Nitin Kumar et al /J. Pharm. Sci. & Res. Vol. 12(1), 2020, 20-27

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Development and Validation of a Discriminatory In-vitro Dissolution Method for Piribedil Prolonged Release Tablets by using High Performance Liquid Chromatography

**Nitin Kumar [*1, 2], D Sangeetha [2], and S Jayapal Reddy[1]**

[1]*Department of Analytical Research and Development, IPDO, Dr Reddy's Laboratories, Hyderabad-500 072, India, E-Mail: hellonitin_2005@rediff.com, Tel No. +918790995689 and*

[2] *Department of Chemistry, SAS, VIT University, Vellore, Tamilnadu, India*

**Abstract:**

Piribedil is available as a Prolonged Release tablet, used in the treatment of Parkinson's disease. Dissolution test is an In-vitro performance test which is an indicator of In-vivo performance of the drug product. The objective of this study was to develop a discriminatory dissolution method which can detect any changes in formulation composition during manufacturing. Dissolution studies were performed in various media and basis that 0.1 N HCl (media volume, 900 mL) was finalized. Dissolution apparatus was chosen as Paddle (50 RPM). Drug release samples were collected at 2, 6, 16, and 24 Hours. HPLC method was developed to perform the assay of drug release samples. Chromatographic separation was achieved by using Hypersil Gold C18 (4.6 x 150) mm, 5µm column, maintained at 30°C. Mobile Phase consisted of 50 mM Phosphate buffer with 0.01% Triethylamine (pH 2.5) and Acetonitrile in the ratio of 80:20 v/v respectively, was delivered at a flow rate of 1.3 mL/minute. Peak responses were quantified at 238 nm. Method was validated and found specific, precise, linear, accurate, rugged and robust. This method is a discriminatory method which can be used a quality control tool to assess the performance of the drug product.

**Keywords:** Piribedil, Tablets, In-vitro, Discriminatory, Dissolution method, HPLC

## INTRODUCTION

Piribedil is a dopamine agonist which stimulates dopamine receptors and the cerebral and peripheral dopaminergic pathways. 2-[4-(1, 3-benzodioxol-5-ylmethyl) piperazin-1-yl] pyrimidine is the IUPAC name of Piribedil and $C_{16}H_{18}N_4O_2$ is the molecular formula (Figure-1). Its molecular weight is 298.346 [1]. Piribedil is available as Prolonged Release tablets in 50 mg strength. Piribidil Prolonged Release tablets are given in the treatment of chronic pathological cognitive and neurosensory deficit in elderly patient and in the treatment of Parkinson's disease [2]. Piribedil Prolonged Release Tablets are available in Germany under the brand name of Clarium which is marketed by Desitin Arzneimittel GmbH. Based on Pharmacokinetic studies, it was found that bioavailability of Piribedil is very low through oral route as compared to the parental route [2]. Maximum daily dose for Piribedil is 250 mg in 3 to 5 divided dose.

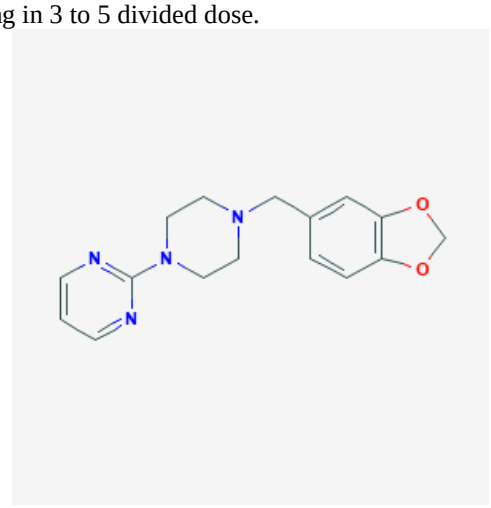



**Figure-1:** Structure of Piribedil

Dissolution is an important tool to assess the performance of the drug product. When an Oral Solid dosage form (Tablet or capsule) is administered, release of the drug from dosage form and absorption of the drug in gastrointestinal tract are the two key aspects for bioavailability of the drug. Drug release is the first step in making the drug available for absorption. Dissolution is the amount of drug which goes in to solution (dissolution media) in the specified time under the given dissolution conditions (e.g. Dissolution media, Volume of media, Apparatus, RPM). Dissolution is even more critical for BCS Class II drugs: Low Soluble and high permeable and Class IV drugs: Low soluble and low permeable, where solubility is the rate limiting step. Dissolution test can be used to predict the in vivo performance of the drug product and even in some case (e.g. BCS Class I Drug: High solubility, High permeability), can replace the in-vivo Bio equivalence studies. It is also very useful tool in identifying the impact of critical material attributes of drug substance (e.g. particle size), critical formulation variables (e.g. grade of excipients), and critical process parameters (e.g. lubrication time). Dissolution test is an economical quality control test which can be used to ensure that correct manufacturing process has been used. It also ensures the performance of the drug product across the shelf life [3].

As per the regulatory note released by United States Food and Drug Administration (USFDA), in 23.3% of the Abbreviated New Drug Applications (ANDA), the most common deficiencies were related to dissolution method and specification. Similarly in 16.6% ANDAs, dissolution analytical method validation and/or report were the other common deficiency. Hence it is very essential to have the right discriminatory dissolution method in place for assessing the performance of drug product [4].

