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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PALIPERIDONE IN BULK AND TABLET DOSAGE FORM BY REVERSE PHASE HPLC METHOD

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ABSTRACT

A simple, precise, rapid, and reproducible reverse phase liquid chromatographic method developed and validated for the quantification of Paliperidone in bulk drug and in Pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a PhenomenaxLunaC_{18} (ODS) column (150 X 4.6 mm i.d., particle size 5µ). The mobile phase consisting of phosphate buffer pH 3.0: Acetonitrile (60:40, v/v). An isocratic elution was achieved at a flow rate of 1 ml/ min at ambient temperature. The detection was carried out at 225nm using Shimadzu UV-Visible detector SpD-10AVP. The retention time of Paliperidone was found to be 5.02 min. The calibration curve was linear in the concentration range of 5-30µg/ ml (r^2= 0.9999).The limit of detection and the limit of quantification were found to be 0.580531 µg/ml and 1.75918µg/ml respectively. The amount of Paliperidone present in the formulation was found to be 99.79 ± 0.8075. The method was validated statistically using the SD, % RSD and SE and the values were found to be within the limits. The recovery studies were performed and the percentage recoveries were found to be 101.10±1.635%. So, the proposed method was found to be simple, specific, linear, and rugged. Hence it can be applied for routine analysis of Paliperidone in the Pharmaceutical formulation.

KEYWORDS

Paliperidone, RP-HPLC Method, Validation and System suitability tests.

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INTRODUCTION

Paliperidone (Figue No.1), (2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7 diisopropyl-8- [4- methoxy- 3- (3-methoxypropoxy) phenyl] octanamide hemifumarate)[1-3] (Figure No.1). The first oral direct renin inhibitor approved for clinical use, exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension.

Paliperidone blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing generation of angiotensin I and angiotensin II.

MATERIALS AND METHODS

Chemicals

Acetonitrile used was of HPLC grade from E. Merck, India. HPLC grade water was obtained using millipore water purification system. Working standard of Paliperidone with potency of 99.67 % was obtained from Dr. Reddy's Laboratories, Hyderabad. Other chemicals were analytical grade of above 99% purity. All volumetric ware was pre-calibrated by the manufacturer (Borosil) and was of grade A. HPLC grade water was obtained using millipore water purification system. Commercial tablets containing Paliperidone (Tekturna-150mg) were procured from the local chemist shop.

Instrumentation

The validated method utilized a Shimadzu HPLC system containing SPD-10 ATVP pump and SPD-10AVP UV-Visible detector with an isocratic elution technique at a flow rate of 1ml/min on a Phenomenax LunaC_{18} column (150 X 4.6 mm i.d., 5µ) at ambient temperature. A rheodyne injector with 20 µl loop was used for injecting the sample. Shimadzu balance[4] was used for weighing purpose in this method.

Chromatographic conditions

The analysis was carried out with UV detection at 293 nm (Figure No.2) using a 20 µl Injection volume. Assay was performed using a C18 reversed-phase column eluted with phosphate buffer pH 3.0: Acetonitrile (60:40, v/v) at a flow rate of 1.0 ml/ min. Chromatography was carried out at ambient temperature. The solvents were mixed, filtered through a membrane filter of 0.45 micron pore and degassed in ultrasonic bath prior to use.

Standard solution preparation[5-11]

Standard stock solutions were prepared by dissolving 10 mg of Paliperidone working standard in 8.0 ml of mobile phase and diluting to 10.0 ml with the same to obtain concentration of 1000 µg/ml. It was filtered through a.22µmembrane filter. The stock solution was protected from light using aluminum foil and stored for 1 week at 40C and was found to be stable during this period.

