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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF ORGANIC IMPURITIES IN PIRIBEDIL PROLONGED-RELEASE TABLETS

K. Thorat[1], M. Bhosale[1], R. Khaire[2,✉], S. Magar[1], M. Sonawane[1], and N. Wani[1]

[1]Pravara Rural College of Pharmacy, At/Po-Pravaranagar, Tal-Rahata, Dist Ahilyanagar, Pin-413736, Maharashtra, India

[2]PRES's College of Pharmacy (For Women), At/Po-Chincholi, Tal-Sinnar, Dist Nashik, Pin-422102,Maharashtra, India

✉Corresponding author: rahuldkhaire@gmail.com

ABSTRACT

A method was developed with a Zodiac Phenyl C18 (250mm× 4.6mm,5 µm) column, comprising Phosphate buffer at pH 2.8 as a mobile phase, methanol, and Acetonitrile. Detection was carried out at 238nm and a flow rate of 1.0 ml/min under gradient conditions. Critical parameters, including retention time, peak shape, baseline stability, and separation of placebo interference, were optimised through multiple trials. Using this method, the total impurity found in the spike sample was 0.48%, and in the test sample, 0.084%. Results confirmed the method's ability to detect impurities at levels as low as 0.1 ppm, with excellent linearity ($r^2 > 0.998$) across the tested range. The method successfully resolved all specified and unspecified impurities with satisfactory peak shapes and reproducibility as per SDG-9: Industry, innovation and infrastructure.

Keywords: Piribedil, RP-HPLC, Organic Impurities, Method Development, Validation, ICH Guidelines, Prolonged Release Tablets, Dopamine Agonist

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INTRODUCTION

As seen in Fig.-1, piribedil is a synthetic non-ergot dopamine receptor agonist that directly activates the limbic route D3 receptor, postsynaptic D2 receptor, and nigrostriatal pathway D1 receptor.[1] It blocks α_2 A/C receptors, enhancing motor control, mood, and cognition by increasing cortical acetylcholine and norepinephrine release. It improves motor symptoms (tremor, rigidity) and non-motor aspects like attention, memory, and mood in Parkinson's patients.[2]

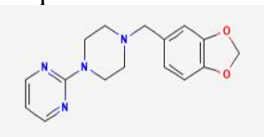


Fig.-1:Structure of Piribedil

Molecular Weight: 298.34g/mol[3]; Dosage form: Tablets; Category of drug: Nonergot Dopamine Agonist; Appearance: Off-white to pale yellow crystalline powder

EXPERIMENTAL

Establishment and authentication of chromatographic conditions for the quantification of Piribedil and its impurities.

Wavelength Selection

The UV spectrum of Piribedil at 50 µg/ml in methanol: 25mM potassium phosphate buffer (60:40v/v) was obtained by scanning between 400 and 200 nm.[5]

Preparation of API Sample Solution (1000ppm)

50.02 mg of Piribedil API was precisely weighed and then put into a 50 mL volumetric flask.

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Preparation of Standard Solution
Weigh 20 mg of KSM-01(piperonal), KSM-02(piperazinyl piperazine), piribedil and 30 mg of PIR stage-1 working reference standard each; transfer to 100ml flask and make up the volume by adding diluent. Take 5 mL of the solution and transfer it to a 50 mL flask to make up the volume.[6]

Preparation of Impurity-Spiked Sample Solution
Crushed the tablets into fine powder with the help of a mortar and pestle, weighed the crushed powder equivalent to 20mg of Piribedil and then transferred it to a 20 mL volumetric flask. Added about 14mL of diluent and stirred the sample solution. Then, the impurity stock solution was added.[7]

Preparation of Impurities
Weigh all Impurity 1,2,3 and 4 as 0.5mg and then it was dissolved in 10ml solvent as Dichloromethane, Dichloromethane, DMSO and Methanol respectively. Pipette out 1 mL of each impurity and further dilute with Diluent in a 25 mL volumetric flask.[8]

