International Journal of Theoretical & Applied Sciences **5**(1): 119-126(2013)

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Determination of Impurity Recovery Through Method Validation of A Related Substances Method for Pramipexole

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(Received 05 March, 2013, Accepted 15 April, 2013)

ABSTRACT: The aim of this work was to develop and validate a Related substances method for Pramipexole in Mirapex tablets using High Performance Liquid Chromatography method. The mobile phase consist 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 drug release was evaluated by High Performance Liquid Chromatography method at 254 nm. The method was validated to meet requirements for a global regulatory filing. The validation included specificity, linearity, precision and accuracy. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard Pharmacopeial methods for the determination and calculation of impurities in Mirapex tablets for Pramipexole using the HPLC systems.

Keywords: Pramipexole, Related substances, Validation, High Performance Liquid Chromatography.

I. INTRODUCTION

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,) 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 in-vitro or in-vivo 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]. Pramipexole can be synthesized from a cyclohexanone [6,7].

II. MATERIAL AND METHODS

A. Drug and reagents

Pure Pramipexole, Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E was obtained 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 and Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E was prepared in diluent having concentration of 2.0 µg/ml for Pramipexole and each impurity 1.5µg/ml. pramipexole sample solution was prepared in the concentration of 1.0 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) .

Further diluted 1 ml of std stock solution and 1 ml of impurity stock solution in to 100 ml volumetric flask and diluted to volume with diluents. . (Standard concentration is 2 µg/ml and each impurity concentration is 1.5 µg/ml).

Sample Preparation: Weighed sample equivalent to 20 mg of pramipexole into 20 ml of volumetric flask added 15 ml of diluent sonicated to dissolved and diluted to volume with diluent. (Sample concentration is 1000 µg/ml).

III. 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. 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.

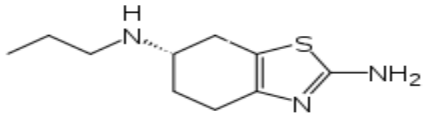
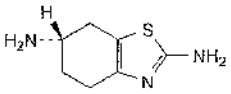
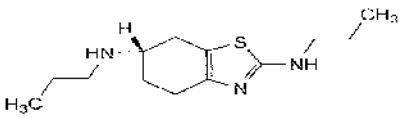


Fig.1 : Pramipexole : (*S*)-N^6-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine.



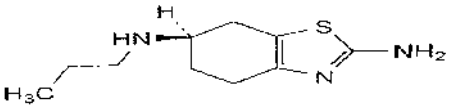
A. (6*S*)-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,

Fig.2 : Pramipexole impurity A.



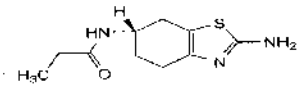
B. (6*S*) *N*,*N*′-dipropyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine,

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.



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

Fig.5 : Pramipexole impurity E.

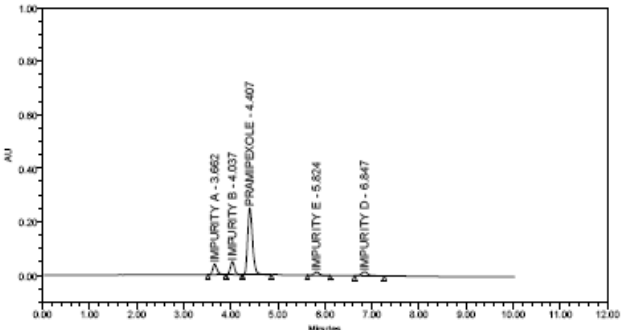


Fig.6. Resolution chromatogram of Pramipexole and its related Impurities.

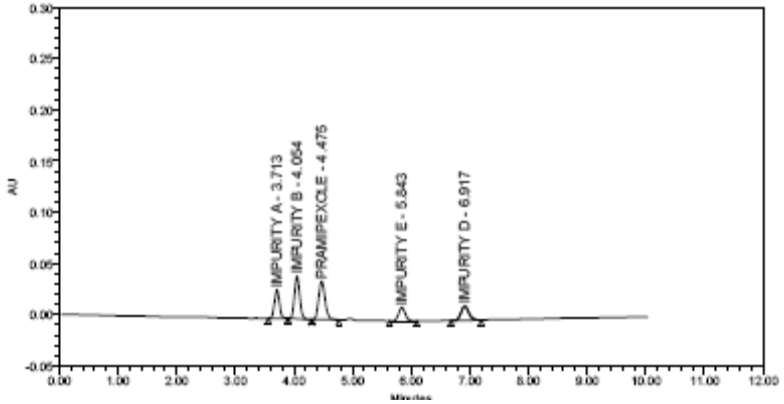


Fig. 7. Standard chromatogram of Pramipexole with impurities.