Procedure for analysis of tablet formulation[12]

20 Tablets of the product under study were weighed, crushed and mixed in a mortar. A portion of powder equivalent to the weight of 100.00mg was accurately weighed and transferred to a dry 100 ml A-grade volumetric flask and 100 ml mobile phase was added. The volumetric flask was sonicated for 20 min to effect complete dissolution of Paliperidone and made up to the volume with mobile phase. Suitable aliquots of solution were filtered through a 0.45 µm nylon filter. This was further diluted with mobile phase to yield concentration of Paliperidone in the range of linearity (15ppm).Each of standard and test preparation was injected into the chromatograph and the responses recorded (Figure No.3).

METHOD VALIDATION[13-17]

Linearity

A series of standard curves were prepared over a concentration range of 5 -30µg/ml by diluting the standard stock solution of Paliperidone (1mg/ml) in mobile phase. The data from peak area versus drug concentration plots were treated by linear least square regression analysis and r^2 was found 0.9999 (Figure No.4). The standard curves were evaluated for intra-day and inter-day reproducibility. Each experiment was repeated in triplicate.

Precision

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 15µg/ml concentration six times.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples Paliperidone (15 µg/ml) were spiked with known amount of standard so as to get three different levels (66.33, 88.33% and 100%) and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate.

Recovery (%), RSD (%) was calculated for each concentration.

Limit of detection and limit of Quantitation

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level (S/N = 3:1) while ten times the noise level gave the LOQ (S/N=10:1).

Ruggedness

The ruggedness of the method was demonstrated by analysis of the samples as for precision study by a second analyst. The RSD of the two sets of data indicates the ruggedness of the method. Further, the t-test was performed on the data and the difference was found to be not significant.

Robustness

The robustness of the method was determined to assess the effect of small but deliberate changes of the chromatographic conditions on the determination of Paliperidone. The different variations are in flow rates by ± 0.1 mL/min, in wavelength by ± 2 nm and in temperature by ± 5 °C. The concentration of the solution analyzed was 15µg/mL.

System suitability tests

The chromatographic systems used for analyses must pass the system suitability limits before sample analysis can commence. The capacity factor (K), injection repeatability, tailing factor (T), theoretical plate number (N) and reference solution (Rs) for the principal peak were the parameters tested on a 15 µg/mL sample of Paliperidone to assist the accuracy and precision of the developed HPLC system.

RESULTS

Linearity

Peak area versus drug concentration was plotted to construct a standard curve for Paliperidone and linearity was shown in concentration range of 5µg/ml to 30µg/ml. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation, $r^2 = 0.9999$.

Precision

System precision is the measure of the method variability that can be expected for a given analyst performing the analysis. Precision of the method was determined with the product. The precision study was carried out by injecting sample preparation of 15µg/ml concentration and assayed in six replicate determinations for each of the six weighing amounts. The results for precision are shown in Table No.1, indicating that acceptable precision was achieved for Paliperidone as revealed by relative standard deviation data (RSD<2.0% in all of the levels)

Accuracy

The % recovery was calculated for triplicate samples and for all levels and mean recovery was calculated. The mean recovery was well within the acceptance limit hence the method was accurate, as depicted in Table No.2.

Limit of detection and limit of Quantitation

The LOD was calculated to be 0.5805µg/ ml and the LOQ was calculated to be 1.7591 of the placebo mixtures with the peak of Paliperidone was observed.

Ruggedness

The % assay and RSD for samples prepared by second analyst was calculated and found within limit. Then RSD of analyst 1 and analyst 2 was calculated and found within limit. This proved that the method is rugged, as depicted in Table No.3.

Robustness

The results of the analysis (% RSD ranged from 0.059 to 1.361 %) of the samples under the conditions of the above variations indicated the nature of robustness of the method.

System suitability tests

The results of the system suitability tests assure the adequacy of the proposed HPLC method for routine analysis of Paliperidone. The capacity factor (k) was found to be 1.905, indicating that the Paliperidone peak is well resolved with respect to the void volume. The RSD of six consecutive injections performed under the precision test (Table No.4) was found to be 0.43% and thus shows good injection repeatability. The tailing factor (T) for Paliperidone peak was found to be 0.7, reflecting good peak symmetry. The theoretical plate number (N) was

found to be 8536, thus demonstrating good column efficiency.

