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A Validated RP-HPLC Method for the Estimation of Paliperidone in Bulk and Tablet Dosage Form

A. S. Manjula Devi*, T. K. Ravi

1. Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore-641 044

ABSTRACT

A novel, precise and selective high performance liquid chromatographic method was developed for the estimation of paliperidone using paracetamol as the internal standard. Separation was achieved on a *LiChrospher® RP-18 HPLC column (5* µ particle size and *25 cm* × 4.6 mm *internal diameter)* using 10 mM ammonium acetate: methanol in the ratio of 10:90 (v/v) as the mobile phase, at flow rate of 0.7 ml/min and the eluate was monitored at 277 nm. The method was validated in compliance with ICH guidelines. The correlation coefficient of the calibration graph was 0.99946±0.00037 over the concentration range 1 to 5 µg mL^{-1}. The limit of detection and limit of quantification were 0.569 µg mL^{-1} and 1 µg mL^{-1}, respectively. Overall percentage recovery of paliperidone ranged between 98.92±0.595 to 100.30±0.693. Relative standard deviations for intra- and inter-day precision studies were <2%. The method was simple, rapid, economic and suitable for the regular quality control of paliperidone in tablet dosage forms.

Keywords: Paliperidone, RP-HPLC, paracetamol, validation, ICH guidelines

* Corresponding Author Email: hari1509@gmail.com

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INTRODUCTION

Paliperidone (PALI) is the major active metabolite of risperidone which is a widely used atypical antipsychotic approved for the treatment of schizophrenia and other psychiatric disorders. Chemically it is (±)-3-[2-[4-(6-fluoro-1,2benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4Hpyrido[1,2-a]pyrimidin-4-one with empirical formula $C_{23}H_{27}FN_4O_3$ and molecular weight 426.49[1]. Literature survey reveals HPLC method for the determination of paliperidone in bulk by external standard method[2], enantioseparation of paliperidone by RP-HPLC[3], stress degradation behavior by RP-HPLC[4], HPTLC method for quantification of paliperidone in formulation[5], and LC-MS-MS method for its determination in human plasma[6]. Internal standard method which is relatively more accurate and precise due its advantage of eliminating errors from sample preparation and run to run variations[7] has not been reported for the estimation of paliperidone. Therefore, the purpose of the present work was to develop a simple, rapid, economic and validated RP-HPLC method for the estimation of paliperidone in bulk and pharmaceutical dosage form using a suitable internal standard.

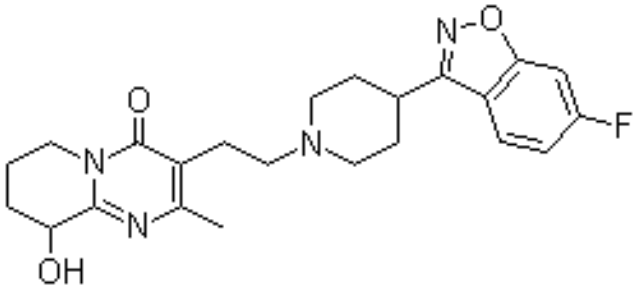

Figure 1: Chemical structure of paliperidone

MATERIALS AND METHODS

Materials and reagents

Reference standards of paliperidone and paracetamol (both having assigned purity >99% w/w) were used to develop the new RP-HPLC method. HPLC grade methanol was obtained from Merck specialties Pvt. Ltd. (Mumbai, India). Other chemicals and reagents were analytical grade. Water for RP-HPLC was prepared using Millipore purification system (Milli-Q, Bangalore, India). Tablet formulation (Palido OD 3, Torrent Pharmaceuticals Ltd, India) containing 3 mg paliperidone was purchased from the local pharmacy.

