World Journal of Chemistry 7 (2): 59-63, 2012
ISSN 1817-3128

DOI: 10.5829/idosi.wjc.2012.7.2.665

A Validated RP-HPLC Method for the Estimation of Pramipexole Dihydrochloride in Pharmaceutical Dosage Form

T. Deepan, K. Paulambethkar, R. Vijayalakshmi and M.D. Dhanaraju

Research Lab, GIET School of Pharmacy, Rajamundry-533294. A.P, India

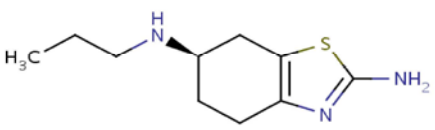
Abstract: A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Pramipexole dihydrochloride in Pharmaceutical Dosage form. Gradient elution at a flow rate of 1.0ml/min was employed on Analytical column C18 (150 mm x 4.6 mm, 5µm) Column at ambient temperature. The mobile phase consisting of buffer and Acetonitrile in the gradient ratio. The UV detection wavelength was 262nm and 20µl sample was injected. The retention time for Pramipexole was 5.47 min. The percentage RSD for accuracy of the method was found to be 0.35%. The method was validated as per the ICH guidelines. The method can be successfully applied for routine analysis of Pramipexole in Pharmaceutical Dosage Form.

Key words: Pramipexole · Acetonitrile · Phosphoric Acid · RP-HPLC · UV Detection · Recovery

INTRODUCTION

Pramipexole (Fig1) is a non-ergotaminedopamine agonistindicated for treating early-stageParkinson's disease (PD) andrestless legs syndrome (RLS). It is also sometimes usedoff-labelas a treatment forcluster headacheand to counteract the problems with sexual dysfunction experienced by some users of theselective serotonin reuptake inhibitor (SSRI) antidepressants. It is designated chemically as (*S*)-2-amino-4, 5, 6, 7-tetrahydro-6 (propylamino) benzothiazole DihydroChloride monohydrate [1, 2]. Pramipexole is a category of Non ergot dopamine receptor agonist.

There are many methods reported in the literature for analysis of Pramipexole dihydrochloride, e.g., spectrophotometric methods [3, 4, 11], LC-MS/MS in human plasma [5, 6], GC-MS in rat plasma [7], capillary electrophoresis [8]. However, no available chromatographic method for the determination of pramipexole dihydrochloride in Pharmaceutical dosage forms. Hence, the objective of the present study is to develop and validate a new RP-HPLC method for the estimation of Pramipexole dihydrochloride in bulk drug and its dosage form.



MATERIALS AND METHODS

Chemicals and Reagents: The reference sample of Pramipexole dihydrochloride (API) was obtained from Derex labs Pvt ltd, Hyderabad. The Formulation was procured from the local market. Acetonitrile, Pottasium dihydrogenphospate and phosphoric acid used were of HPLC grade and purchased from Merck Private Limited, Mumbai, India.

Instruments: Peak HPLC containing LC 20 AT pump, variable wavelength programmable UV/VIS detector and Rheodyne injector was employed for the investigation. The chromatographic analysis was performed on Analytical C18 column (150 mm x 4.6 mm, 5µm) column. Degassing of the mobile phase was done by using sonicator.

Chromatographic Conditions: Pramipexole dihydrochloride was analyzed by reverse phase columns. Among C8 and C18, Analytical C18 column (150 mm × 4.6 mm, 5µm) was selected. Various combinations of, acetonitrile, phosphoric acid and potassium dihydrogen phosphate were tested. Mixture of buffer: acetonitrile in gradient ratio was selected as mobile phase and the pH was adjusted to 3.0 using phosphoric acid. The concentrations of the buffer: acetonitrile were optimized to give symmetric peak with short run time. The following are the finalized chromatographic

Corresponding Author: T. Deepan, Research Lab, GIET School of Pharmacy, Rajamundry-533294. A.P, India.

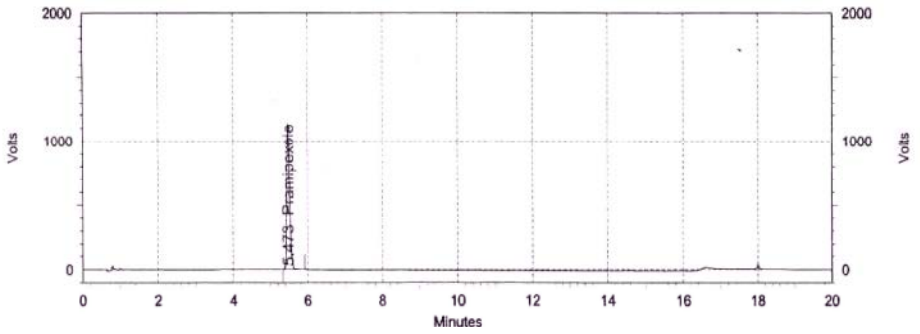


Fig. 2: Chromotogram for standard.

parameters. UV Detection wave length was 262nm, Flow rate was 1.0ml/min, Injection volume was 20µl, with Ambient temperature. The Retention time was found to be 5.47 min and the resulting HPLC Chromatogram shown in Fig. 2.