Based on literature search there are few research article reported on the estimation of Piribedil in bulk drug and tablet dosage form by using HPLC [5,6]. There are few other reported analytical method for estimation of Piribedil in Pharmaceutical preparation, urine by potentiometric method [7,8] and in plasma by HPLC method [9] There is another article on estimation of Piribedil in rat plasma and brain tissues by using PDA and fluorescence technique [10]. A micellar electro kinetic capillary chromatography is also reported for estimation of Piribedil in formulations [11]. There are various article reported on the enhancement of dissolution pattern of Piribedil [12] , Optimization of Piribedil Mucoadhesive Tablets [13], characterization of Piribedil buccal tablets [14] and In-vivo evaluation of Piribedil solid lipid micro- and nanoparticles [15]. Piribedil Prolonged Release Tablets are not official in any of the Pharmacopeia also [16]. No method is reported for estimation of drug release in Piribedil Prolonged Release tablets. Hence it becomes necessary to develop a discriminatory dissolution method which can be used to assess the performance of the Piribedil Prolonged Release Tablet. The objective of the current work was to develop a discriminatory dissolution method by using Reversed phase HPLC method and subsequent validation of the method.

## MATERIALS AND METHODS

### Materials

Potassium dihydrogen orthophosphate, Triethylamine, Ortho phosphoric acid were sourced from Merck, Mumbai. Acetonitrile was procured from Rankem. All these reagents were of HPLC grade. For preparation of dissolution medium, Hydrochloric Acid was purchased from Rankem, Gurugram. Syringe filters (0.45 µm PVDF membrane) for sample filtration, were procured from Merck-Millipore. Piribedil Reference standard (Purity ≥99.0%) and Piribedil Prolonged Release tablets were provided by Dr. Reddy's Laboratories, Hyderabad.

### Methods

### Instruments

Dissolution analysis was performed by using Electrolab 8 stations (Model: EDT-08LX) dissolution tester with auto sampler. Dissolution samples were analyzed for estimation of % drug release by using Waters Alliance HPLC with quaternary pump, auto sampler and UV detector (Waters Corporation, Milford, USA). For data acquisition and processing Waters Empower 3 networking software was used. Hypersil Gold C18 (4.6 x 150) mm, 5 µm HPLC column was used for chromatographic separation.

### Dissolution conditions

Dissolution studies were performed on six individual units by using 0.1 N Hydrochloric acid in water as a dissolution medium. Dissolution medium volume was optimized as 900 mL. USP Apparatus-2 (Paddles) was selected and dissolution was run at 50 RPM. Temperature of dissolution medium during dissolution was maintained at 37±0.5ºC. Samples were withdrawn at 2 Hours, 6 Hours, 16 Hours and 24 Hours' time intervals during dissolution run.

### Chromatographic conditions

50 mM Phosphate buffer with 0.01% Triethylamine (pH 2.5) and Acetonitrile in the ratio of 80:20 v/v respectively were used as mobile phase. Hypersil Gold C18 (4.6 x 150) mm, 5µm column was used for separation. Column flow was maintained at 1.3 mL/minute and column oven temperature was kept as 30°C. 5 µL was injected for standard and sample solutions. Detection wavelength was selected as 238 nm.

### Dissolution medium preparation

85 mL of Hydrochloric acid (38%) was diluted to 10,000 mL with water. Dissolution media was degassed by heating till 41 °C and degassed under vacuum.

### Standard Preparation

Standard solution was prepared by dissolving Piribedil reference standard in dissolution medium (Diluent) to get the final solution with 55 µg/mL.

### Sample Preparation

900 mL of preheated (equilibrated at 37±0.5ºC) dissolution medium was transferred to each dissolution vessel. One tablet was dropped into each dissolution vessel and dissolution was run as per the specified conditions. 10 mL of sample was withdrawn from each dissolution vessel at the specified tie intervals by using the auto sampler and equal volume (10 mL) of the dissolution medium was replaced to each dissolution vessel.

### Method validation

HPLC method was validated as per ICH guideline *Validation of analytical procedures: Text and Methodology Q2 (R1).* Method was validated for Placebo interference, Method precision, Intermediate Precision, Recovery, Linearity, Solution stability and filter validation (robustness).

### System Suitability

To verify the adequacy of the HPLC instrument for analysis, five replicates of standard solution were injected and USP Peak tailing, USP Plate counts and %RSD of peak area were evaluated against a set specification; Peak tailing not more than 2.0, Plate counts not less than 2000 and %RSD not more than 2.0 respectively.

### Method precision

Method precision was performed by doing dissolution analysis on six tablets. Samples were collected at the specified time intervals and % drug release was estimated by assaying the samples by using HPLC method. % Drug release at each time point was assessed against a set specification. % RSD was also evaluated for time points where >50% drug release was observed.

Intermediate precision was performed by analyzing six units of Piribedil Prolonged Release Tablets 50 mg on different day (Day-2), different analyst (Analyst-2), and by using different Dissolution and HPLC instrument. % Drug release was determined at the specified time points. %RSD was calculated for six units from intermediate precision.

### Specificity

Specificity study was performed to ensure that there is no interference at the retention time of Piribedil due to excipients. Placebo (excipients) solutions were prepared in duplicate as per the test preparation and injected into HPLC.

