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DEVELOPMENT AND VALIDATION OF NEW HPLC METHOD FOR THE ESTIMATION OF PALIPERIDONE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple, rapid, sensitive, accurate and precise HPLC method has been developed and validated for the estimation of Paliperidone in bulk and its pharmaceutical dosage forms. The method was carried out using Thermosil Symmetry C18 (100 x 4.6 mm I.D., 5 µm particle size) column and mobile phase comprised of ammonium acetate buffer pH 4.0 and acetonitrile in proportion of ratio 50:50 v/v and degassed in ultrasonic water bath. The flow rate was 0.8 mL/min and the detection wavelength was at 275 nm. The linearity was observed in the range of 5-30 µg/mL with a correlation coefficient of 0.999. The retention time of Paliperidone was 2.458 min. The method was validated as per the ICH guidelines for its linearity, precision, accuracy, specificity, limit of detection, limit of quantitation and by performing recovery studies. The percentage recovery of the drug Paliperidone was 98.5% to 101.3% from the tablet formulation. The proposed method is suitable for the routine quality control analysis for the estimation of Paliperidone in bulk and pharmaceutical dosage form.
Keywords: Paliperidone, Estimation, RP-HPLC, Validation.

INTRODUCTION

Paliperidone is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives, indicated for the treatment of schizophrenia[1]. Chemically Paliperidone is, (±)-3-[2-[4-(6-fluoro-1,2benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one (Fig. 1). Paliperidone is the major active metabolite of risperidone[2]. The mechanism of action of Paliperidone, as with other drugs having efficacy in schizophrenia, is unknown, but it has been proposed that the drug's therapeutic activity in schizophrenia is mediated through a combination of central dopamine Type 2 (D_2) and serotonin Type 2 ($5HT_{2A}$) receptor antagonism[3].

A few HPLC[4-6], RRLC[7], UPLC[8] and HPTLC[9-10] methods were reported earlier for the estimation of Paliperidone in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of Paliperidone in bulk drug and in tablet dosage forms.

EXPERIMENTAL

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Thermosil Symmetry C18 (100 x 4.6 mm I.D., 5 µm particle size), a 2695 binary pump, a 20 µL injection loop, auto sampler and a 2487 dual absorbance DAD or UV detector and running on Waters Empower software.

Chemicals and solvents

The reference sample of Paliperidone was provided as gift sample from Sumages Pharma Pvt. Ltd., Bhimavaram, India. Paliperidone tablets were purchased from local market. HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai, India. Ammonium acetate and glacial acetic acid of AR

Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC grade water obtained from Milli Q water purification system was used throughout the study.

Preparation of ammonium acetate buffer pH 4.0
6.0 grams of ammonium acetate was weighed into a 1000 mL beaker, dissolved in 400 mL HPLC water. Diluted to 1000 mL with HPLC water and pH adjusted to 4.0 with glacial acetic acid.

Preparation of mobile phase and diluent
500 ml of the ammonium acetate buffer was mixed with 500 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

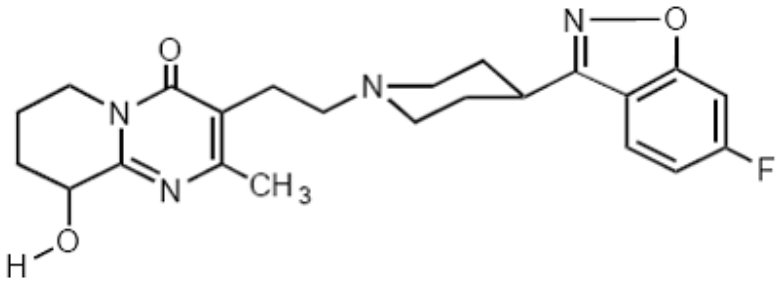

Fig.-1: Chemical structure of Paliperidone

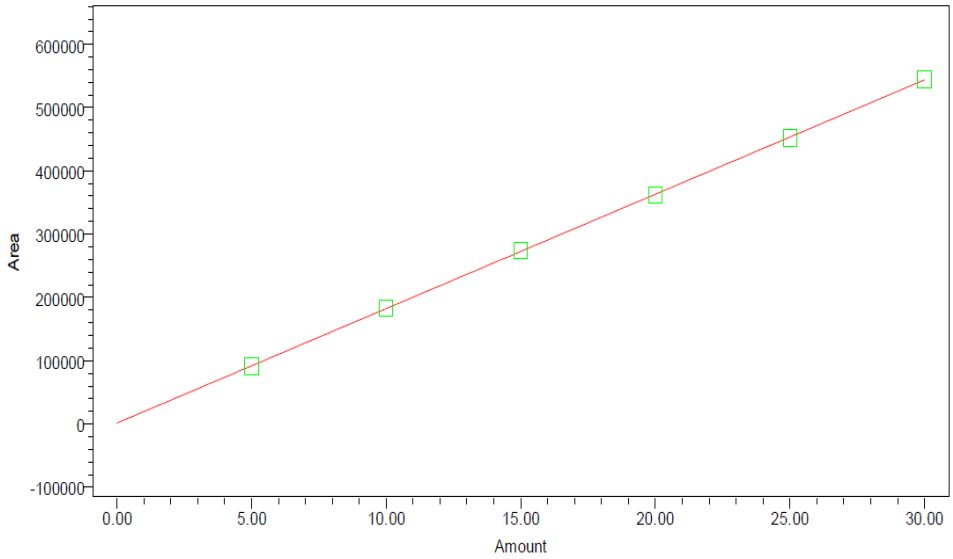

Fig.-2: Calibration curve of Paliperidone

Preparation of standard stock solution
Accurately weigh and transfer 10 mg of Paliperidone working standard into a 10 mL volumetric flask, add about 7 mL of diluent, sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.4 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Preparation of sample solution
Weigh 20 Paliperidone tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Paliperidone into a 10 mL volumetric flask. Add about 7 mL of diluent, sonicate to

dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Calibration plot

About 10 mg of Paliperidone was weighed accurately, transferred into a 10 mL volumetric flask and dissolved in 7 mL of a 50:50 v/v mixture of ammonium acetate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a stock solution. From this, a working standard solution of the drug (20 µg/mL) was prepared by diluting 0.2 mL of stock solution to 10 mL of diluent in a volumetric flask. Further dilutions ranging from 5-30 µg/mL were prepared from the solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 0.8 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area (Fig. 2) was found to be linear in the concentration range of 5-30 µg/mL of the drug. The relevant data are furnished in Table 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Paliperidone in tablet dosage forms.