Procedure
Inject 20 µl of the sensitivity solution, test solution, standard solution (in six duplicates), placebo solution, and blank (diluent) separately into the chromatographic apparatus. Keep track of the chromatograms and calculate the peak area counts for each peak.[9] Ignore the other unidentified degradation product whose area is smaller than the sensitivity solution's area. Look for any extraneous peaks in the chromatograms of the blank and placebo and ignore them in the chromatogram of the test solution.[10]

RESULTS AND DISCUSSION

Development of Analytical Method
All peaks were well separated, and the baseline was smooth. A better peak shape of Piribedil was observed.

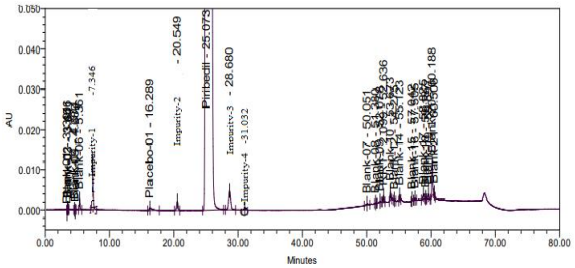


Fig.-2: Optimized Chromatogram for Piribedil

Validation of RP-HPLC Method
Filtration Study
As shown in Table-1 Area of 0.45µ PVDF filter had matched to Centrifuge sample, hence used 0.45µ PVDF filter.

Table-1: Filter Suitability Study

Peak Name	Area				
	Centrifuge Sample	0.45µ Millipore PVDF	0.45µ VWR PVDF	0.45µ + PTFE (mdi)	0.45µ Nylon (VWR)
Impurity-1 (RRT-0.29)	154927	155470	155957	157849	154403
Impurity-2 (RRT-0.82)	30425	30220	29986	30665	30156
Impurity-3 (RRT-1.14)	75411	74668	75601	75171	74481
Impurity-4 (RRT-1.24)	110695	110234	109813	112601	111281

Linearity Study
As seen in Table-2, Table-3, and the graph in Fig.-3, nine levels of linearity were carried out between 1% and 200% of the working concentration.

Table-2: Linearity Study of Piribedil

Level No.	%w.r.t.standard concentration	Concentrations(mcg/ml)	Average peak area
1	1	0.010	910
2	2	0.021	3084
3	10	0.103	11059
4	25	0.275	29430
5	50	0.515	51932
6	85	0.858	84388
7	100	1.030	11158
8	150	1.545	169138
9	200	2.060	224761
		Slope	109008.8130
		Intercept:	-1502.1725
		Correlation coefficient	0.99916

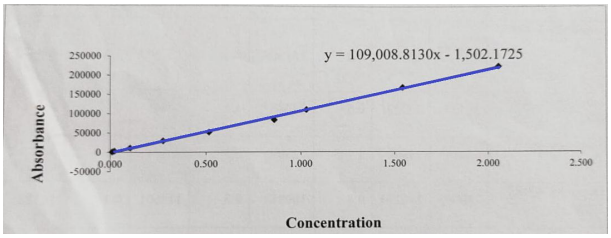


Fig.-3: Linearity Graph of Piribedil

Table-3: Results of Linearity

S. No.	Drug	Slope	Regression coefficient
1	Piribedil	109008.813	851.2978
2	Impurity-1	134018.72	665.1632
3	Impurity-2	29114.46	453.1398951
4	Impurity-3	81558.5132	579.00145
5	Impurity-4	95237.5752	984.8365

LOD and LOQ

The LOD and LOQ were determined using the formula based on the values in Table-4.

