Biological Forum – An International Journal **5**(1): 78-83 (2013)

ISSN No. (Print) : 0975-1130
ISSN No. (Online) : 2249-3239

Determination of Assay Evaluation of Pramipexole in Parkinsone's Disease by RP-HPLC Method

M.H. Patil* and M.D. Rokade*

Jagdishprasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu Churu, (RJ) India

(Received 05 March, 2013, Accepted 25 April, 2013)

ABSTRACT: An isocratic reversed-phase HPLC method with PDA detector has been developed for the assay evaluation of Pramipexole in bulk drug. The analysis was performed using peerless Basic AQ C18 (250 x 4.6mm, 5µ) as a stationary phase with column oven temperature 40°c and UV detection at 254nm. The separation was achieved using isocratic program of buffer (Buffer used was prepares as dissolved 4.5 gm of potassium phosphate and 2.0 gm of 1-Octane sulphonate sodium salt in to 2000 ml of water and pH adjusted to 3.0 with diluted orthophosphoric acid) and Acetonitrile in the ration 70:30. The method was optimized based on the peak shapes and resolution of Pramipexole impurity A, Pramipexole impurity B, Pramipexole, Pramipexole impurity E and Pramipexole impurity D. The method was validated as per International Conference of Harmonization (ICH) guidelines in terms of linearity, precision, accuracy, specificity, robustness and solution stability. The sample concentration were injected was 2 mg/ml for Pramipexole. The method is linear within the range of 100 to 300 µg/ml for Pramipexole.

Keywords: Pramipexole, Assay , RP-HPLC,

I. INTRODUCTION

Development of analytical methods for bulk drug and their formulations is an important aspect in the drug product development as it helps to maintain the quality and efficacy of the drug product right from the product development process till its ultimate therapeutic use. Highly specific and sensitive analytical technique holds the key to the design, development, standardization and quality control of medicinal products [1].

The efficacy and safety of a medicinal product can be assured by analytical monitoring of its quality. It is important that analytical procedure proposed of a particular active ingredient or its dosage form should be systematically sound under the condition in which it is to be applied.

Parkinson's disease: Parkinson's disease is a neurodegenerative disease affecting the substantia nigra, a component of the basal ganglia. The substantia nigra has a high quantity of dopaminergic neurons, which are nerve cells that release the neurotransmitter known as dopamine. When dopamine is released, it may activate dopamine receptors in the striatum, which is another component of the basal ganglia. When neurons of the substantia nigra deteriorate in Parkinson's disease, the striatum no longer properly receives dopamine signals. As a result, the basal ganglia can no longer regulate body movement effectively and motor function becomes impaired. By acting as an agonist for the D_2, D_3, and D_4 dopamine receptors, pramipexole may directly stimulate the under functioning dopamine receptors in the striatum, thereby restoring the dopamine signals needed for proper functioning of the basal ganglia.

Pramipexole (**Mirapex**, **Mirapexin**, **Sifrol**) is a non-ergoline dopamine agonist indicated for treating early-stage Parkinson's disease (PD) and restless legs syndrome (RLS). It is also sometimes used off-label as a treatment for cluster headache and to counteract the problems with sexual dysfunction experienced by some users of the selective serotonin reuptake inhibitor (SSRI) antidepressants. Pramipexole has shown robust effects on pilot studies in a placebo-controlled proof of concept study in bipolar disorder. It is also being investigated for the treatment of clinical depression and fibromyalgia [2,3].

Pramipexole dihydrochloride [4] is chemically (S)-2-amino 4, 5, 6, 7-tetra hydro -6-(propylamino) benzothiazole dihydrochloride. It is a non-ergot dopamine receptor agonist used for symptomatic treatment of Parkinson's disease. Pre-clinical studies reveal that nano molar concentrations of Pramipexole protect dopaminergic neurons invitro or invivo by a receptor-dependent pathway mediated by the high selectivity of the drug for D_3-receptors. At higher concentrations, the drug has been shown to be neuroprotective invitro independent of the dopaminergic agonism [5]. Pramiprexole can be synthesized from a cyclohexanone derivative by the following route [6,7].