Instrumentation and chromatographic conditions

Chromatography was performed under ambient conditions with liquid chromatography mass spectrometer (Shimadzu LCMS-2010EV) equipment comprising of a binary gradient pump (LC-20AD), degasser (DGU-$20A_3$), and a variable wavelength programmable PDA detector (SPD-

M20A) with auto sampler system (SIL-20AC). The instrumentation was controlled by Shimadzu LCMS Solution Software. Chromatographic separation was achieved on a *LiChrospher® RP-18 HPLC column (5* µ particle size and *25 cm* × 4.6 mm *internal diameter)* using the mobile phase composed of 10 mM ammonium acetate: methanol in the ratio of 10:90 v/v at flow rate of 0.7ml/min. The mobile phase was filtered through 0.45 µ nylon filter and degassed by sonication prior to use. Column temperature was maintained at ambient and the run time for all the tests was set at 10 min. The column was equilibrated for 30-40 min with mobile phase prior to injection of the analyte. The volume of injection was 20µL. Detection wavelength was set at 277 nm since both the components showed reasonable absorbance at this wavelength. The optimum wavelength of detection was determined by scanning standard solutions of the analyte and internal standard from 200 to 400 nm using Jasco V-630 UV/VIS Spectrophotometer.

Preparation of Standard Solutions

Stock solutions of paliperidone and paracetamol were separately prepared by dissolving 10mg each in 100 ml methanol. Varying concentrations of paliperidone (1 to 5 µg mL^{-1}) were then prepared by taking 0.1 ml to 0.5 ml and diluting to 10 ml with mobile phase. Constant volume (0.1 ml) of paracetamol (1 µg mL^{-1}) was added as the internal standard (IS) before diluting with mobile phase. Each sample solution was injected six times into the HPLC column to find out the retention time.

Procedure for pharmaceutical formulation

Twenty tablets (Palido OD 3) were weighed, powdered and a powder mass equivalent to 3 mg of paliperidone was transferred to a 50 ml volumetric flask containing methanol. The mixture was then sonicated for 20 min to dissolve the material completely and centrifuged at 3,000 rpm for 5 min. An aliquot (0.5 ml) of supernatant solution was transferred to 10 ml volumetric flask, fixed volume (0.1 ml) of standard solution of IS was added and diluted to 10 ml using the mobile phase to get a solution containing 3 µg mL^{-1} of paliperidone and 1 µg mL^{-1} of IS. The resulting solution was analyzed by the proposed method. All determinations were carried out in six replicates.

Method Validation

The developed RP-HPLC method was validated as per ICH guidelines[8].

Linearity and range

Stock solution of paliperidone was suitably diluted with the mobile phase to get concentrations in the linear range of 1 to 5 µg mL^{-1}. Then 1 µg mL^{-1} of paracetamol (IS) was added to each dilution and 20 µl was injected into the column, the peak area and retention times were recorded.

The calibration curve for paliperidone was constructed by plotting the ratio of the peak area of paliperidone to the peak area of the internal standard (Y) against concentration (X) and linearity was evaluated by linear regression equation. The slope, intercept and correlation coefficient values were recorded. Each experiment was performed in six replicates.

Accuracy

Accuracy, which is the measure of closeness of the experimental value to the true value, was determined by standard addition method. To a pre-analyzed sample formulation a known quantity of standard was added at three levels (50, 100 and 150% of the assay concentration). A constant concentration (1 µg mL^{-1}) of internal standard was added to all the samples and the mixtures were analyzed by the developed method. The experiment was performed in six replicates. The % RSD and % recovery were calculated for all the concentrations.

Precision

System precision was evaluated by measuring the peak area and retention time of six determinations using standard paliperidone. Method Precision was determined in terms of repeatability (intra-day) and intermediate precision (inter-day) studies. Repeatability was performed by six repeated injections of homogeneous samples from single batch under the same experimental conditions on the same day. This experiment was performed at 3 different concentrations (2.5, 3 and 3.5 µg mL^{-1}) of the sample containing fixed concentration (1 µg mL^{-1}) of IS. Intermediate precision of the method was evaluated by performing the analysis on three different days for three different concentrations of paliperidone containing constant concentration of IS. Precision was expressed as %RSD.

