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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF PIRIBEDIL IN BULK DRUG

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ABSTRACT

Objective: A simple, precise, fast, economic, accurate, robust, and stability indicating isocratic reverse phase high-performance liquid chromatographic method was developed for the analysis of Piribedil.

Method: The chromatographic conditions were standardized using Unisol C-18 (4.6 × 150 mm × 3.0 µ) column with UV detection at 244 nm, and the mobile phase composed of methanol:acetate buffer-pH 5.0 (85:15, v/v).

Results: The retention time of Piribedil was found to be 3.4 minutes. The calibration curve was linear with correlation coefficient of 0.999 over a concentration range of 20-100 µg/ml with linear regression equationy=74,69,224.37x–39,46,924.90. The limit of detection and limit of quantitation were found to be 0.04 and 0.4 µg/ml, respectively.

Conclusion: The proposed method has been validated according to the ICH guidelines. Piribedil was subjected to stress conditions including acidic, alkaline, oxidation, photolysis, and thermal degradation. Piribedil is more sensitive to photolytic stress. There are no interfering peaks from degradation products at analyte retention time, and thus the method is specific for the estimation of Piribedil in the presence of degradation products. Thus, the proposed method can be successfully applied in the routine quality control and stability samples of Piribedil in bulk drug.

Keywords: Piribedil, Validation, Stability indicating, Reverse phase high-performance liquid chromatographic.

INTRODUCTION

Piribedil chemically 2-[4-(benzo[1,3]dioxol-5-ylmethyl)piperazin-1-yl]pyrimidine (Fig. 1), is an antiparkinsonian agent and piperazine derivative which acts as D2 and D3 receptor agonist. It also has alpha-2 (α_2) adrenergic antagonist properties [1]. Piribedil is a non-ergot dopamine D2 agonist that stimulates cerebral and peripheral dopamine receptors and corrects dopamine deficiency. It is used largely, as an adjunct to levodopa therapy (80-140 mg daily) or as monotherapy (150-250 mg daily in divided doses) in the treatment of Parkinson's disease [2], which is a neurodegenerative disorder that affects dopaminergic neurons and causes symptoms including muscle rigidity, tremors and changes in speech and gait. After diagnosis, treatments can help relieve symptoms, but there is no cure [3].

Only a few studies were conducted to determine Piribedil in human serum, urine, and pharmaceutical dosage form by LC-DAD [4] and its p-hydroxylated, catechol, and N-oxide metabolites in plasma by high-performance liquid chromatographic (HPLC) [5]. Hence, so far no single stability indicating method (SIM) by HPLC has been reported for the estimation of Piribedil in bulk drug. The objective of the present work was to develop a stability indicating reverse phase-HPLC method for the estimation of Piribedil in bulk form and validate it according to ICH guidelines. The objective of the present work was to develop a stability indicating reverse phase-HPLC method for the estimation of Piribedil in bulk form and validate it according to ICH guidelines [6-9].

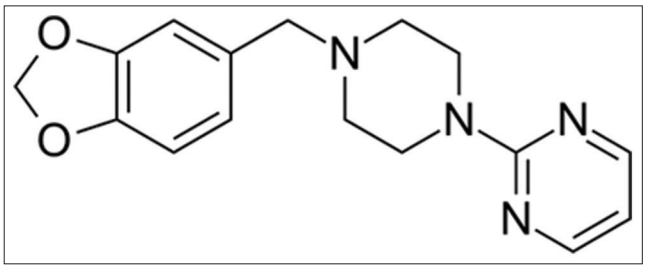

Fig. 1: Structure of Piribedil

METHODS

Instrumentation and chromatograph

The chromatograph used was Agilent 1260 infinity series equipped with a 1260 quaternary pump, 1260 infinity auto sampler unit, 1290 infinity PDA detector with OpenLab CDS EZChrom Ed. workstation. All weighings for analysis were performed on Mettler-Toledo analytical balance Me-204.

Chemicals

The working standard used was Piribedil, which was supplied by Dr. Reddy's laboratories Pvt Ltd., Hyderabad, as a gift sample. Methanol, ammonium acetate, hydrochloric acid, sodium hydroxide, hydrogen peroxide and HPLC grade water were procured from Merck Speciality Pvt. Ltd., Mumbai, Maharashtra, India, and Glacial acetic acid from Qualikems Fine Chem Pvt. Ltd., Vadodara, India.

Preparation of standard and sample solutions

Accurately weighed and transferred 100 mg of Piribedil working standard into a 100 ml clean and dry volumetric flask. Dissolved it in 10 ml of methanol by sonication and the volume was made with methanol to get 1000 mg/ml. From the above solution, 10 ml was pipetted out and transferred into a 100 ml clean and dry volumetric flask and volume were made with diluent (60:40v/v – methanol:water) to get 100 mg/ml. Sample solutions were prepared by appropriate dilution of standard solutions with the diluent.