**Linearity**

Linearity of detector response was performed from 1% of the target concentration (55 µg/mL) to 150% of the target concentration. Seven solutions with different concentration of Piribedil were prepared across the method range and injected into HPLC. Linearity graph was plotted by mapping the Peak response against the concentration. Correlation coefficient, slope and intercept were determined.

**Recovery**

Recovery studies were performed by adding the known amount of drug in placebo (excipients). Recovery was performed at five different levels by varying the amount of drug added and keeping the placebo weight constant as per the tablet weight. Levels for recovery included 5%, 20%, 50%, 100% and 150% of the target concentration (55 µg/mL). Samples were prepared in triplicate for each level and % recovery was calculated for all the samples.

**Filter validation**

Dissolution samples may contain few undissolved particles of excipients, hence to make the solution clear before injecting in to HPLC, dissolution samples were filtered by using 0.45 µm membrane syringe filters. Filter validation studies were performed to select the suitable filter for filtration. Dissolution samples were collected from dissolution apparatus. These samples were centrifuged and filtered by using 0.45 µm PVDF and Nylon filters. % Assay was compared between centrifuged and filtered samples and % difference was calculated.

**Solution stability**

Standard preparation and Test preparation stability was performed by storing the solutions on bench top (at 25±2°C and 75±5% RH) and in refrigerator (5±3°C). Stored standard and test preparations were injected after 24 Hours and after 48 Hours. For test preparation, % difference was calculated from the initial assay value. Similarly, similarity factor was calculated for stored standard against freshly prepared standard.

## RESULTS AND DISCUSSION

**Method development**

**Development of dissolution conditions**

The main objective of this study was to develop a discriminatory dissolution method which can identify any change in the manufacturing of drug product as well as it can be used as an indicator of In-vivo performance of the drug product. Selection of dissolution medium was the first step of dissolution method development for which saturation solubility studies were performed at 37 °C in various pH buffers ( e.g. 0.01 N HCl, 0.1 N HCl, pH 4.5 Acetate buffer, pH 6.8 Phosphate buffer, Water and pH 7.4 Phosphate buffer) across the physiological range. Piribedil was found having pH dependent solubility. It is having the highest solubility in 0.1 N HCl (Table-1). After solubility studies, comparative dissolution studies in different media (e.g. 0.01 N HCl, 0.1 N HCl, pH 4.5 Acetate buffer and pH 6.8 Phosphate buffer) were performed for In-house formulated drug product, by using USP Apparatus-2 (Paddle) at 50 RPM. In 0.01 N HCl, pH 4.5 Acetate buffer and pH 6.8 Phosphate buffer, incomplete drug release was observed (Figure-2) which was echoing with the solubility study outcome. In 0.1 N HCl, complete extent of drug release was observed because drug is having comparatively better solubility in this media.

Based on the solubility studies and dissolution in various pH dissolution media, 0.1 N HCl was selected as dissolution media. Sink condition was determined and based on that media volume was finalized as 900 mL. As dosage form is tablet, dissolution apparatus was selected as USP Apparatus-2 (Paddles) and RPM was optimized as 50. Temperature of dissolution bowl was maintained at 37±0.5°C. Piribedil is a Prolonged Release tablet hence dissolution sampling time points were finalized as 2 Hours, 6 Hours, 16 Hours and 24 Hours. In the finalized condition, comparative dissolution studies were performed for marketed formulation and In-house formulated drug product (Figure-3).

To check the discriminatory power of the dissolution medium, drug product batches with different coating build-ups (Lower, target and higher coating build-up) were analyzed in final conditions. Dissolution method was able to discriminate between three batches with different coating build ups. In higher coating build-up batch, % drug release was much slower as compared to batch with target and lower coating build-up (Figure-4). It shows that the proposed method is a discriminatory method and is able to detect the changes in the formulation composition.

Table-**1 Saturation Solubility**

| Buffer name | Mean Solubility (in mg/mL) |
|---|---|
| 0.01 N HCl | 2.98 |
| 0.1 N HCl | 13.59 |
| pH 4.5 Acetate Buffer | 4.84 |
| pH 6.8 Phosphate Buffer | 0.19 |
| Water | 0.10 |
| pH 7.4 Phosphate Buffer | 0.08 |

**Chromatographic condition development**

For performing the assay of the drug release samples, a simple and rapid reversed phase HPLC method was developed. Based on the absorption maxima of Piribedil, detection wavelength was selected as 238 nm. For initial scouting of chromatographic conditions, Mobile phase was prepared by using 10 mM of Potassium dihydrogen ortho phosphate and Methanol. Piribedil is having pKa value of 6.91, hence pH of the buffer was selected as 3.0. C18 columns of various brands were screened such as Waters Xterra RP18 (4.6 x 150) mm 5 µm, Inertsil ODS 3V (4.6 x 150) mm 5 µm, and Hypersil Gold C18 (4.6 x 150) mm 5 µm. Retention times (Waters Xterra: 2.44 Minutes, Inertsil ODS 3V: 2.99 minutes, Hypersil Gold: 3 minutes) were comparable in all the columns but in Inertsil ODS 3 V column peak tailing was high (USP tailing: 1.5). In Waters Xterra RP18 column, plate counts were observed very low (<2000) so for further trials Hypersil Gold C18 column was selected. To optimize the injection volume, 5 µL and 10 µL of standard solution were injected and with 5 µL injection volume, peak area was found adequate to quantify it precisely. Various pH buffers (pH 3.0 and pH 8.0) were also screened; in pH 8.00, Piribedil peak was not

eluted till 10 Minutes, hence it was decided to go with lower pH. For improving the USP plate count, organic modifier (Methanol) of the mobile phase was replaced with Acetonitrile and Triethylamine was also added in mobile phase buffer (0.01% v/v).