Table-4: Result LOQ and LOD

No.	Drug	Slope	Residual SD	LOD (mcg/ml)	LOD %	LOQ (mcg/ml)	LOQ %
1	Piribedil	109008.813	851.2978	0.005	0.0258	0.078	0.016
2	Impurity-1	134018.72	665.1632	0.003	0.0164	0.050	0.010
3	Impurity-2	29114.46	453.1398951	0.010	0.0514	0.156	0.031
4	Impurity-3	81558.5132	579.00145	0.005	0.0234	0.071	0.014
5	Impurity-4	95237.5752	984.8365	0.007	0.0341	0.103	0.021

Accuracy (Recovery %)

Recovery of the impurity spike in the placebo is in the range of 0.02% to 0.4% of the sample concentration calculated.

Precision

Standard deviation or relative standard deviation are common ways to express an analytical method's precision. On the test sample, precision was carried out.

Repeatability

Weigh at least 20 tablets to calculate average weight. After weighing and transferring the crushed tablet powder equivalent to 50 mg of piribedil into a 50 mL volumetric flask, add 30 mL of methanol, and

sonicate for 30 minutes at roughly 10°C while shaking occasionally. Then add 10ml of buffer, mix and allow the solution to cool at room temperature for approximately 20 minutes. Filter the solution via 0.45µ PVDF (Make: Avantor, provided by VWR, 30mm) syringe filter, discarding the first 4ml of filtrate. The Repeatability data results are displayed in Table-5.

Table-5: Results of Repeatability

S. No.	Sample	Test sample	Total Impurities %
1	Sample-1	50.1mg	0.34%
2	Sample-2	50.2mg	0.39%
3	Sample-3	50mg	0.41%
4	Sample-4	49.99mg	0.38%
5	Sample-5	49.50mg	0.49%
6	Sample-6	50.01mg	0.48%

Robustness

Following changes made under Robustness:

(a) Changes made in the mobile phase flow +/-0.2ml

(b) Changes made in the column temperature by +/-5C

(c) Changes made in the mobile phase (Buffer) +/- 0.2units

The robustness investigation was carried out by estimating the retention time, area, and impurity of the analyte, as indicated in Table-6.

Table-6: Results of Robustness

Peak name	As such	-flow	+flow	- temp	+ temp	- pH	+ pH
Impurity-1	7.346	8.094	6.692	7.671	6.985	7.294	7.446
Impurity-2	20.549	22.037	19.314	21.586	19.583	20.651	20.643
Piribedil	25.073	26.703	23.920	26.553	23.899	25.061	25.730
Impurity-3	28.680	30.695	26.980	30.401	27.064	28.833	28.844
Impurity-4	31.032	32.486	29.247	32.573	28.924	30.590	31.095

Method Development Strategy

The suitable chromatographic conditions were selected to achieve separation of Piribedil and its related impurities, including four key impurities identified during manufacturing and stability studies: Impurity-1 (2-(4-(Chloromethyl)piperazin-1-yl)pyrimidine), Impurity-2 (1-Piperonylpiperazine), Impurity-3 (1,4-Di(pyridin-2-yl)piperazine), and Impurity-4 (N-oxide derivative). The initial trials based on the Drug Master File (DMF) method were inadequate due to peak interference from excipients (placebo) and unacceptable baseline instability.[12] Extensive optimisation trials were subsequently conducted involving variations in column chemistry (e.g., C18, phenyl), composition of the mobile phase, pH, rate of flow, and gradient profile.

The mobile phase with a pH 2.8 buffer solution and an organic mixture of methanol and acetonitrile (in gradient mode) demonstrated optimal selectivity and peak performance. The low pH was essential to enhance retention and sharpen the peak shape of basic compounds like Piribedil and its piperazine-containing impurities. Detection was performed at 238 nm, corresponding to the λmax of Piribedil, thereby enhancing sensitivity without compromising baseline stability.[13]

Suitability and Specificity

Theoretical plates (N) for Piribedil were consistently less than 2000.

Tailing factor remained within acceptable limits.