Standard Preparation: Transferred an accurately weighed amount of 10 mg of Pramipexole DihydroChloride mono hydrate reference standard (or) working standard in 100 ml volumetric flask add 60 ml of diluents, sonicate to dissolve and dilute to volume with diluent and mix well.

Sample Preparation: Transferred an accurately weighed 10 tabs in 100 ml volumetric flask. Add about 60ml of diluent, shake on rotatory shaker for 30 min at 200 rpm and sonicate for 45 min and dilute to volume with diluent.

Method Development: According to the Literature Review done and different trials made the finalized mobile phase is as follows:

Mobile Phase: Dissolved 9.1g of Potassium dihydrogen phosphate and 5.0g of Sodium 1-octane sulfonate monohydrate in 1000ml of water and adjusted to pH 3.0 with phosphoric acid. Filter through 0.45um PVDF filter. Degassed in a sonicator for about 10 minutes.

The peak of maximum absorbance wavelength was observed. The spectra of the Pramipexoledihydrochloride were showed that a wavelength was found to be 262 nm. RP-HPLC Chromatogram of standard solution is given in Fig 2.

Method Validation: The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification.

System Suitability: Based on the evaluation of system suitability parameters at each validation parameter, system suitability criteria can be established.

Acceptance Criteria: As per the test method assay should be (98% to 102%) and % RSD NMT 2.0%.

Accuracy: Accuracy parameters were evaluated as per the test method. Based on the evaluation of accuracy parameters at each validation parameter, accuracy criteria can be established. Acceptance Criteria: Co efficient of co relation should be NLT 0.999.

Precision: Samples were prepared as per test method in 6 replicates and by different analysts also has to be done, for intermediate precision.

Acceptance Criteria:

- %RSD of assay results should be NMT 2.0%
- Assay should be in the range of test method

Linearity and Range: The analyte solutions was prepared from limit of quantification level to 300% (or as per protocol) of the target concentration and establish the calibration curve by analyzing the sample as per test method. Linearity shall be established across the range. The co-efficient of co-relation, Y-intercept and slope of regression line was calculated.

Acceptance Criteria: Co efficient of co relation should be NLT 0.999.

Preparation of Standard Stock Solution: 235.7 mg of Pramipexole Dihydro Chloride mono hydrate was dissolved in 500 ml volumetric flask with diluent.

Ruggedness: Establish the stability of standard and test solution on bench top for a period of 2 days and in refrigerator for a period of about 5 to 7 days. Acceptance Criteria:

The%Assay of Standard and Test Preparation should not deviate by more than 10.0 from initial value. The standard is considered 'Stable' if the Similarity Factor is in the range of 0.90 to 1.10.

Robustness: Standard solution was prepared as per test method. Filtered the standard solution through individual filters. Inject unfiltered standard solution and filtered standard solution into the HPLC system under the test condition. Calculate the average area of duplicate injections of individual standard solutions and determine similarity factor of filtered standard solution against unfiltered standard.

Acceptance Criteria: The similarity factor of test solution against unfiltered standard solution should be in the range of 0.98 to 1.02.

RESULTS AND DISCUSSIONS

The solutions of pramipexole dihydrochloride workingstandards were injected into the HPLC system and run in different solvent systems as mobile phases. Representative chromatogram of mixed standard of AML and OLM is shown in Figure 2 and 3. The results are found to be within the limits.

Table 1: System Suitability

Sample No.	Area	%Assay
1.	4222289	101.4
2.	4245606	102.3
3.	4263126	101.6
4.	4244915	100.8
5.	4210228	100.5
6.	4237236	100.3
Average		101.1
Standard Deviation		0.7789
% RSD		0.8

The Plot of Linearity Concentration versus Area shown in Fig 4.

The retention time for Pramipexole was 5.47(Fig 2). Regression analysis of the calibration for Pramipexole shown between peak area and concentration could be represented by equation y = 40882x + 12046 (Fig 4). It was found to be linear with coefficient correlation of 0.9998. Theresults were found to be linear and precise as per the ICH guidelines[9] .The repeatability of peak area was checked and%RSD were found within limit as per guidelines[10]. The accuracy was checked by mean recovery of drug and was found within 99.5% (Table 2). The results are fount be within the limits as per ICH guidelines[9]

While checking robustness of method it was seen that retention time for Pramipexole varied in the range 5.85±0.1 (Table 6, 7) With change in mobile phase composition there were no marked changes in the area and asymmetry values which demonstrate that method developed is robust. The developed method was validated for its intraday and interday precision in the range of 99.4% (Table 3).

