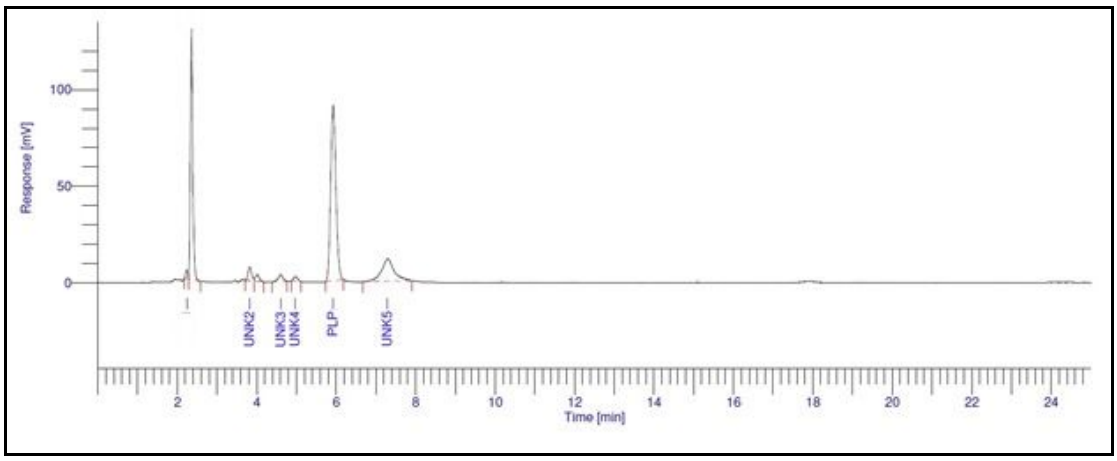

Fig. 4 Alkaline degradation of PALI (0.1 M NaOH, 8h at RT)

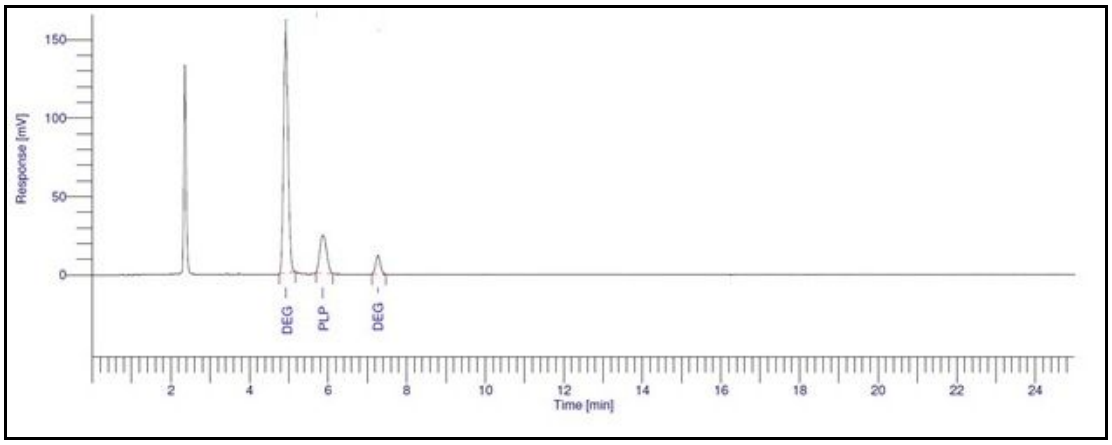

Fig. 5 Oxidative degradation of PALI (1% H_2O_2 for 7h at room temperature)

Validation

Linearity and range

The linearity of PALI**(Fig. 6)** in mobile phase was plotted in the range of 40-200 µg/ml and range was studies in 80- 120 % of its test concentration (50 µg/ml). The method was found to be linear in the selected concentration range with regression coefficient of 0.9992, slope and intercept values being 29939 and 90097, respectively.

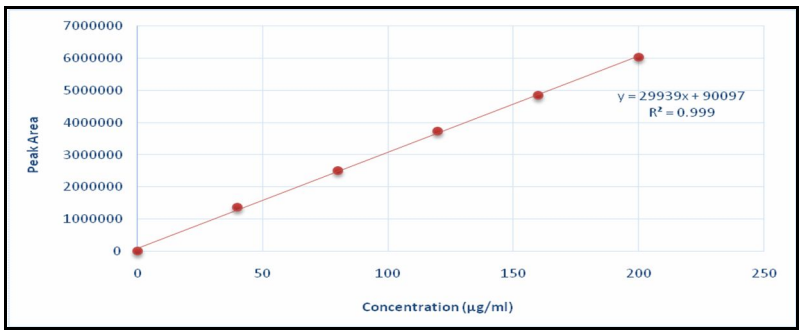

Fig. 6 Linearity graph of PALI

LOD and LOQ

LOD and LOQ were found to be 1.72 and 5.35 µg/ml, respectively, computed by using ANOVA analysis.

Precision

In repeatabilitystudy, the % RSD of six replicates of test solutions of PALI was found to be 0.78 indicating the method is precise. Whereas in interday study, the RSD of the drug content of all 12 samples (6 from day 1 and 6 from day 2) was found to be 1.15, which is below 2% confirming that the method was precise.

Accuracy

An excellent recovery of 98-100% with RSD value of 0.87 was obtained at all the experimental levels (80, 100 and 120%). Hence, it was inferred that there was no interference in quantitative estimation of PALI and proposed HPLC method is proved to be accurate.

Robustness

Deliberate change of mobile phase pH and flow rate of the column showed % RSD values of less than 2 reflecting robustness of the method **(Table 2)**.

Table 2. Effect of change in pH and flow rate on % assay of PALI

Condition	RT*	Mean Area*	Symmetry factor*	Assay (%)*
Unaltered condition	6.21	1549785.36	1.04	99.38
Altered condition				
pH: 5.8	6.221	1612467.15	1.210	98.19
pH: 6.2	6.214	1556375.72	0.946	100.24
Flow rate 0.8 ml/ min	7.83	1532438.05	0.810	101.86
Flow rate 1.2 ml/ min	5.509	1495631.43	0.816	100.42

* Values are mean of three determinations (n=3)

From the above table it can be seen that there was no significant change in the symmetry and % assay of PALIindicating that the method is robust.

4. Conclusion

A simple, rapid, accurate and precise stability indicating HPLC analytical method has been developed and validated for the routine analysis of PALI in bulk. The results of the stress testing reveal that the method is selective and stability indicating. The proposed method has the ability to separate these PALI from their degradation productscan be applied to the analysis of samples obtained during accelerated stability studies.

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