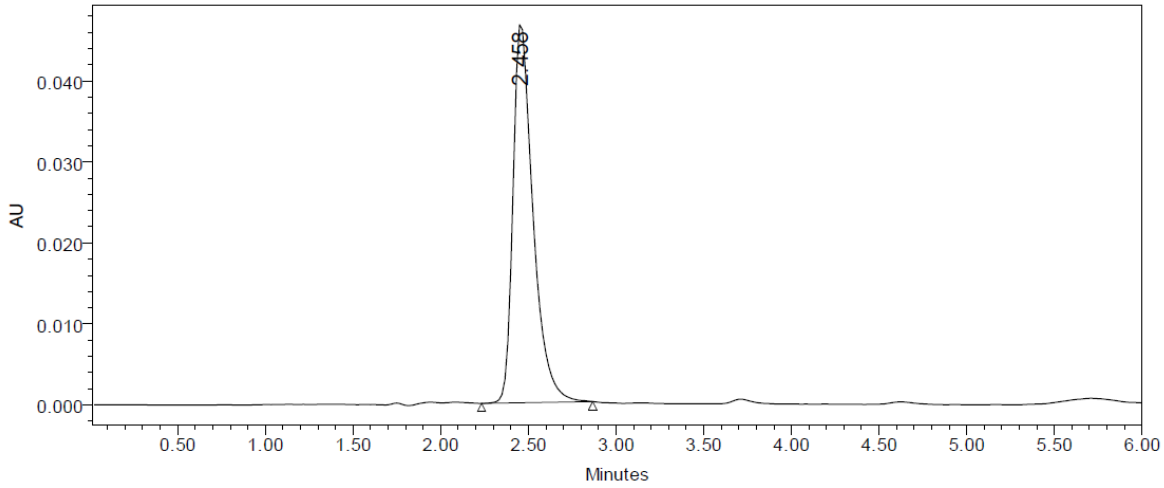

Fig.-3: Typical chromatogram of Paliperidone

Procedure

A mixture of ammonium acetate buffer pH 4.0 and acetonitrile in the ratio of 50:50 v/v was found to be the most suitable mobile phase for ideal separation of Paliperidone. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.8 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. Inject 20 µL of the standard, sample solutions into the chromatographic system and measure the area for the Paliperidone peak. The detection of the drug was monitored at 275 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drug Paliperidone was 2.458 min. A typical chromatogram showing the separation of the drug is given in Fig. 3.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines for the estimation of Paliperidone[11]. Solution containing 20 µg/mL solution of

Paliperiodne was subjected to the proposed HPLC analysis to check precision of the method and the results are furnished in Table 2. The accuracy of the HPLC method was assessed by analyzing solutions of Paliperidone at 50%, 100% and 150% concentration levels by the proposed method. The results are furnished in Table 3. The system suitability parameters are given in Table 4.

Table-1: Calibration data of the method

Concentration (µg/mL)	Mean peak area (n=6)
5	91429
10	182859
15	274086
20	362217
25	452109
30	544931

Table-2: Precision data of the proposed HPLC method

Concentration of Paliperidone (20 µg/mL)	Peak area
Injection-1	359401
Injection-2	361272
Injection-3	360628
Injection-4	360947
Injection-5	361664
Average	360782
Standard Deviation	862.5
%RSD	0.24

Table-3: Accuracy studies

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	370067	5.0	5.0	101.3%	99.9%
100%	730879	10.0	10.0	100.0%	
150%	1072183	14.9	14.6	98.5%	

Table-4: System suitability parameters

Parameter	Result
Linearity ((µg/mL)	5-30
Correlation coefficient	0.999
Theoretical plates (N)	2287
Tailing factor	1.5
LOD (µg/mL)	0.05
LOQ (µg/mL)	0.20

Table-5: Assay studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation-1	10	10.03	99.67

Estimation of Paliperidone in tablet dosage forms

Commercial formulations of Paliperidone tablets were chosen for testing the suitability of the proposed method to estimate Paliperidone in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of Paliperidone was transferred into a 10

mL volumetric flask and dissolved in 5 mL of a 50:50 v/v mixture of ammonium acetate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 3 mL of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. This solution of Paliperidone was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Paliperiodne was found to be 2.458 min. Quantification was linear in the concentration range of 5-30 µg/mL. The regression equation of the linearity plot of concentration of Paliperidone over its peak area was found to be y=1599.4+18076.52x (r^2=0.999), where x is the concentration of Paliperidone (µg/mL) and y is the corresponding peak area. The number of theoretical plates calculated was 2287, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.05 µg/mL and 0.20 µg/mL respectively, which indicate the sensitivity of the method. The use of ammonium acetate buffer pH 4.0 and acetonitrile in the ratio of 50:50 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Paliperidone by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, accurate and precise for the determination of Paliperidone and can be reliably adopted for routine quality control analysis of Paliperidone in its tablet dosage forms.

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