MATERIAL AND METHODS

A. Drug and reagents

Pure Pramipexole, Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E was obtained as sample from Cipla ltd Research Laboratories (Mumbai, India). Analytical reagent (AR) grade Potassium phosphate and Octane 1-sulphone sodium salt was purchased from Fluka (Banglore, India) and Acetonitrile from sigma Aldrich (Mumbai, India). Water for HPLC studies was obtained from milipore water purifying system.

B. Apparatus and equipment

LC was carried out on Waters HPLC system (Model no. 2690) with photodiode array detector (Make-996). The output signal was monitored and processed using Empower software . In all the studies, separations were achieved on a peerless Basic AQ C18 (250 mm x 4.6 mm i.d., particle size 5 µm) procured from LCGC (Banglore, INDIA). Other small equipment were PCI sonicator (22L500/CC/DTC made in), precision analytical balance (Mettler Toledo, Schwerzenbach, Switzerland).

C. Chromatographic conditions

The separation was achieved using Isocratic program of solution A (i.e Solution A used Contains Buffer prepared as by dissolved 4.5 gm of potassium phosphate and 2.0 gm of 1-Octane sulphonate sodium salt in to 2000 ml of water and pH adjusted to 3.0 with diluted orthophosphoric acid): and Solution B is Acetonitrile in the ratio of 70:30 v/v. the flow rate was set at 1.0 ml/min and column was maintained at 40^{o}C. The injection volume was set 5µl and detector was set at a wavelength of 254 nm.

D. Preparation of sample during method development and Validation

The diluent was selected for dissolving pramipexole and its impurities was mixture of buffer and Acetonitrile (in ration of 70:30 v/v). Standard solution of pramipexole was prepared in diluent having concentration of 0.2 mg/ml . pramipexole sample solution was prepared in the concentration of 0.2 mg/ml and injected.

E. Preparation of Resolution solution

Preparation of impurity stock solution: Weighed 1.5 mg of each Pramipexole impurity standard (Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E) into 10 ml of volumetric flask added 7 ml of diluent sonicated to dissolved and diluted to volume with diluent. (concentration of each impurity is 150 µg/ml).

Weighed 20 mg of pramipexole standard and into this added 1 ml of impurity stock solution into 100 ml of volumetric flask added 70 ml of diluent sonicated to dissolved and diluted to volume with diluent. (Standard concentration is 200 µg/ml and each impurity concentration is 1.5 µg/ml)

Standard Preparation: Weighed 20 mg of pramipexole standard into 100 ml of volumetric flask added 70 ml of diluent sonicated to dissolved and diluted to volume with diluent. (Standard concentration is 200 µg/ml)

Sample Preparation: Weighed sample equivalent to 20 mg of pramipexole into 100 ml of volumetric flask added 70 ml of diluent sonicated to dissolved and diluted to volume with diluent. (Sample concentration is 200 µg/ml)

METHOD DEVELOPMENT AND COLUMN SELECTION

Chemical structure of Pramipexole, Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E are shown in (fig.1 to 4). The sample of Pramipexole procured from market which was selected for validation studies. Different mobile phase and stationary phases were employed to developed a suitable LC method for the quantitative determination of Pramipexole in their respective formulations. A number of column containing various packing materials such as waters symmetry C18 (150 x 4.6mm, 5.0µm), phenomenex luna C18 (250 x 4.6mm, 5.0µm), Inertsil ODS (250 x 4.6mm, 5.0µm) supplied by different manufacturers and different mobile phase composition such as Phosphate buffer, acetonitrile and methanol (50:25:25 v/v/v), Phosphate buffer, acetonitrile and methanol (50:30:20 v/v/v), 0.1% Phosphate buffer in water: Acetonitrile and Methanol(70:15:15 v/v/v) were tried to get good peak shapes and selectivity for the impurities present in pramipexole. The separation was achieved using isocratic program of Buffer (A Buffer prepared as by dissolved 4.5 gm of potassium phosphate and 2.0 gm of 1-Octane sulphonate sodium salt in to 2000 ml of water and pH adjusted to 3.0 with diluted orthophosphoric acid): Acetonitrile. The method was optimized based on the peak shapes and resolution of Pramipexole (fig. 1), Pramipexole impurity A (fig.-2), Pramipexole impurity B (fig.-3), Pramipexole Impurity D (fig. 4) and Pramipexole impurity E (fig. 5) and for resolution chromatogram refer fig. 6.