Specificity

In the current study, purity of the peaks of paliperidone and IS were assessed by comparing the individual spectrum of standard and sample at three regions i.e. peak start, peak apex and peak end. Interferences due to common excipients present in tablet formulations were studied. Peak purity index was also noted.

Sensitivity

Sensitivity of the method was determined from limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were determined using the calibration curve.

LOD = 3.3xD/S and

LOQ = 10 x D/S,

where,

D = standard deviation of y intercept of regression line

S = slope of the calibration curve

Robustness

Robustness of the method was studied to evaluate the effect of small but deliberate variations of the chromatographic conditions on the method parameters. Robustness was determined by changing individually the flow rate (0.7±0.1ml/min), organic solvent (90±1%) and ionic strength of buffer (10mM±5 mM). Their effects on resolution (R_s), retention time (t_R), and tailing factor *(T*) were studied.

System suitability tests

The test was carried out by making six replicate injections of a standard solution containing 2.5 µg mL^{-1} and 1 µg mL^{-1} of PALI and IS, respectively, and analyzing each solute for their peak area, theoretical plates (*N*), resolution (R_s), tailing factor (*T*), capacity factor (*k'*), and asymmetric factor (A_s).

Stability Studies

A suitably diluted solution of paliperidone and IS in methanol was stored under laboratory bench conditions for 48 hours and under refrigeration (8±0.5ºC) upto 5 days. The solutions were then assayed by the proposed method.

RESULTS AND DISCUSSION

Method development

The proposed method describes a new RP-HPLC method for the determination of PALI in bulk and tablet dosage form using paracetamol as internal standard (IS) on a *LiChrospher® RP-18 HPLC column (5* µ particle size and *25 cm* × 4.6 mm *internal diameter)* with PDA detection at 277 nm. 10 mM ammonium acetate: methanol in the ratio of 10:90 (v/v) was employed as mobile phase at a flow rate of 0.7 ml/min. The final decision on mobile phase composition and flow rate was made on the basis of peak shape (peak area, asymmetry, tailing factor), baseline drift, time required for analysis, and cost of solvents. Various mobile phase composition were tried to achieve better separation and resolution between paliperidone and IS. Initially, water: methanol, water: acetonitrile, ammonium formate: methanol and ammonium acetate: methanol were tried in the ratio of 50:50 v/v. Relatively better peak shapes and resolution were observed with ammonium acetate: methanol. Various ratios and ionic strength of the later were tried to optimize separation between the analyte and IS. Increasing the flow rate to 1.5 ml/min has shown poor resolution between the analyte and IS. The retention times of IS and paliperidone was 3.97 and 5.3 min, respectively. Figure 2 shows the overlay spectrum of drug and IS. The typical

chromatogram of paliperidone (bulk) with IS is shown in Figure 3. Among the various internal standards tried, paracetamol was chosen as it eluted before the analyte with the peak showing good symmetry and resolution. In addition, paracetamol is less costly and easily available.