Linearity and Range

Linearity was checked across 0.01–2.0 µg/mL, equivalent to 1–200% of target impurity concentration. A total of nine levels were analysed for each impurity, yielding $R^2 > 0.998$ for all four impurities. This strong linear relationship validates the method's quantitative reliability over a wide analytical range, which is crucial for detecting trace impurities as well as higher degradation levels in stability studies.

Accuracy and Recovery

The Recovery studies were conducted by spiking known amounts of each impurity into the placebo matrix at four concentration levels (10%, 50%, 100%, and 200% of target impurity level). Each level was analysed in triplicate. The average recoveries for all impurities ranged from 95.6% to 109.3%. The RSD values were consistently below 5%, indicating low variability. These results demonstrate that the method is not only accurate across the full quantitation range but also robust in the presence of matrix effects, which often pose a challenge in pharmaceutical formulations.

Precision and Repeatability

At two levels, the developed method's precision was evaluated:
Intraday Precision: Six replicate preparations of the Piribedil sample were analysed on the same day.
Inter-day and analyst variability: The test was repeated on different days with different analysts using separate instruments. The %RSD of impurity content across all replicates was < 2%, confirming that the method yields reproducible results irrespective of operational variability. These findings meet the ICH criteria for acceptable precision in quantitative impurity methods.

Robustness

Robustness was assessed by deliberately varying:
Change within rate of flow +/- 0.2ml/min
Change within temp of column +/- 5°C
Change in pH of mobile phase +/- 0.2units

LOD and LOQ

LOD and LOQ for the four impurities were found to be within 003–0.06µg/ml and 0.1–0.2µg/ml respectively. These values correspond to impurity levels well below the ICH threshold of 0.1%, thereby enabling the method to detect even trace levels of degradation or process-related impurities. Furthermore, the signal-to-noise ratio for LOQ solutions was ≥10, and for LOD solutions ≥3, meeting established analytical benchmarks. Such high sensitivity makes this method particularly advantageous for stability studies and batch release testing where detection of early degradation is critical.

Solution Stability

Stability of the solution was evaluated by analysing solutions of the standard and the sample at the 0, 12, and 24 hours under ambient laboratory conditions. No significant change in peak area or shape was observed, and the % difference from initial values remained below 2%. This indicates that prepared solutions are stable over a 24-hour period, which is practical for routine use, especially in high-throughput laboratories where delayed analysis may occur.

Filtration Study

Filtration studies were conducted to ensure that the choice of syringe filters (PVDF and Nylon, 0.45 µm) does not introduce variability. No significant difference in impurity area counts was observed between centrifuged and filtered samples, indicating filter compatibility. Additionally, no analyte loss due to adsorption or filter leaching was detected, reinforcing the reliability of sample preparation procedures.[14]

CONCLUSION

This study reports the development and validation of a simple, accurate, and precise RP-HPLC method for the determination of organic impurities in Piribedil prolonged-release tablets. The literature review indicated only one reported method; hence a new sensitive and reliable method was developed. Separation of the analyte and its impurities was achieved using a gradient program with mobile phases consisting of 25 mM phosphate buffer: methanol (95:5) (MP-A) and buffer:methanol:acetonitrile (30:40:30) (MP-B). The detection wavelength was 238 nm. System suitability parameters were within acceptable limits. The method was validated for specificity, linearity, accuracy, precision, robustness, LOQ, LOD, solution stability and the filtration study. The results showed excellent linearity ($r^2 \geq 0.999$), acceptable recovery, and precision (%RSD < 2%). Thus the method is robust, reproducible, and suitable for routine pharmaceutical analysis.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-9: Industry, Innovation and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Kaushal Thorat https://orchid.org/0009-0005-5787-2031
Mayur Bhosale https://orchid.org/0000-0003-2390-8620
Rahul Khaire https://orchid.org/0000-0002-7323-839X
Sagar Magar https://orchid.org/0000-0001-8467-849X
Manisha Sonawane https://orchid.org/0009-0001-1534-0521
Nilima Wani https://orchid.org/0009-0004-9280-4918

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