Specificity

The chromatograms obtained showed separation of the analyte from the excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis. No interference.

DISCUSSION

A simple, selective, rapid and precise RP-HPLC method for the estimation of Paliperidone in bulk material and in pharmaceutical formulation has been developed and validated. The linearity range was determined by external standard calibration method in the concentration range of 5-30µg/ml. The correlation co-efficient was found to be 0.9999 indicated that the concentrations of Paliperidone had good linearity. The LOD and LOQ were found to be 0.580531 µg/ml and 1.75918 µg/ml, respectively. The system suitability parameters like capacity factor, asymmetric factor, tailing factor, HETP and number of theoretical plates were calculated and it was observed that all the values are within the limits. The percentage of Paliperidone present in formulation was found to be 99.79 ± 0.8075. Further the precision of the method was confirmed by the repeatable analysis of formulation. The % RSD was found to be 0.8077. It indicated that the method has good precision. The percentage recovery of Paliperidone present in formulation was found to be 101.10±1.635and the percentage RSD value was found to be 0.8700. The low percentage RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed.

Table No.1: Precision of developed method at working level

S. No	No. of Injection	% Assay*
1	1	99.48
2	2	99.23
3	3	100.35
4	4	99.68
5	5	99.85
6	6	99.11
Mean		99.79 ± 0.8075
SD		0.8075
% RSD		8077

Table No.2: Recovery studies of Paliperidone

S. No	Amount Present (µg/ml)	Amount added* (µg/ml)	Amount found* (µg/ml)	% recovery*	Average ± S.D	% RSD
1	19.96	2.003	21.97	101.35	101.10± 1.635	1.654
2	19.96	6.009	26.06	101.84		
3	19.96	10.015	29.97	100.13		

(* n=6)

Table No.3: Ruggedness Analysis

S.No	Analyst 1 Sample	% Assay	Analyst 2 Sample	% Assay
1	1	100.04	1	99.85
2	2	100.22	2	98.91
3	3	99.84	3	100.45
4	4	100.35	4	100.03
5	5	100.67	5	99.47
6	6	100.41	6	99.49
*Mean		100.25	Mean	99.75
SD		0.283	SD	0.193
RSD		0.271	RSD	0.159

(*n=6)

Table No.4: System Suitability Parameter

S. No	Parameter	Suitable Values
1	Retention Time	5.02
2	Tailing factor	0.7
3	Asymmetrical factor	1.23
4	Theoretical plates	8536
5	Capacity factor	1.905
6	HETP	0.03256