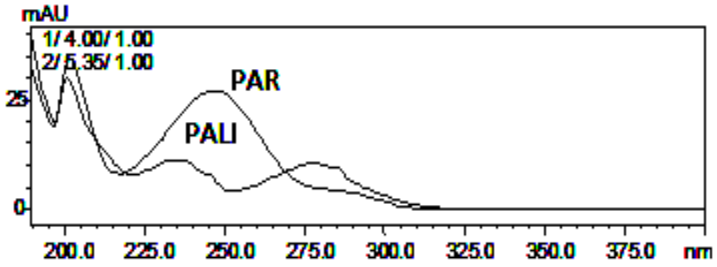

Figure 2: Overlay spectrum of paliperidone and internal standard

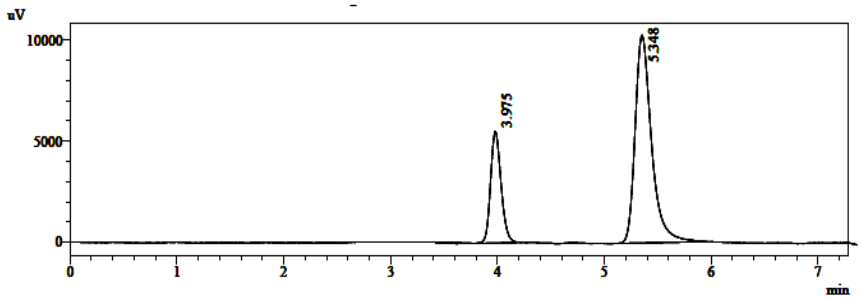

Figure 3: RP-HPLC chromatogram of standard solution of paliperidone (6 µg mL^{-1}) with internal standard (1 µg mL^{-1}) at 277 nm

Validation of the method

The developed method was validated in terms of specificity, linearity and range, accuracy, intra-day and inter-day precision, LOD, LOQ, robustness and system suitability by following the recommendations of ICH guidelines.

The specificity of the method was demonstrated by the fact that the UV spectra obtained for each analyte peak in the sample solution were matching with the corresponding standard analyte peaks. There appeared to be no interferences from excipients present in the formulation. Complete separation of the peaks of paliperidone and IS was observed with peak purity index close to 1.

The standard calibration curve was linear over the concentration range 1 – 5 µg mL^{-1} with a good correlation between analyte peak area and concentration (r=0.99946±0.00037), the corresponding linear regression equation being y = 0.8002x - 0.0646 (figure 4). In the linear range of 1-5 µg mL^{-1} LOD was found to be 0.3303 µg mL^{-1}and LOQ was 1 µg mL^{-1}, indicating the sensitivity of the method.

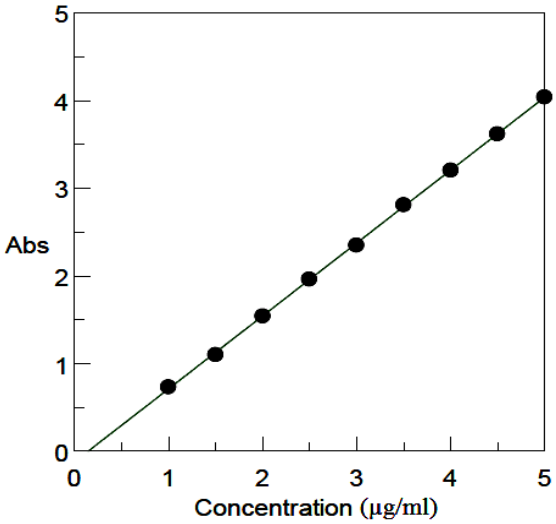

Figure 4: Linear graph of paliperidone

Recovery experiments demonstrated satisfactory accuracy with small relative standard deviations (< 0.691%). The mean percentage recovery ranged from 100.30±0.6931 to 98.92±0.5953. Recovery values indicate the proposed method for the determination of paliperidone from tablet dosage form is highly accurate. Details of results obtained are shown in table 1.

Table 1: Recovery data of paliperidone

Conc. of drug taken (µg/ml)	Conc. of standard added (µg/ml)	Mean Conc±SD[a]	Mean Recovery (%) ±SD	%RSD
1.012	0.5	1.5089±0.00578	100.3±0.6931	0.6910
1.012	1.00	2.0012±0.0059	98.93±0.5953	0.6018
1.012	1.5	2.5134±0.00667	100.1±0.4464	0.4460

[a] Mean ± SD, n = 6

The developed RP-HPLC method was found to be precise since the %RSD values met the acceptance criteria (<2) for both system precision and method precision studies. The %RSD of system precision was 0.8988 and that of repeatability (intra-day) and intermediate precision (inter-day) ranged from 0.1517 to 0.4549 and 0.2710 to 1.2759 respectively.