Various ratio of Buffer and Acetonitrile were studied at different column temperature (25ºC, 30ºC and 35ºC) with different mobile phase flow rates (0.8 mL/minute, 1.0 mL/minute, 1.2 mL/minute and 1.3 mL/minute). In mobile phase ratio (Buffer: Acetonitrile, 80:20 v/v), at 35ºC with 1.3 mL/minute flow rate; retention time, USP peak tailing and USP Plate counts of Piribedil were observed as 3.326 minutes, 1.2 and 9793 respectively. During this study shift in retention time between multiple runs, was observed, hence buffer strength was optimized to 50 mM and pH was changed to 2.50. Based on all optimization trials, chromatographic conditions were finalized. Hypersil Gold C18 (4.6 x 150) mm, 5 µm HPLC column was used. Column oven was maintained at 30 °C. 50 mM Phosphate buffer containing 0.01% Triethylamine (pH 2.5) and Acetonitrile in the ratio of 80:20 v/v respectively were used as mobile phase. Mobile phase was delivered at a flow rate of 1.3 mL/minute. Injection volume was optimized as 5 µL and detection wavelength was chosen as 238 nm.

**Method Validation**

Method was validated as per ICH Guideline for validation of analytical procedures for Specificity, Precision, Linearity, Recovery, solution stability and filter validation. Precision data is given in Table-2. Linearity results are summarized in Table-3. Recovery, solution stability and filter validation results are given in Table-4, Table-5 and Table-6 respectively.

Table **2 Precision and Intermediate Precision**

| **Time** | **Precision** | | | | **Intermediate Precision** | | | |
|---|---|---|---|---|---|---|---|---|
| | **% Drug Release** | | | | **% Drug Release** | | | |
| (In hours) | Min | Max | Mean | [%RSD (n=6)] | Min | Max | Mean | [%RSD (n=6)] |
| 2 | 30 | 35 | 33 | 5.3 | 27 | 38 | 30 | 6.0 |
| 6 | 60 | 65 | 63 | 2.7 | 62 | 66 | 64 | 2.5 |
| 16 | 94 | 97 | 95 | 1.3 | 92 | 96 | 94 | 1.7 |
| 24 | 99 | 102 | 100 | 1.0 | 97 | 101 | 99 | 1.2 |

Table **3 Linearity**

| Level | Relative to target Concentration (in %) | Concentration (in µg/mL) | Peak Area | Correlation Coefficient | Slope | Intercept |
|---|---|---|---|---|---|---|
| Level-1 | 1 | 0.5538 | 10972 | | | |
| Level-2 | 20 | 11.0755 | 222818 | | | |
| Level-3 | 50 | 27.6888 | 561551 | | | |
| Level-4 | 75 | 42.0870 | 850713 | 0.9999 | 20226 | 80.6 |
| Level-5 | 100 | 55.3776 | 1119341 | | | |
| Level-6 | 125 | 68.6682 | 1391903 | | | |
| Level-7 | 150 | 84.1740 | 1700369 | | | |

Table **4 Recovery**

| **Recovery Level[a]** | **“mg” added** | **“mg” recovered** | **% Recovery** | **Mean% Recovery** |
|---|---|---|---|---|
| 5%-Sample-1 | 2.411 | 2.430 | 100.8 | |
| 5%-Sample-2 | 2.411 | 2.435 | 101.0 | 101.1 |
| 5%-Sampke-3 | 2.411 | 2.450 | 101.6 | |
| 20%-Sample-1 | 10.046 | 9.895 | 98.5 | |
| 20%-Sample-2 | 10.046 | 10.006 | 99.6 | 99.2 |
| 20%-Sample-3 | 10.046 | 10.006 | 99.6 | |
| 50%-Sample-1 | 25.114 | 25.214 | 100.4 | |
| 50%-Sample-2 | 25.114 | 25.240 | 100.5 | 100.5 |
| 50%-Sample-3 | 25.114 | 25.290 | 100.7 | |
| 100%-Sample-1 | 50.228 | 50.379 | 100.3 | |
| 100%-Sample-2 | 50.228 | 50.379 | 100.3 | 100.2 |
| 100%-Sample-3 | 50.228 | 50.278 | 100.1 | |
| 150%-Sample-1 | 75.342 | 75.643 | 100.4 | |
| 150%-Sample-2 | 75.342 | 75.493 | 100.2 | 99.8 |
| 150%-Sample-3 | 75.342 | 74.363 | 98.7 | |

a: Accuracy level with respect to target concentration

Table **5 Solution stability**

| **Time Point (Hours)** | **% Drug Release** | | | | | | **%Difference from Initial** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Initial** | | **Day-1** | | **Day-2** | | **Day-1** | | **Day-2** | |
| | **T1** | **T2** | **T1** | **T2** | **T1** | **T2** | **T1** | **T2** | **T1** | **T2** |
| 2 | 34 | 30 | 35 | 30 | 35 | 31 | 1 | 0 | 1 | 1 |
| 6 | 64 | 60 | 65 | 61 | 65 | 62 | 1 | 1 | 1 | 2 |
| 16 | 94 | 94 | 94 | 93 | 96 | 95 | 0 | 1 | 2 | 1 |
| 24 | 99 | 100 | 99 | 100 | 100 | 101 | 0 | 0 | 1 | 1 |