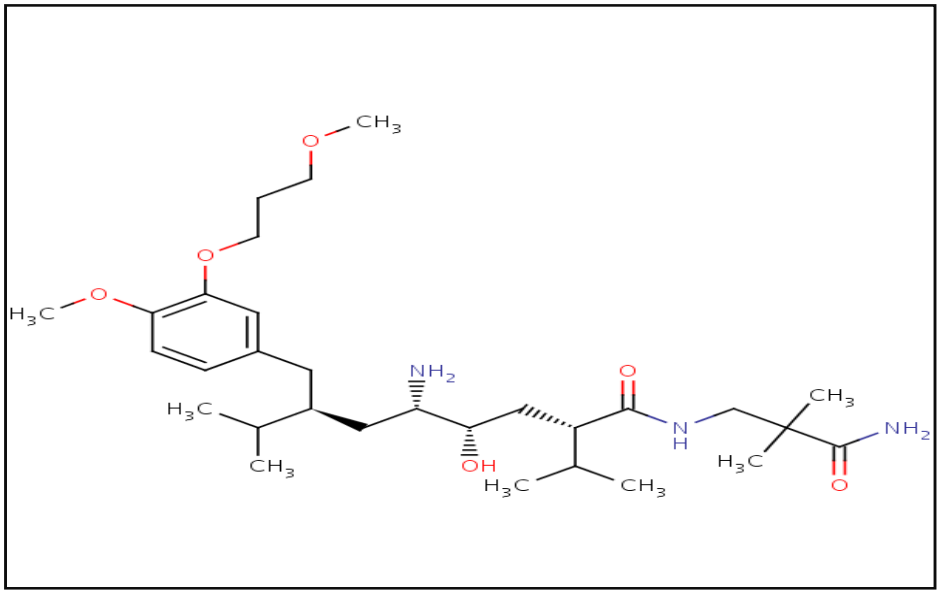

Figure No.1: Chemical structure of Paliperidone

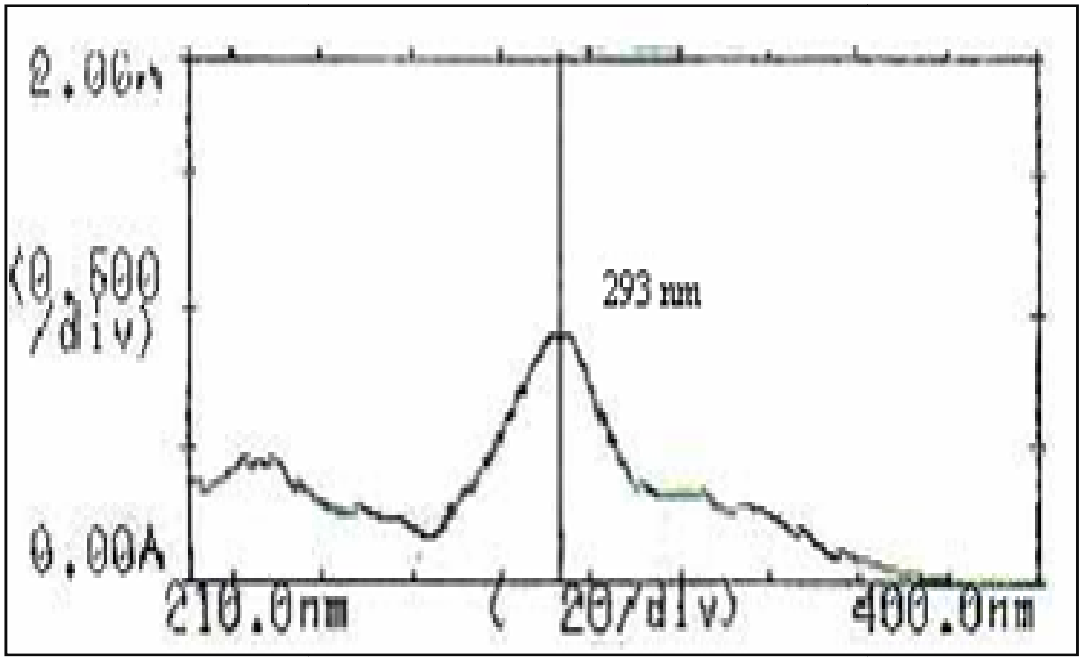

Figure No.2: UVAbsorbance Spectrum for Paliperidone

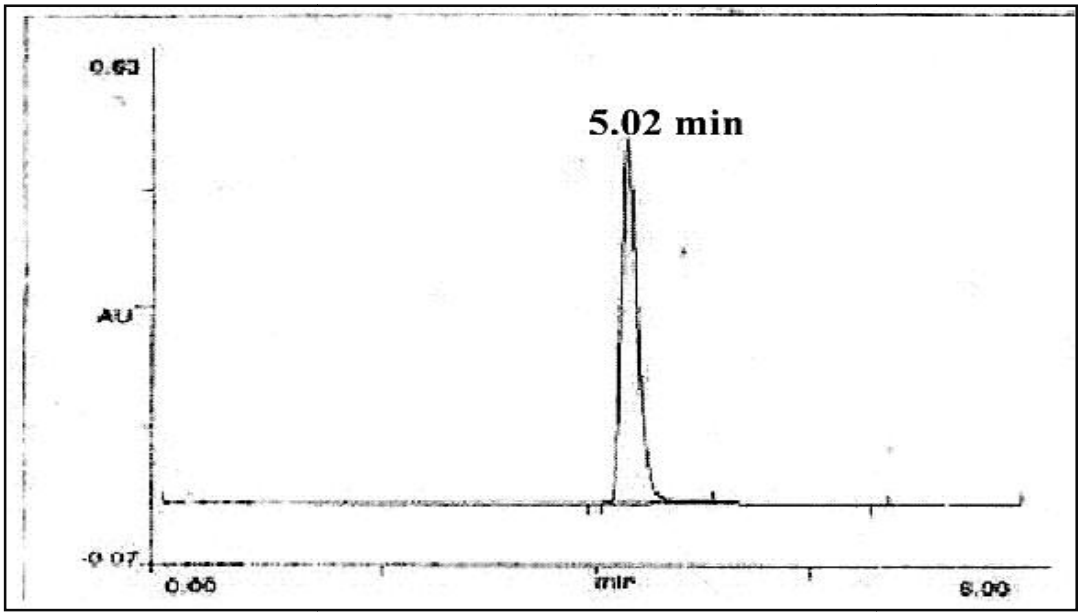

Figure No.3: Typical RP-HPLC Chromatogram of Paliperidone by the proposed method

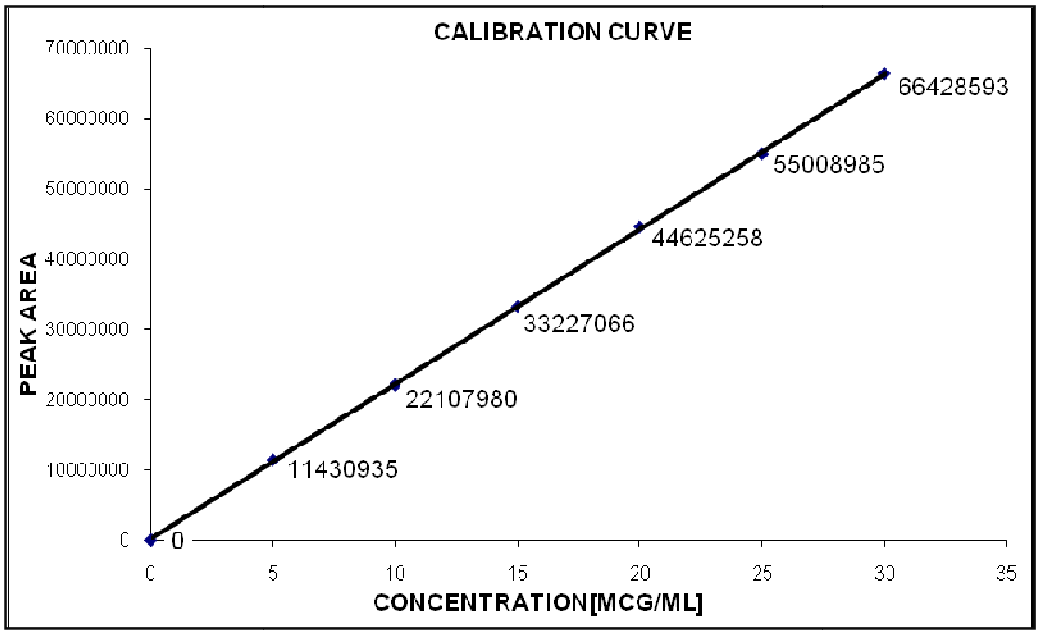

Figure No.4: Calibration curve of Paliperidone

CONCLUSION

The developed RP-HPLC method was validated and the system suitability studies were performed and all parameters combined with the simplicity and ease of operation ensures that the validated method can successfully used for routine analysis of Paliperidone in bulk and tablet dosage formulation.

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