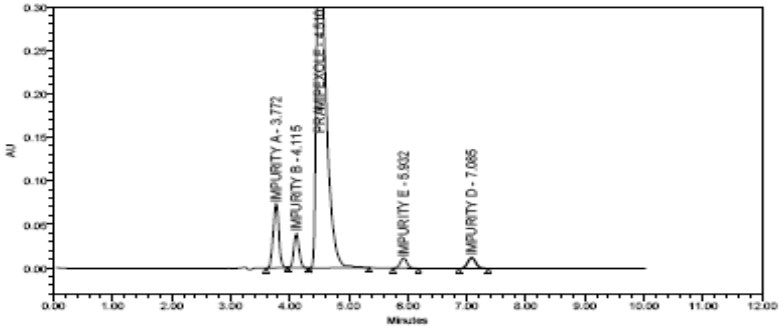


Fig. 8. Sample chromatogram of Pramipexole with spiked impurities at 100% Level.

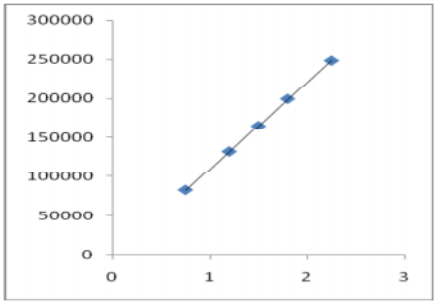


Fig. 9. Linearity curve of impurity A.

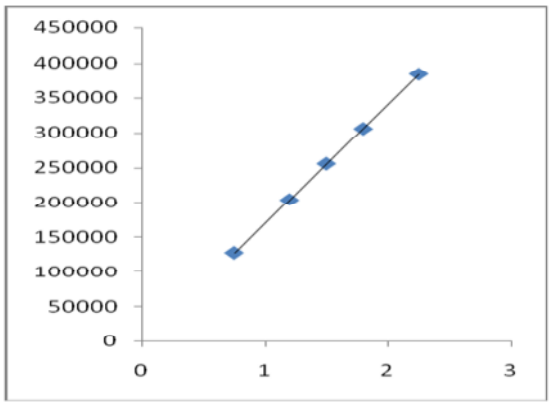


Fig.10 Linearity curve of Impurity B.

IV. 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 1 ppm (Level 1) to 3 ppm (Level 5) for Pramipexole and 0.75 µg/ml (Level 1) to 2.25 µg/ml (Level 5) of each impurity concentration. 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, Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E was found to be 1.00, which indicates good linearity (Fig. 9 to 13).

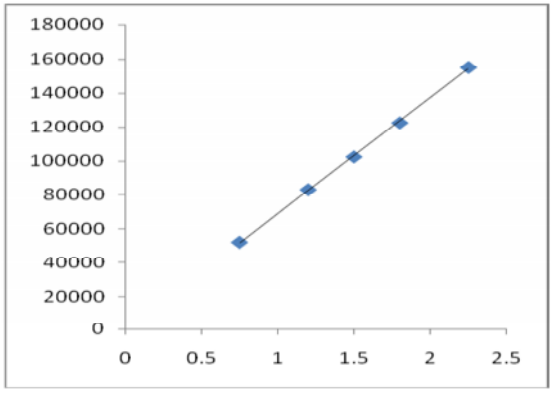


Fig. 11. Linearity curve of Impurity E.

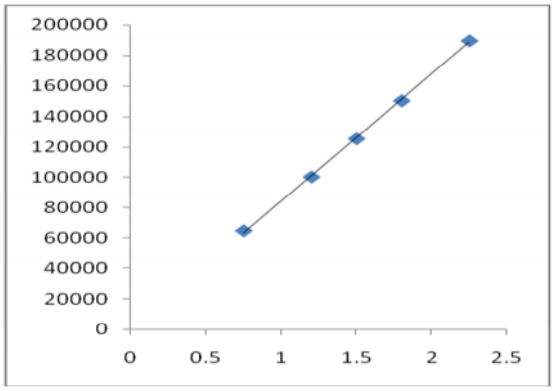


Fig.12. Linearity curve of Impurity D.

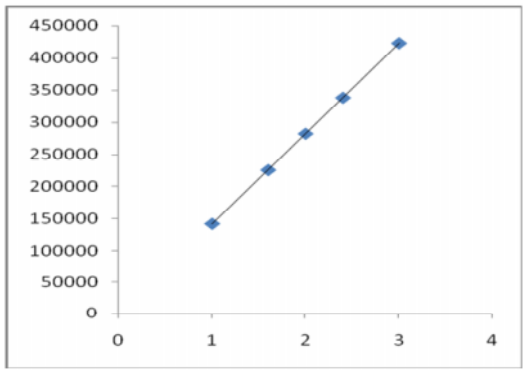


Fig.13. Linearity curve of Pramipexole.

Accuracy. Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E were spiked in Sample solution at LOQ Level, 50%, 100% and 150%. Each spiked solution was prepared in triplicate and injected. The recovery percentage and %RSD were calculated for each impurity. Recovery of Pramipexole impurity A, Pramipexole impurity B, Pramipexole Impurity D and Pramipexole impurity E ranged from 98.0-102.0% for 50%, 100% and 150% and for LOQ level it is found in between 85.0% to 115.0%. The results are shown in Table 1.