T1-Test Sample-1
T2-Test Sample-2

Table **6 Filter validation**

| Sample | % Drug Release | | %Difference from Initial | |
|---|---|---|---|---|
| | T1 | T2 | T1 | T2 |
| Centrifuged | 99.8 | 98.7 | NA | NA |
| 10 µm full Flow filter | 100.0 | 98.7 | 0.2 | 0.0 |
| 0.45 µm PVDF | 99.9 | 98.8 | 0.1 | 0.1 |
| 0.45 µm Nylon | 99.9 | 99.0 | 0.1 | 0.3 |

T1-Test Sample-1
T2-Test Sample-2

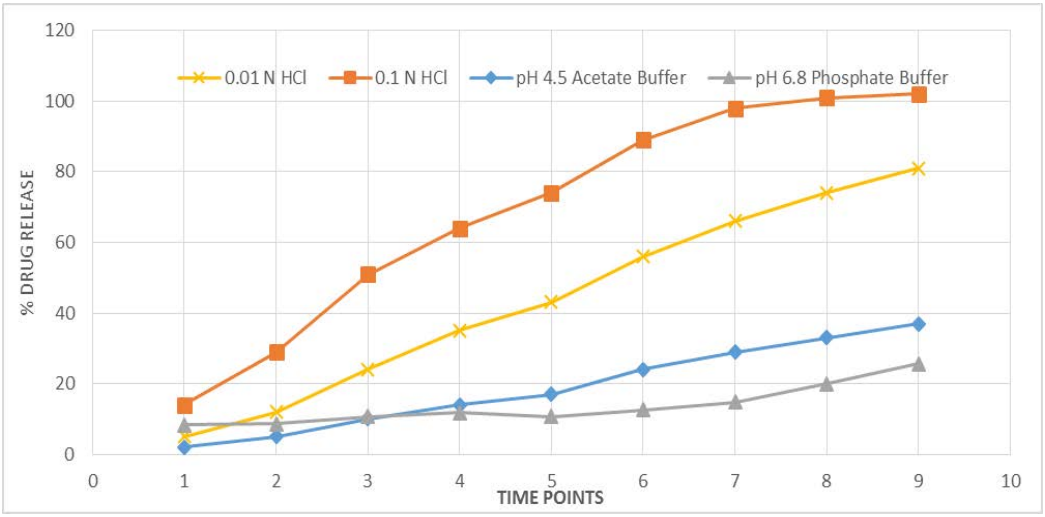



**Figure-2:** Dissolution profile of Piribedil Prolonged Release tablet in different dissolution media

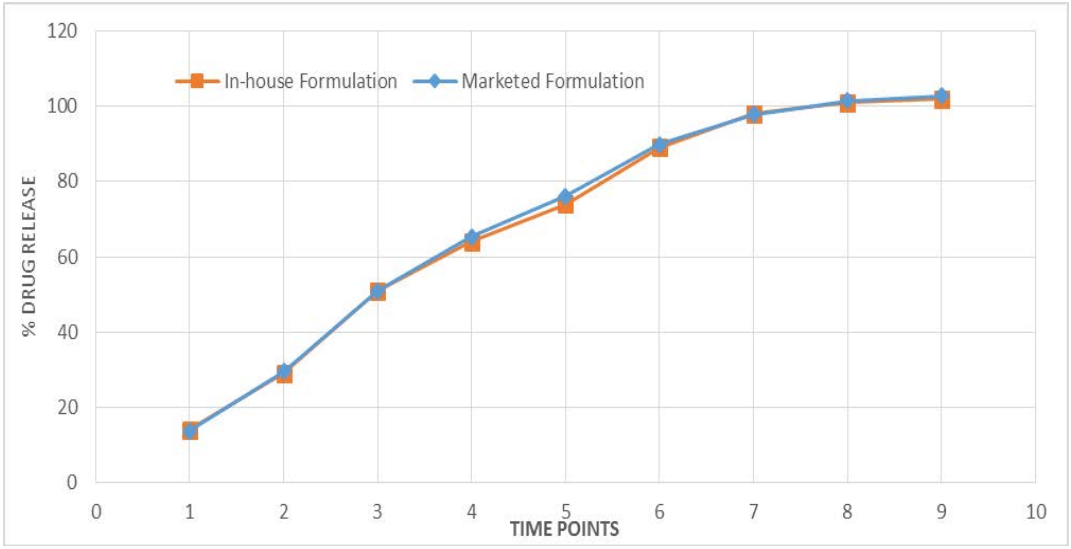



**Figure-3:** Comparative dissolution profile of Piribedil Prolonged Release tablet (In-house formulation) and marketed drug product