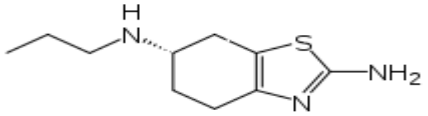
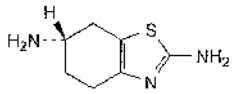
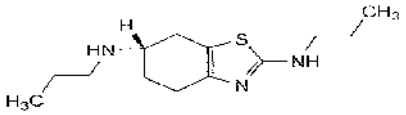


Fig.1 : Pramipexole : (*S*)-*N*^{6}-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine.



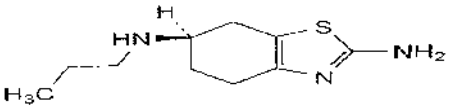
A. (6*S*)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,

Fig.2 : Pramipexole impurity A.



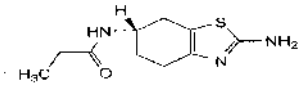
B. (6*S*) *N*,*N*′-dipropyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,

Fig.3 : Pramipexole impurity B.



D. (6*R*)-6-*N*-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,

Fig.4 : Pramipexole impurity D.



E. *N*-[(6*S*)-2-amino-4,5,6,7-tetrahydro-1,3-benzothiazol-6-yl]propanamide.

Fig.5 : Pramipexole impurity E.

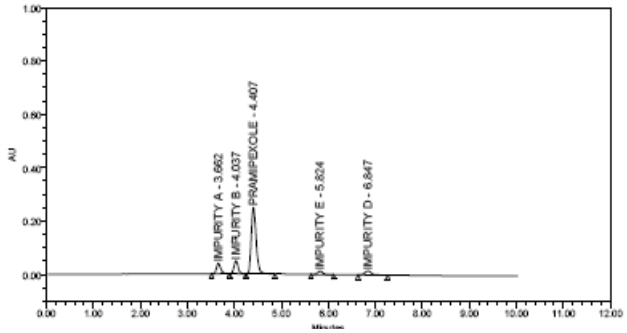


Fig.6. Resolution chromatogram of Pramipexole and its related Impurities.

RESULTS AND DISCUSSION

A. Method validation

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Assuring specificity is the first step in developing and validating a good method. If specificity is not assured, method accuracy, precision and linearity all are seriously compromised. Method specificity should be reassessed continually during validation and subsequent use of the method.

Linearity: Linearity of the method was checked by preparing solutions at five concentration levels of 100 ppm (Level 1) to 300 ppm (Level 5) for Pramipexole. Level 1 and level 5 was injected six times were as level 2 to level 4 was injected two times. The mean responses recorded for each analyte were plotted against concentration. The correlation coefficient for pramipexole was found to be 1.00, which indicates good linearity.

Accuracy: Pramipexole analytes were spiked in placebo solution at 50%, 100% and 150%. Each spiked solution was prepared in triplicate and injected. The recovery percentage and %RSD were calculated for each analyte. Recovery of Pramipexole ranged from 99.46-100.85%. The results are shown in Table 1.

Table 1. Accuracy Results of Pramipexole.

Added (µg)	Recovered (µg)	% Recovery (Limit NLT 98.0% and NMT 102.0%)	Mean Recovery
100.0	99.5	99.5 %	99.50 %
	99.8	99.8 %	
	99.2	99.2 %	
200.0	202.0	101.0 %	100.85 %
	201.5	100.75%	
	201.6	100.8 %	
300.0	299.2	99.73 %	99.46 %
	298.7	99.57 %	
	297.2	99.07 %	

System and method precision: The system for the impurities in Pramipexole was checked. The sample was prepared by dissolving tablets in diluent of target analyte concentration and injected six times. The %RSD was found to be less than 2.0% for system precision.