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 5.0% for system precision. No impurities were detected in sample solution hence at limit level of impurities were spiked in sample solution and %RSD were checked for the same. The results are shown in Table 2.

Stability in analytical solution. A solution of Pramipexole sample was prepared with spiked impurities 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.

B. Sample preparation of Pramipexole for routine analysis

Weighed 10 tablets of mirepex (containing 10 mg of Pramipexole) sample in 10 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. 8).

Table 1: Results of Accuracy and Recovery.

Accuracy Level	Sr.No.	Impurity A	Impurity B	Impurity E	Impurity D
Loq Level	1	95.1	96.8	97	97.9
	2	96.4	97.5	96.2	98.9
	3	95.8	97.9	97.9	97.5
	Mean	**95.75**	**97.15**	**96.60**	**98.40**
	RSD	**99.95**	**99.23**	**98.67**	**100.92**
50% Level	1	98.9	99.5	100.9	100.5
	2	99.4	99.4	100	100.9
	3	99.8	99.9	99.2	100.4
	Mean	**99.42**	**99.38**	**99.86**	**100.77**
	RSD	**99.62**	**99.48**	**100.66**	**100.37**
100% Level	1	100.3	101.4	98.7	98.1
	2	100.8	100.9	98.3	99.8
	3	101.1	100.5	99.9	99.4
	4	99.9	101.9	100.5	100.9
	5	99.5	99.4	100.2	101.5
	6	100.9	99.8	99.1	100.2
	Mean	**100.20**	**100.60**	**99.71**	**100.01**
	RSD	**99.31**	**100.80**	**100.62**	**99.81**
150% Level	1	101.7	100.1	100.9	98.5
	2	100.6	99.2	100.5	99.2
	3	101.3	98.2	99.5	100.8
	Mean	**100.54**	**100.03**	**100.67**	**99.17**
	RSD	**99.25**	**101.87**	**101.18**	**98.38**

Table 2: Results of Precision.

Precision	Sr.No.	Impurity A	Impurity B	Impurity E	Impurity D
	1	100.3	101.4	98.7	98.1
	2	100.8	100.9	98.3	99.8
	3	101.1	100.5	99.9	99.4
	4	99.9	101.9	100.5	100.9
	5	99.5	99.4	100.2	101.5
	6	100.9	99.8	99.1	100.2
	Mean	**100.32**	**100.82**	**99.52**	**99.94**
	RSD	**99.43**	**101.02**	**100.42**	**99.74**

Table 3: Results of Solution Stability.

Spl No.	Impurity A	Impurity B	Impurity E	Impurity D
0th Hr	98.2	100.1	101.9	99.5
2nd Hr	99.1	100.9	100.2	99
4th Hr	98.5	101.8	101.6	101.8
8th Hr	98.9	101.3	101.4	101.1
12th Hr	100.7	99.2	99.2	100.2
16th Hr	100.2	98.1	98.4	101.6
20th Hr	101.8	98.7	100.6	101.7
24th Hr	101.3	99.6	101.7	99.5
Mean	**99.84**	**99.96**	**100.63**	**100.55**
SD	**1.35**	**1.30**	**1.28**	**1.14**
RSD	**1.35**	**1.30**	**1.27**	**1.13**

VI. 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.

REFERENCES

[1]. Shankar M.B., Mehta F.A., Bhatt K.K., Mehta R.S., and Geetha M. (2008), An Introduction To Analytical Method Development For Pharmaceutical Formulations,*Indian Journal of pharmaceutical sciences*, **65(2)**, 167-170.

[2]. Lattanzi L., Dell'Osso L., Cassano P., Pini S., Rucci P., Houck P., Gemignani A., Battistini G., Bassi A., Abelli M., Cassano G. (2002), "Pramipexole in treatment-resistant depression: a 16-week naturalistic study.", *Bipolar Disord.* **4 (5)**, 307–314.

[3]. Holman A., Myers R. (2005), "A randomized, double-blind, placebo-controlled trial of pramipexole, a dopamine agonist, in patients with fibromyalgia receiving concomitant medications.", *Arthritis Rheum*, **52 (8)**, 2495–2505.

[4]. Sweetman S., editor. Martindale: The Complete Drug Reference, 35th ed. London, Pharmaceutical press 2007, 739-756.

[5]. Ramirez A., Wong S., Menniti F.(2003), Pramipexole inhibits MPTP toxicity in mice by dopamine D3 receptor dependent and independent mechanisms, *Eur. J. Pharmacol*, **475 (1),** 29-35.

[6]. Schneider C., Mierau J. (1987), "Dopamine autoreceptor agonists: resolution and pharmacological activity of 2,6-diaminotetrahydrobenzothiazole and an aminothiazole analog of apomorphine". *Journal of Medicinal Chemistry* **30 (3),** 494–498.

[7]. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use: Harmonised Tripartite Guideline on Stability Testing of New Drug substances and Products, Q1A (R2), **(2006)**.