LCMS METHOD DEVLOPMENT AND VALIDATION OF PALIPERIDONE IN FORMULATION DOSAGE

P.V.V. Satyanarayana*, Alavala Siva Madhavi

Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India,
Contact Mail id: sivamadhavi@rocketmail.com

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Paliperidone tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitril: Water 60: 10: 30 v/v, (P^H 4.8). The UV detection wavelength was 237nm and 20µl sample was injected. The retention time for Paliperidone was 4.8min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Paliperidone tablet dosage form and bulk drug.
Key Words: Paliperidone, RP-HPLC, UV detection, recovery, precise, 237nm

INTRODUCTION

Paliperidone (trade name Invega), also known as 9-hydroxyrisperidone, is a dopamine antagonist of the atypical antipsychoticclass of medications.paliperidone is theprimary active metabolite of the older antipsychotic resperidone.[1] . Paliperidone is used to treat mania and at lower doses as maintenance for bipolar disorder. It is also used for schizophrenia and schizoaffective disorder.

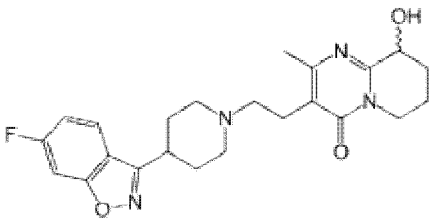

Figure.1

A few common paliperidone side effects include drowsiness, headaches, and a rapid heart rate. Less common side effects of this medicine (occurring in less than 5 percent of people) can include fatigue, shakiness, and dry mouth. Certain side effects of paliperidone are more serious and should be reported to your healthcare provider. These more serious side effects can include large or rapid weight gain, anxiety, and very severe constipation.

EXPERIMENTAL

Materials

Working standard of Paliperidone was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC [6-11] system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Paliperidone were scanned in the range of 200 -400 nm against mobile phase as a blank. Paliperidone showed maximum absorbance at 237nm. So the wavelength selected for the determination of Paliperidone was 237nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of PALIPERIDONE an isocratic PEAK HPLC instrument with Chromosil C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Acetonitril: Water 60: 10: 30 v/v. Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 237nm with 10min runtime.

Standard and sample solutions

A 10 mg amount of Paliperidone reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (INVEGA) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Paliperidone was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 20ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In allcases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Api concentrations	20ppm
Mobile phase	Methanol: Acetonitril: Water 60: 10: 30 v/v
Wavelength	237nm
Column	C_{18} Column
PH	4.8
Concentration	20ppm
Retention Time	4.8min
Run Time	10min
Area	152200
Th. Plates	8265
Tailing Factor	1.11
Pump Pressure	9.0 Mpa

Table.1 System suitability parameters of PALIPERIDONE

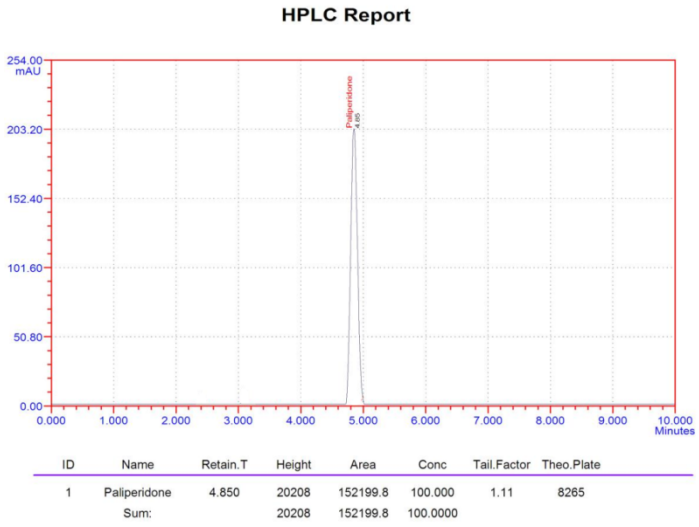

Figure.2: Standard chromatogram of Paliperidone

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 5,10,15,20and25for Paliperidone. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 7452+ 3739x (r. 0.9995) Linearity values can shown in Table: 2

S.No	Concentration (µg/ml)	Area
1	5	43511
2	10	79547
3	15	116993
4	20	152200
5	25	191445
6	30	224903
	Slope Intercept Cc	7451.643 3739.5 0.999586