To determine the method precision six independent solutions were prepared with respect to target analyte concentration. Each solution was injected once. The variation in the results for the two analytes were expressed in terms of % RSD. The values calculated were found to be below 2.0% RSD for analytes, indicating satisfactory method precision. The results are shown in Table 2.

Table 2. Method Precision Results of Pramipexole.

Sr. No.	**% Assay of Pramipexole**
Sample-1	99.3
Sample-2	99.8
Sample-3	99.0
Sample-4	100.6
Sample-5	97.8
Sample-6	99.6
Mean	99.4
SD	0.93
%RSD	0.94

Stability in analytical solution: A solution of Pramipexole was prepared and kept at room temperature. This solution was injected at intervals of 0, 2, 4, 8, 12, 16, 20 and 24hr. Area of all the Analytes were nearly identical to that obtained at 0h and additional peaks were not observed which indicate solution stability. The results are shown in Table 3.

Table 3: Solution Stability Results of Pramipexole.

Sr. No.	% Assay of Pramipexole
0th Hr Sample	98.3
2nd Hr sample	97.6
4th Hr Sample	98.8
8th Hr Sample	97.8
12th Hr Sample	97.6
16th Hr Sample	98.4
20th Hr Sample	98.9
24th Hr Sample	98.1
Mean	98.2
SD	0.51
%RSD	0.51

Sample preparation of Pramipexole for routine analysis: Weighed 10 tablets of mireapex (containing 10 mg of pramipexole) sample in 50 ml volumetric flask, dissolved in diluents and dilute up the volume with diluents. Injected this solution into HPLC to determine the amount of analyte present in the sample. The chromatogram obtained after the analysis was shown in (fig. 7).

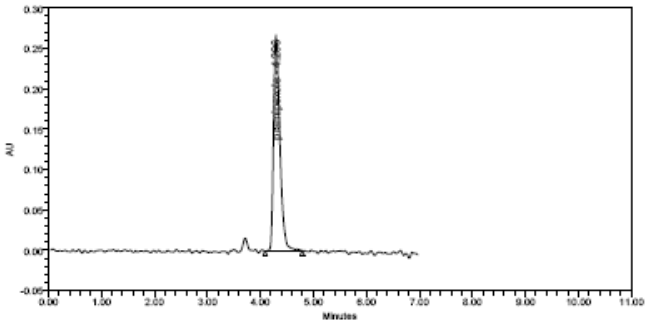


Fig.7. Standard chromatogram of Pramipexole.

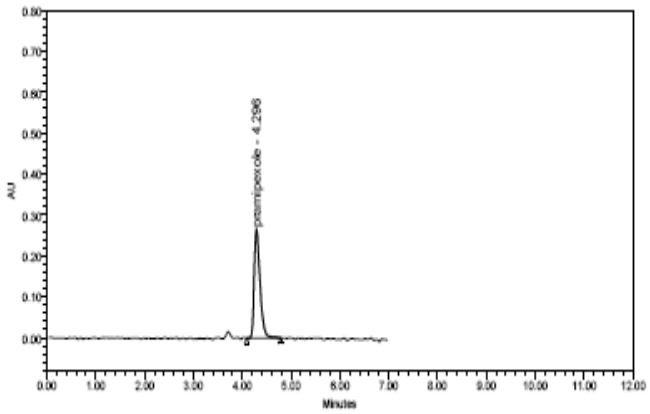


Fig. 8. Typical Sample chromatogram of Pramipexole.

CONCLUSION

The proposed LC method is selective for the quantification of Pramipexole present in Mirapex tablets. Hence this method is useful for the detection Pramipexole in routine analysis.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Manoj Rokade for reviewing and giving suggestions for the manuscript. The authors are grateful to Dr. Manoj Rokade their encouragement and support to carry out this work.

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