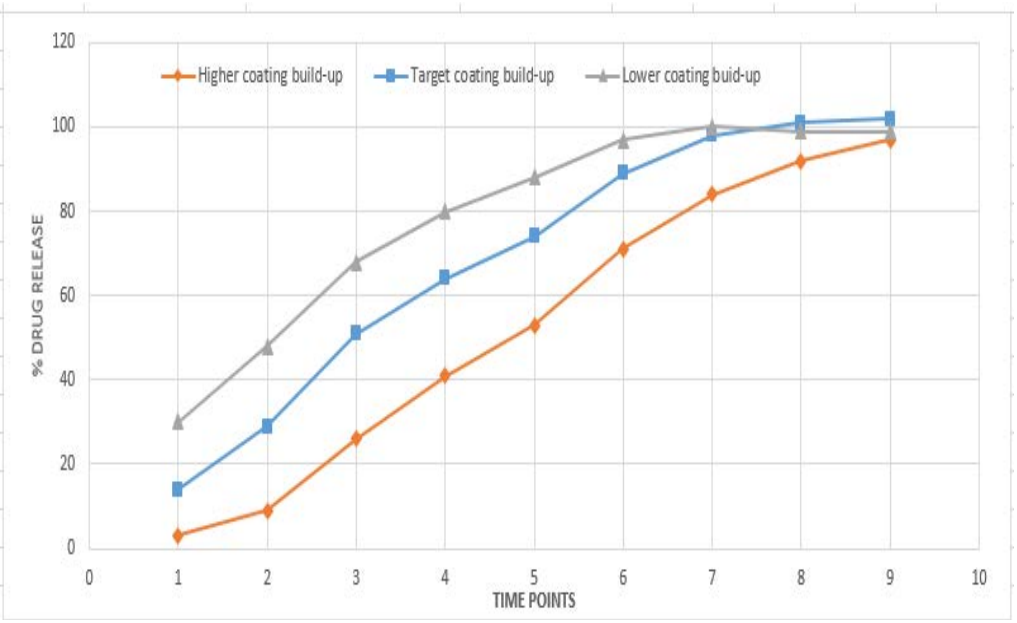
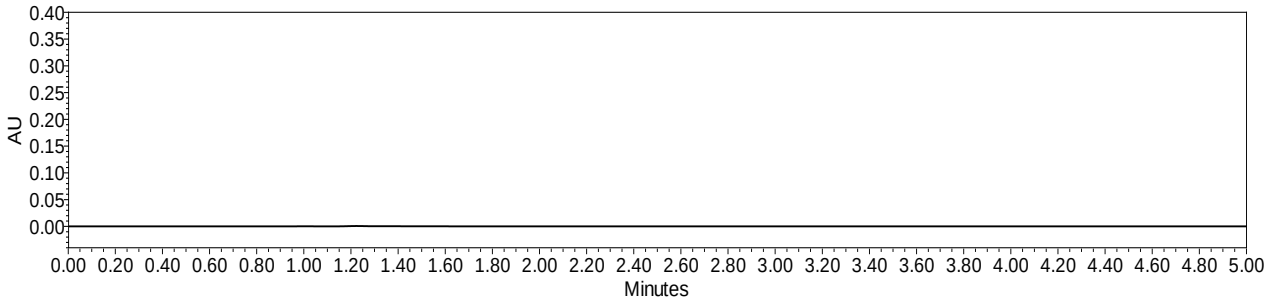
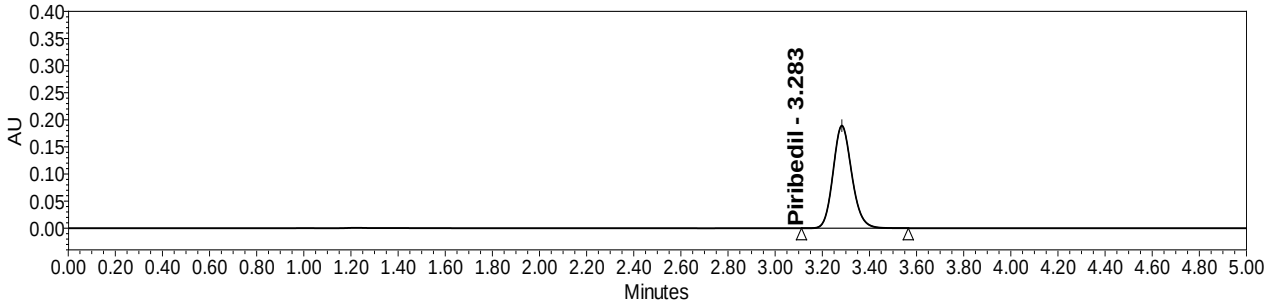



**Figure-4:** Dissolution profile of In-house Drug product with three different coating build-ups (Lower coating buildup, target coating build up and higher coating build up)




[a] Blank preparation




[b] Standard Preparation

**Figure-5.** Representative chromatograms of Piribedil Prolonged Release Tablet (a) Blank preparation (b) Standard preparation