Small but deliberate changes in experimental conditions did not alter peak symmetry. There was no significant change in the retention time of paliperidone and IS during these experiments. The %RSD for each method parameter was calculated and was found to be <2. The results of robustness evaluation are presented in table 2. The low values of RSD indicated the robustness of the method. The regression characteristics, such as standard deviation of slope (*Sb*), the standard deviation of intercept (*Sa*), and correlation coefficient (*r*) for paliperidone by the proposed method and results of validation are summarized in table 3.

Table 2: Robustness evaluation of the method

Factor	Level	Asymmetric Factor	No. of theoretical plate	Resolution
Flow Rate (mL min^{-1})				
0.6	-0.1	1.372	7190	5.924
0.7	0	1.361	7198	5.888
0.8	+0.1	1.373	7201	5.858
%B of mobile phase(methanol)				
89	-1	1.351	7188	5.831
90	0	1.360	7198	5.888
91	+1	1.376	7194	5.758
Ionic strength of ammonium formate (mM)				
5	-5	1.342	7213	5.861
10	0	1.361	7198	5.888
15	+5	1.347	7186	5.593

Table 3: Regression parameters and validation parameters of paliperidone by RP-HPLC method

Parameter	Data
Concentration range	1-5 µg mL^{-1}
Slope[a]	0.80026±0.0321
Intercept[a]	-0.06461±0.0801
Correlation coefficient (r)	0.99946±0.00037
LOD (µg mL^{-1})	0.3303
LOD (µg mL^{-1})	1.000
Recovery (%)[a]	100.3±0.6931
Intra-day precision (RSD, %)[a]	0.15-0.45
Inter-day precision (RSD, %)[a]	0.27-1.27

[a] Mean ± SD, n = 6

Adequacy of the proposed RP-HPLC method for routine analysis of paliperidone was assured by system suitability tests. The capacity factor (*k'*) was found to be 12.23 and 16.85 for IS and drug respectively, signifying that both the IS and analyte peaks were well resolved with respect to the void volume. The tailing factor (*T*) of drug and IS peak reflects good peak symmetry. The resolution (*Rs*) for the principle peak and internal standard was found to be 5.888, showing good separation. The theoretical plate number (*N*) for drug and IS was found to be 7198 and 6014 respectively, demonstrating good column efficiency. The results of system suitability tests are shown in table 4.

The results of assay were in good compliance with the labeled claim, indicating the suitability of the method. The assay method proposed in the present study was found to be more reliable and accurate. The amount of paliperidone present in the commercial formulation (Palido OD 3) was

found to be 100.36±0.1664% of the labeled claim (table 5). Excipients present in the tablet did not interfere with the analyte peaks, as shown in figure 5.

Table 4: System suitability parameters of the proposed RP-HPLC method

Parameter	Peak	
	Drug	IS
Retention time (t_R)	5.348	3.975
Peak purity index	1	1
Asymmetry	1.361	1.256
Tailing factor (T)	1.383	1.267
Capacity factor (k')	16.83	12.25
Theoritical plates (N)	7198	
Selectivity (α)	1.345	
Resolution (R_s)	5.888	

Table 5: Assay of paliperidone in formulation by RP-HPLC method

Drug	Labeled amount (mg/tablet)	Amount found (mg)[a]	Assay (%)	RSD (%)
Paliperidone (Palido OD3)	3	3.0107±0.004912	100.36	0.16640

[a] Mean ± SD, n = 6

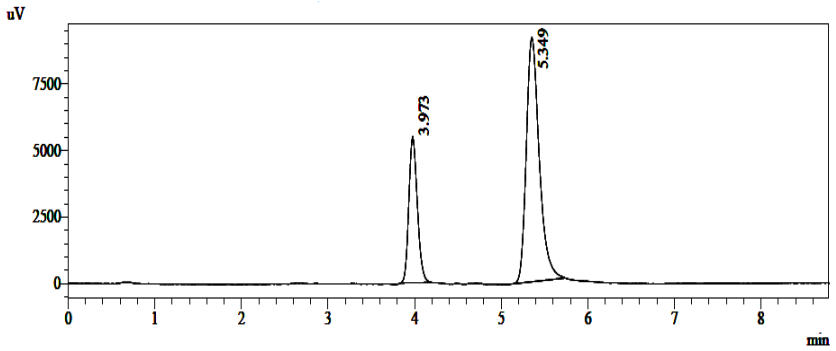

Figure 5: RP-HPLC chromatogram of formulation of paliperidone (3 µg mL^{-1}) with internal standard (1 µg mL^{-1}) at 277 nm