Preparation of buffer

Accurately weighed quantity (1.54 g) of Ammonium acetate was transferred into a 1000 mL volumetric flask. About 900 mL of HPLC grade water was added and degased by subjecting to sonication for 5 minutes and final volume was made with double distilled water to get 20 millimolar solution. The pH of the solution was adjusted to 5.0 with glacial acetic acid. The buffer was filtered through 0.45 µ filter paper under vacuum before use.

Method development and optimization

The dilutions were chromatographed by a set of conditions on Agilent Infinity 1260 series. A mixture of methanol: acetate buffer (pH 5.0) 85:15 v/v was used as a mobile phase for the elution of Piribedil on Unisol C-18 (4.6 × 150 mm × 3.0 µ) at 1 ml/minute flow rate and at 20°C column temperature. Piribedil was eluted at 3.4 minutes with a run time of 5 minutes, and detection was performed by photodiode array (PDA) detector at 244 nm.

Method validation

The method was validated for analytical procedures according to ICH guidelines to determine the system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

System suitability

System suitability parameters were analyzed to check the system performance consistency. 20 µl of standard solution (60 µg/ml) was injected in six replicates under optimized chromatographic conditions, and column performance characteristics such as tailing factor, the number of theoretical plates, retention time and area were observed. Percentage of relative standard deviation (%RSD) were calculated and listed in Table 1.

Forced degradation studies/specificity

Stress testing of the drug substance can help identify the likely degradation products, which can, in turn, help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating the power of the analytical procedures used [6].

Stress testing was carried out on the standard solution of Piribedil having the concentration of 10 µg/ml by exposing to acidic, basic, oxidative, thermal and photolytic stress conditions.

Acid degradation was carried out by using 1M HCl and alkaline degradation by 1M NaOH for 24 hrs at 60°C. After cooling, the solutions were neutralized and diluted accordingly with mobile phase.

Oxidative stress testing was done using 3% H_2O_2 at 60°C for 24 hrs. The sample solution was cooled and diluted.

For thermal stress testing, the drug solution (10 µg/ml) was heated in a hot air oven at 80°C for 48 hrs and the solution was cooled and used.

Photostability testing was demonstrated by exposing the drug solution (10 µg/ml) to direct sunlight for 48 hrs.

All the above solutions including diluent and blank/mobile phase were filtered before injection and analyzed to evaluate the stability indicating properties and specificity of the method.

The results were shown in Figs. 2-5 and tabulated in Table 2.

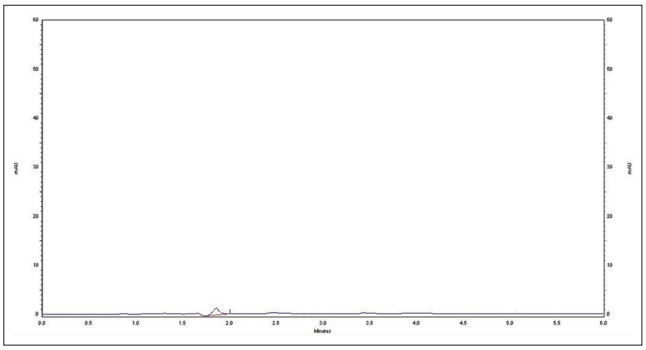

Fig. 2: Representative chromatogram of diluent

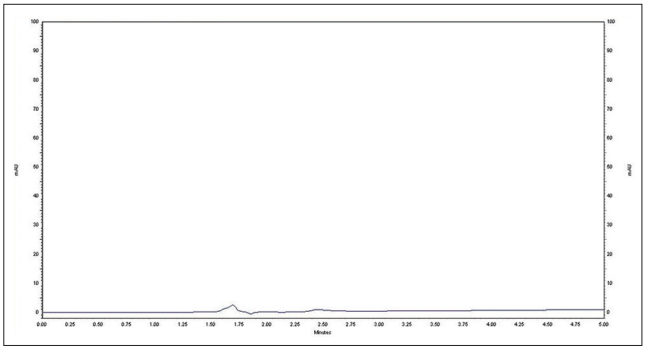

Fig. 3: Representative chromatogram of blank/mobile phase

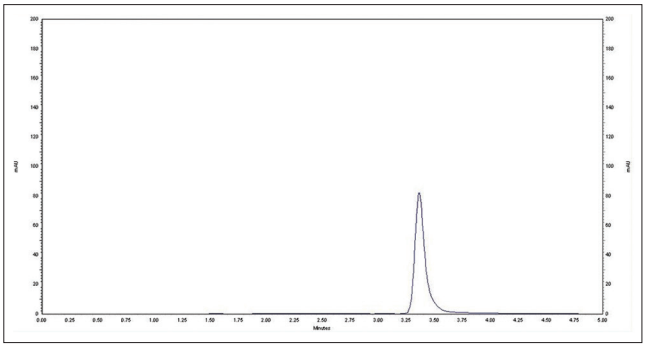

Fig. 4: Representative chromatogram of standard (10 µg/ml)