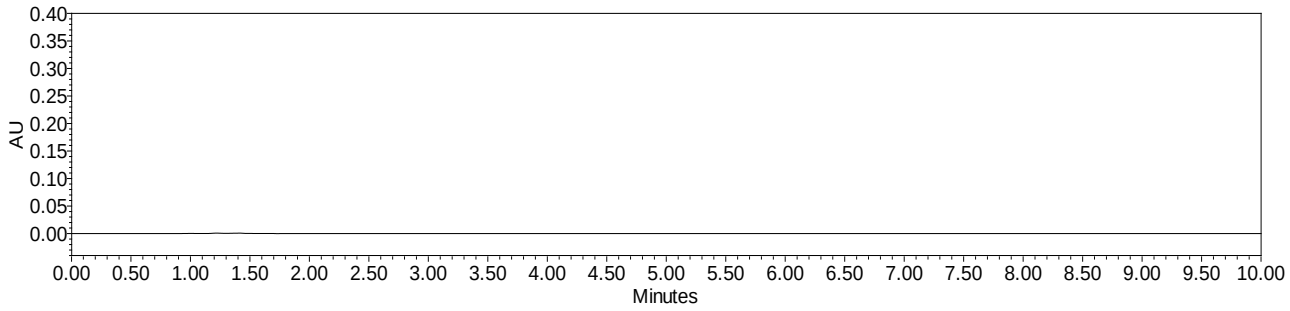



**Figure-6.** Representative chromatograms of Piribedil Prolonged Release Tablet Placebo preparation

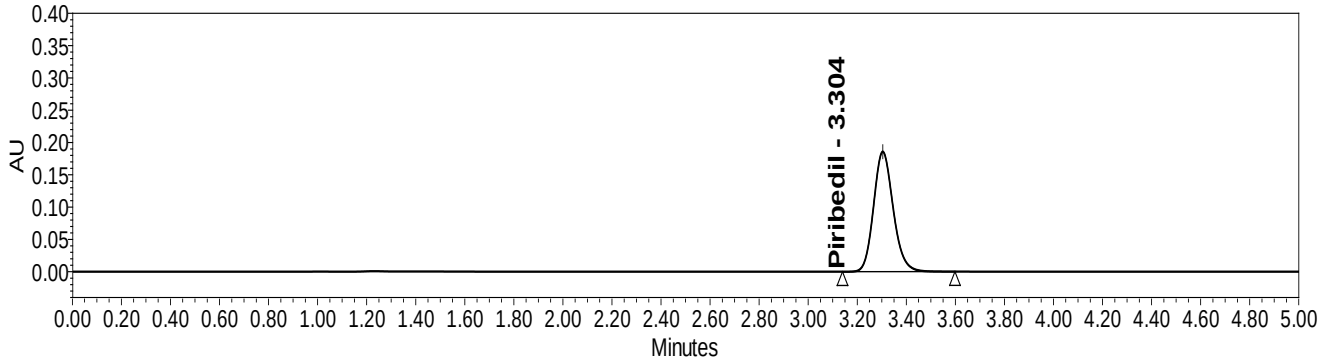



**Figure-7.** Representative chromatograms of Piribedil Prolonged Release Tablet Test preparation

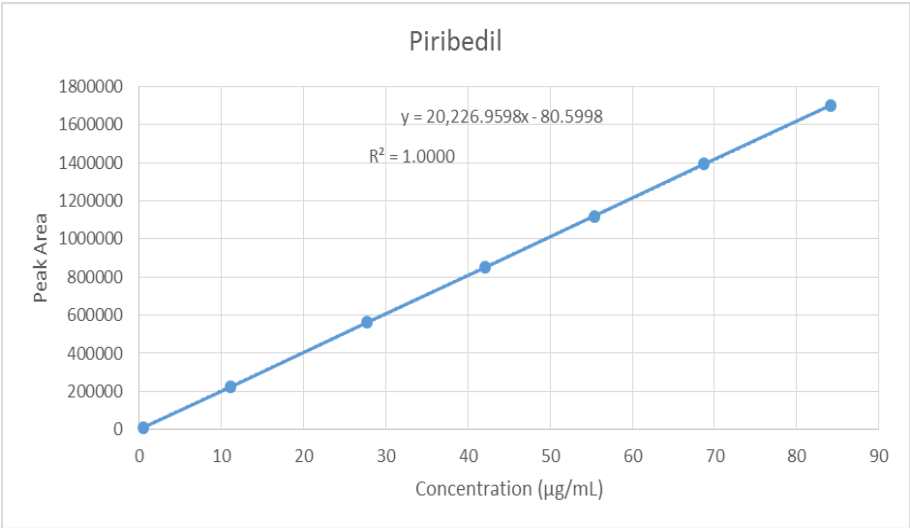



**Figure-8.** Linearity plot of Piribedil

**System suitability**

System suitability parameters were assessed against a predefined acceptance criteria and were meeting the acceptance criteria. USP peak tailing was observed as 1.1 against a limit of not more than 2.0. Plate count was observed as 5000 and % RSD for five replicate injection of standard was found 0.7% against a limit of not less than 2000 and not more than 2.0% respectively (Figure-5).

**Specificity**

Placebo interference was performed by injecting placebo (excipients) preparation in duplicate. No interference was observed at the retention time of Piribedil which demonstrated that method is specific for estimation of Piribedil (Figure-6).