Solution stability studies demonstrated a good stability of standard and sample solutions for 40 hours when stored under ambient light at room temperature and upto 5 days under refrigeration, thereby, confirming the applicability of the method for routine analysis. The results indicating peak area, amount of drug remaining and %RSD are summarized in table 6 and 7.

A major advantage of the current method is that it shortens the overall assay time by eliminating the laborious and time consuming steps of mobile phase preparation that was used by the HPLC method reported in the literature (2). In addition, the current assay is superior in sensitivity compared with previously published HPLC method and the internal standard is readily available.

Table 6: Results of stability studies of paliperidone (Bench top)

Time (hrs)	Conc of drug		Ratio of peak area	% Amount	%RSD (n=6)
	Paliperidone	IS			
0	3	1	2.3741	100	0.4263
8			2.3504	99.01	0.9018
16			2.3487	98.93	0.6319
24			2.2682	95.54	0.3330
32			2.2319	94.01	0.7891
40			2.1607	91.02	1.2106
48			2.0232	85.23	1.3212

Table 7: Results of stability studies of paliperidone (Refrigeration)

Time (Days)	Conc of drug		Ratio of peak area	% Amount	%RSD (n=6)
	Paliperidone	IS			
0	3	1	2.3739	100	0.4368
1			2.3412	98.62	0.6521
2			2.3400	98.57	0.2889
3			2.3195	97.70	0.9812
4			2.2902	96.47	1.2911
5			2.2517	94.85	1.0365

CONCLUSION

The HPLC method developed was accurate, precise, reproducible and specific. The method is economical and utilizes a mobile phase which can be easily prepared. The method is less time consuming. All these merits make this method suitable for quantification of paliperidone in bulk and pharmaceutical dosage forms without interference.

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REFERENCES

1. Martindale: The Complete Drug Reference. 36th ed., Ed. S. C. Sweetman. London: Pharmaceutical Press, The Royal Pharmaceutical Society; 2009: 1028-29.
2. Mathew J, Joshi CK, Aghera J. Analytical methods for the estimation of paliperidone. Int J Res Pharm Sci 2011; 2: 158-161.
3. Danel C, Barthelemy C, Azarzar D, Robert H, Bonte JP, Odou P, Vaccher C. Analytical and semipreparative enantioseparation of 9-hydroxyrisperidone, the main metabolite of risperidone, using high-performance liquid chromatography and capillary electrophoresis. J Chromatography A 2007; 1163:228-236.
4. Sanjay AJ, Shashikant BL, Choudhari MC, Pavankumar VS, Saroj R, Bembalkar R, Vijayavitthal TM. Stress degradation behaviour of paliperidone, an antipsychotic drug,

and development of suitable stability-Indicating RP-LC method. Chromatography Res. Int 2011; 3: 1-10.

5. Rashmin BP, Mrunali RP, Kashyap KB, Bharat GP. HPTLC method development and validation: Quantification of paliperidone in formulations and *in vitro* release study. Anal. Methods 2010; 2: 525-531.
6. Tan MS, Breau A, Bugge CJ. Quantitative determination of paliperidone in human plasma by LC-MS-MS. Available from: http://www.wwctrials.com/UserFiles/Docs/Paliperidone.pdf; accessed on 30-03-2011.
7. Snyder LR, Kirkland JJ and Glajch JL. Practical HPLC Method Development. 2nd ed., New York: John Wiley and Sons; 1997: 184-85.
8. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline – Validation of Analytical Procedures:Text and Methodology Q2(R1), Current Step 4 version. London; 2005.