Table 1: System suitability parameters

Injection number	Response (area) of Piribedil	Theoretical plates	Tailing factor	Rt
1	440949836	11990	1.05	3.43
2	442189400	11978	1.04	3.44
3	441459973	11897	1.05	3.44
4	442127725	11901	1.03	3.44
5	441333462	11992	1.05	3.44
6	441609209	11945	1.05	3.44
Mean	441611600833	11950	1.045	3.438
Standard deviation	477184.79	43.316	0.008366	0.00408
% RSD	0.11	0.36	0.80	0.12
Acceptance criteria	NMT 2.0	NLT 2000	NMT 2.0	

Linearity

Linearity was evaluated by analyzing different concentrations of the standard solutions of Piribedil. The response was a linear function of concentration over a range of 20-100 µg/ml which was used as working a range of the method. Peak area and concentration were subjected to statistical methods to calculate the calibration equation and correlation coefficient, which were shown in Table 3 and Fig. 6.

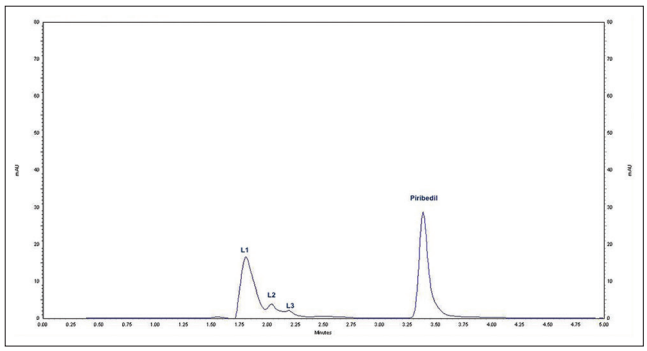

Fig. 5: Representative chromatogram of photolytic degradation

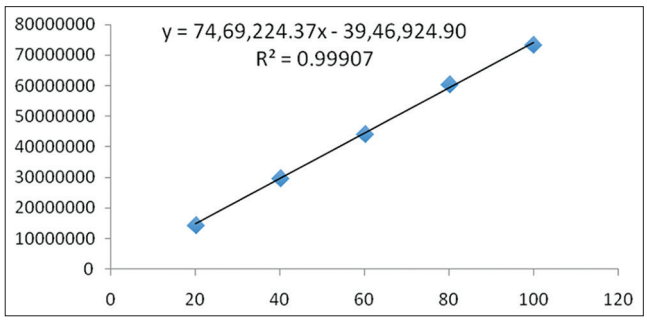

Fig. 6: Linearity chart

Table 2: Summary of forced degradation studies

Stress condition	Time	Assay of active substance %	Remarks
Acid hydrolysis (1M HCL)	24 hrs	99.37	No degradation
Base Hydrolysis (1M NaOH	24 hrs	98.92	No degradation
Oxidation (3% H_2O_2)	24 hrs	99.94	No degradation
Thermal (80°C)	48 hrs	99.12	No degradation
Photolytic degradation	48 hrs	81.76	Degraded

Table 3: Linearity of detector response

Concentration (µg/ml)	Area response of Piribedil
20	142777358
40	295840522
60	442458842
80	604671815
100	735284148

Accuracy

Accuracy was determined by recovery method at three different concentration levels, i.e., 50%, 100% and 150% by using standard addition method and injecting each concentration in triplicate. The results are tabulated in Table 4.

Precision

Intra- (repeatability) and inter-day (intermediate precision) precision studies for Piribedil were done by injecting the standard solution (60 µg/ml) on the same day and different days. This was performed with replicates of the standard solution under the same experimental conditions. The results are shown in Tables 5 and 6.

LOD and LOQ

LOD and LOQ were determined by injecting various concentrations of standard solutions ranging from 0.03 to 0.1 µg/ml, and signal to noise (s/n) ratio of the peaks were recorded, prior to this the mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained.