**Precision**

Method precision and Intermediate precision were performed by performing the dissolution analysis on six tablets of Piribedil Prolonged Release Tablets 50 mg. % Drug release was estimated at 2 Hours, 6 Hours, 16 Hours and 24 Hours. %RSD of drug release for six units at 6 Hours, 16 Hours and 24 Hours (where > 50% release was observed) were found < 3.0, < 2.0 and < 1.5 respectively. Difference between the mean % drug release between Precision and Intermediate precision was found < 2% at 6 Hours, 16 Hours and 24 Hours (Table-2). Typical chromatogram of Piribedil Prolonged Release Tablet 50 mg is given in Figure-7.

**Linearity**

Seven solutions of Piribedil with concentration ranging from 1% of target concentration to 150% of target concentration, were injected and linearity graph was plotted (Figure-8). Correlation coefficient was found 0.9999. Linearity details are given in Table-3.

**Recovery**

Recovery study was performed at 5%, 20%, 50%, 100% and 150% of the target concentration (55 µg/mL). % Individual recovery values from all the spike levels were found between 98.5% to 101.6%. Individual and Mean % Recovery values for all the levels are given in Table-4

**Solution stability**

Standard and Sample solution stability studies were carried out at controlled room temperature and under refrigerated conditions (5± 3 °C). Standard solution was found stable at room temperature for 48 Hours. Sample preparation was also found stable at room temperature for 48 Hours. Solution stability results are summarized in Table-5.

**Filter validation**

Filter validation was performed by using 0.45 µm Nylon and PVDF membrane filters. % difference in assay between centrifuged and filtered samples were found <

1.0%. Hence both the filters were found suitable for Filteration. Filter validation results are given in Table-6.

## CONCLUSION

For assessing the performance of Piribedil Prolonged Release Tablets 50 mg, a simple rapid reversed phase, discriminatory In-vitro dissolution method was developed. This method can be used as a quality control tool to assess the product performance.

## ACKNOWLEDGEMENT

The author would like to thank Management of Dr. Reddy's laboratories for providing the facility to perform the research work.

## REFERENCES

[1] Substance information, Piribedil. Available at: https://echa.europa.eu/substance-information/-/substanceinfo/100.020.695. Accessed December 7, 2019.

[2] Patrick, Wuthrich, Herve, Rolland, Marc, Julien, Orally Dispersible Pharmaceutical Piribedil composition, *United States Patent Application Publication*, US 2005/0085485 A1, 21 Apr 2005.

[3] Mubarak, Nasser, Al, Ameri, Nanda, Nayuni, K.G., Anil, Kumar, David, Perrett, Arthur, Tucker, Atholl, Johnston, *Results in Pharma Sciences*. 2012, 2 , 1–8.

[4] Qing Liu, Barbara, M., Davit, Svetlana, A., Cherstniakova, Suman, Dandamudi, Johnetta, F., Walters, Christina, H., Lee, Kimberly, W., Raines, Ke, Ren, Leeh, N., Williamso, Dale, P., Conner, *The AAPS Journal*. 2012, 14, 19-22.

[5] Kandala, Bhaskara, Veera, Rohith, Venkata, Ramana, G., Madhavi, Latha, N., Supriya, P., Harini, U., Pawa,r Akm, *Asian Journal of Pharmaceuticals and Clinical Research*. 2016, 9, 342-346.

[6] T.S.S., Jagan, Mohan, Datla, Peda, Varma, Khagga, Bhavyasri, Kancherla, Prasa, *Asian Journal of Chemistry*. 2017, 29 (5), 1113-1118.

[7] Hosny, Ibrahim, *Journal of Pharmaceutical and Biomedical Analysis*. 2005, 38 (4), 624-32.

[8] Y.M, Issa, M.M, Hassouna, F.M, Abdel-Gawad, E.M, Hussien, *Journal of Pharmaceutical and Biomedical Analysis*. 2000, 23 (2-3), 493-502.

[9] S., Sarati, Giovanna, Guiso, Silvio, Caccia, *Journal of chromatography B: Biomedical sciences and applications*. 1991, 563 (2), 323-332.

[10] Chandra, Teja, Uppuluri, Avantika, V. Dalvi, Ekta, Prasanthi, Bommireddy, Punna, Rao Ravi, *Biomedical Chromatography*. 2018, 32(10), 4303.

[11] Ceren, Yardimci, Incilay, Suslu, Nuran, Ozaltın, *Analytical and Bioanalytical Chemistry*. 2004, 379(2), 308–311.

[12] Demirel M, Buyukkoroglu G, Kalava BS, Yazan Y. , *Methods and Findings in Experimental and Clinical Pharmacology*. 2006, 28 (2), 83-8.

[13] Celik B, Ozdemir S, Barla Demirkoz A, Uner M., *Drug Development and Industrial Pharmacy*. 2017, 43(11), 1-29.

[14] Burak, Celik, Melike, Uner, Formulation and characterization of Piribedil buccal tablets. 9th Annual European Pharma Congress, Madrid, Spain, 2017.

[15] M., Demirel, Y., Yazan, R.H., Muller, F., Kilic, B., Bozan, *Journal of Microencapsulation Micro and Nano Carriers*. 2008, 18(3), 359-371.

[16] U.S. Pharmacopeia; 41st Ed., U.S. Pharmacopeial Convention, Rockville, MD, 2010. Available at: www.uspnf.com.. Accessed December 7, 2019.