Table.2: Linearity results of Paliperidone

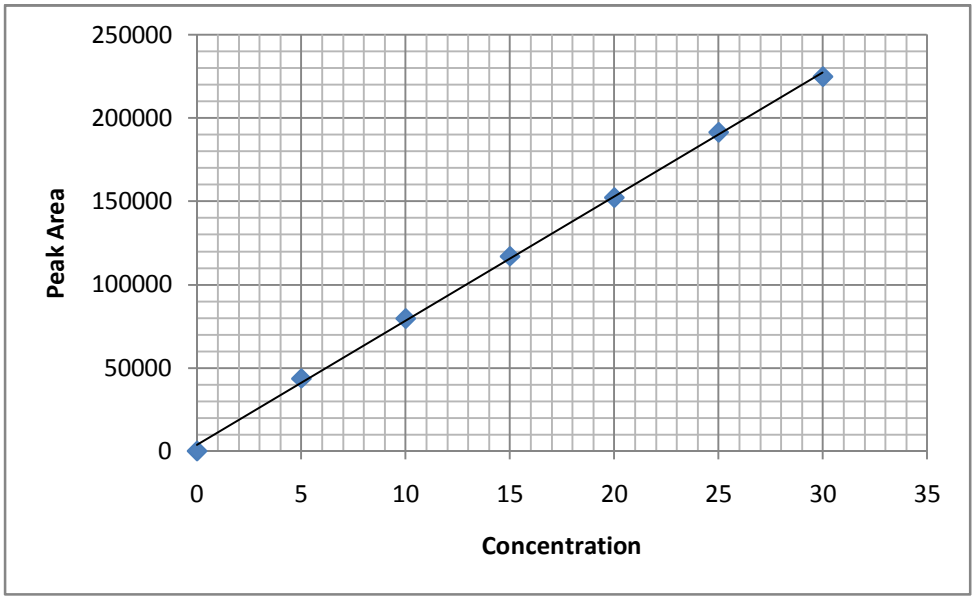

Figure 3: Calibration curve of Paliperidone

Precision

To study precision, six replicate standard solutions of Paliperidone (20ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Table 3: Intraday Precision Results for Paliperidone:

Sample (µg/ml)	Area
1	152200
2	152709
3	152010
4	151415
5	150354
6	156596
RSD	1.40352

Table 4: Inter day Precision results of Paliperidone

Sample (µg/ml)	Area
1	157875
2	156769
3	159241
4	156974
5	156023
6	157066
RSD	0.705987

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.175ppm dilution Peak was not clearly observed, based on which 0.175ppm is considered as Limit of Detection and Limit of Quantification is 0.6ppm.

Table.5: LOD and LOQ results of Paliperidone

Parameter	**Measured Value**
Limit of Quantification	0.6ppm
Limit of Detection	0.175ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Paliperidone at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 6.

S.NO	**Parameter**	**change**	**area**	**%of change**
1	**standard**	--------	152200	--------
2	**Mp**	Methanol: Acetonitril: Water 55: 10: 35 v/v	155087	1.896846
3		Methanol: Acetonitril: Water 65: 10: 25 v/v	151951	-0.1636
4	**pH**	**5.0**	154083	1.237188
5		**4.6**	152242	0.027595
6	**wl**	**239**	154802	1.709593
7		**235**	154138	1.273325

Table.6: Robustness results of Paliperidone

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

SAMPLE	CONC (PPM)	INJECTION NO	PEAKS AREA	R.S.D (Acceptance criteria ≤ 2.0%)
Paliperidone	20	1	154083	0.731532
		2	154251	
		3	155767	
		4	153919	
		5	152920	
		6	155811	

Table.7: Ruggednessresults of Paliperidone

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 15ppm, 20ppm, 25ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.64 to 101.76 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.8

% Recovery	Paliperidone				
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	10	5	15	14.95192	99.67947
50%	10	5	15	14.7973	98.64864
50%	10	5	15	14.90333	99.35552
100%	10	10	20	19.9138	99.56899
100%	10	10	20	20.19737	100.9869
100%	10	10	20	20.28936	101.4468
150%	10	15	25	24.91734	99.66936
150%	10	15	25	25.3659	101.4636
150%	10	15	25	25.44177	101.7671

Table.8: Recovery results of Paliperidone

Formulation	**Dosage**	**Concentration**	**Amount found**	**% Assay**
Invega	3mg	20ppm	19.80	99.00

Table.9: Formulation Analysis

CONCLUSION

The proposed method for the assay of Paliperidone in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could

effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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