Robustness

The robustness of the analytical procedure was done by injecting the standard solution in six replicates and recording the system suitability parameters after the introduction of small deliberate changes in flow

Table 5: Intra day precision studies

Injection number	Area	Observation	Acceptance criteria
1	441964329	Mean	%RSD not more than 2.0
2	440815237	441561550.83	
3	441493682	SD	
4	442012613	559972.10	
5	440976134	%RSD	
6	442107310	0.13	

SD: Standard deviation, RSD: Relative standard deviation

Table 6: Inter day precision studies

Injection number	Area	Observation	Acceptance criteria
1	441328151	Mean	%RSD not more than 2.0
2	442013210	441685091.0	
3	441757824	SD	
4	440821310	514370.75	
5	442107632	%RSD	
6	442082419	0.12	

SD: Standard deviation, RSD: Relative standard deviation

Table 4: Recovery studies of Piribedil

Serial number	% Level	Spiked amount (µg/ml)	Standard amount (µg/ml)	Amount found (µg/ml)	% Recovery	Mean recovery
1	50	20	40	59.89	99.82	Mean=99.703
		20	40	59.70	99.51	SD=0.168
		20	40	59.86	99.78	RSD=0.17%
2	100	40	40	79.71	99.64	Mean=99.70
		40	40	79.87	99.84	SD=0.121
		40	40	79.69	99.62	RSD=0.12%
3	150	60	40	100.01	100.01	Mean=99.85
		60	40	99.71	99.71	SD=0.156
		60	40	99.83	99.83	RSD=0.15%

RSD: Relative standard deviation, RT: Retention time

Table 7: Robustness data or Piribedil

Parameter	Change level	RT	% RSD of peak area	Tailing factor	USP plate count
Flow rate (±0.2 ml/minute)	0.8 ml/minute	4.27	0.09	1.07	14022
	1.2 ml/minute	2.85	0.17	1.00	10238
Column temperature variation	15°C	3.44	0.09	1.07	15062
	25°C	3.41	0.14	1.01	10572
Mobile phase composition	70:30	6.22	0.19	1.01	9872
	80:20	4.33	0.17	1.01	10672
Buffer pH	pH 4.5	3.48	0.10	1.04	15938
	pH 5.5	3.42	0.13	1.07	10879

RSD: Relative standard deviation, RT: Retention time

Table 8: Solution stability data

Serial number	Sampling time	Area obtained	Amount found	% Assay
1	Standard (fresh solution)	442312107	60	100
2	12 hrs	441300392	59.86	99.76
3	24 hrs	440827185	59.79	99.65
4	36 hrs	441732147	59.92	99.86
5	48 hrs	442148362	59.97	99.95

rate (±0.2 ml), temperature (±5°C), mobile phase composition and buffer pH (±0.5). The results obtained are shown in Table 7.

Solution stability study

Stability in solution was evaluated for the standard solution. The solutions were stored at ambient temperature, without the protection of light and tested after 12, 24, 36, and 48 hrs. The responses for the aged solution were evaluated by comparison with freshly prepared solution. The results are shown in Table 8.

RESULTS AND DISCUSSION

System sutablity studies

The RSD values for system suitability parameters like retention time, tailing factor, and theoretical plate count were less than 2% indicating that the present conditions were suitable for the analysis of Piribedil.

Specificity

Degradation was not observed when the drug solutions were subjected to stress conditions like acid hydrolysis, base hydrolysis, oxidation, and heat.

Approximately Piribedil showed moderate degradation of about 18% (sum of 3 degradants L1, L2, L3) after exposure to sunlight for 48 hrs. The UV spectra of pure Piribedil and undegraded Piribedil were compared and found to be similar with regard to λ_{max} and appearance. The absence of co-eluting peak and well separation of degraded products from parent peak of Piribedil indicated that the developed method is specific for the estimation of Piribedil in the presence of degradation products.

Linearity

The response was found to be linear in the concentration range of 20-100 µg/ml and the correlation coefficient was found to be 0.999.

Accuracy

The values of percentage recoveries of the three concentrations were within the limits of 100±2% and the mean percentage recovery values close to 100%, and low %RSD values indicate the high accuracy of the analytical method.

Precision

The low values of the %RSD showed that the repeatability and intermediate precision of the method were within the acceptable value.

LOD and LOQ

The LOD value was found at 0.04 µg/ml concentration where the signal to noise ratio was found to be 3:1 and the LOQ value was found at 0.14 µg/ml with a signal to noise ratio of 10:1.

Robustness

The low levels of %RSD indicate that the method is robust.

Solution stability

During the study of the stability of stored solutions of standards preparation for assay determination, the solutions were found to be stable for up to 48 hrs. Assay values obtained after 48 hrs were identical with the initial value without a measurable loss.

CONCLUSION

A new stability indicating isocratic reverse phase liquid chromatographic method was developed and validated according to the ICH guidelines. It was simple, fast, precise, accurate, robust and economic. Degradation products resulting from the stress studies were well resolved from the analyte peak with a significant difference in Rt values.

Even though no attempt has been made for the identification of the degraded products, the proposed method can be used as a SIM for determination of Piribedil in bulk drug.

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