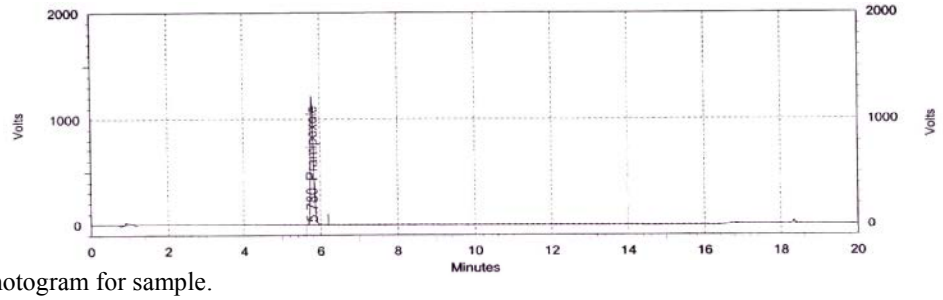


Fig. 3: Chromotogram for sample.

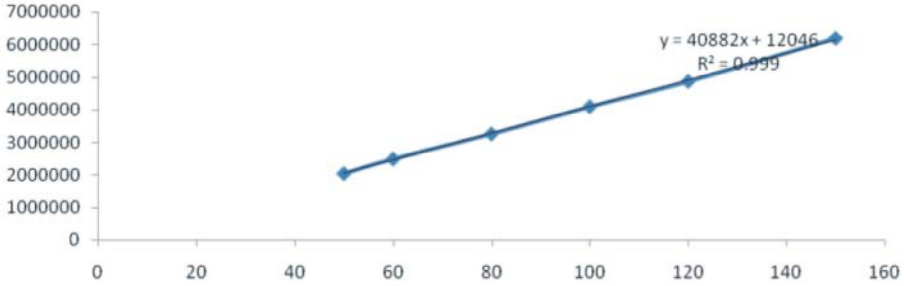


Fig. 4: Plot of Linearity Concentration versus Area

Table 2: Accuracy

Spike level (%)	Weight of Placebo added (mg)	Weight of API Added (mg)	Make up to (ml)	Area	Average mg added (API)	mg Found	Avg. mg Found	% Recovery
50	140.9	5.2	100	2015141	5.23	5.1	5.16	99.3
	139.4	5.3		1991659		5.2		
	141.6	5.2		2001406		5.2		
100	140.2	10.3	100	4056275	10.36	10.2	10.3	98.9
	139.0	10.5		4041142		10.5		
	141.9	10.3		4035978		10.2		
150	140.5	15.5	100	6111493	15.53	15.3	15.36	98.7
	138.1	15.6		6129646		15.5		
	137.5	15.5		6147261		15.3		

Table 3: Precision

Sample No.	Area	%Assay
1.	4153351	100.2
2.	4145981	100.6
3.	4130340	100.5
4.	4115882	99.9
5.	4156838	100.8
6.	4123611	100.0
Average		100.3
Standard Deviation		0.35
% RSD		0.35

Table 4: Linearity

S No.	Concentration(ppm)	Peak Area
1.	50	2055989
2.	60	2499083
3.	80	3264390
4.	100	4093925
5.	120	4869274
6.	150	6183656
Intercept		12046
Slope		40882
Correlation Coefficient		0.9995

Table 5: Bench top stability standard

S. No.	Time Period	Standard Wt taken Trial I	Standard Wt taken Trial II	Std area Trial I	Std area Trial II	Similarity Factor
1	Initial	10.0	10.2	4163502	4139127	1.01
2	After 24hrs	10.2	10.3	4167839	4167254	1.01
3	After 48hrs	10.1	10.5	4142544	4143065	1.01

Table 6: Effect of variation in flow rate

S.No.	Flow Rate	Area	Tailing Factor
1.	1.3 ml/min	4846763	1.7
2.	1.5ml/min	4422230	1.7
3.	1.7ml/min:	3666323	1.7

Table 7: Effect of Column Temperature

S.No.	Column Temperature	Area	Tailing Factor
1.	35°C	4188298	1.7
2.	40°C	4169756	1.7
3.	45°C	4024470	1.7

Table 8: Effect of pH

S.No.	pH	Area	Tailing Factor
1.	2.8	4123156	1.7
2.	3.0	4167318	1.7
3.	3.2	4297679	1.5

The average drug content of Pramipexole was found to be 0.8 with RSD 99.9 and is in good agreement to the labeled amount. No interfering peaks were found in chromatogram, indicating that the tablet excipient did not interfere with estimation of drug by the proposed HPLC method. The developed method was validated based on ICH guidelines[10]

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Pramipexole in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. The above method is suitable for the routine analysis of Pramipexole in pharmaceutical dosage forms. So it could be used for the rapid and reliable determination of Pramipexole in tablet formulations.

REFERENCES

1. http:// drug bank.ca/ drugs/ DB01076, DB01149, DB00413
2. http:en.wikipedia.org/wiki/analytical chemistry.
3. Gurupadayya, B.M., V. Vishwagith and N. Srujana, 2009. Developed a validated Spectrophotometric methods for the estimation of pramipexoledihydrchloride in pharmaceutical formulations. World Journal of Chemistry, 4(2): 157-160.
4. Lavudu, P., C. BalaSekaran, N. Gayathri, T. Srinu and A. Premilarani, 2011. Analysis of Pramipexoledihydro chloride by Vis-Spectrophotometric methods; Journal of Pharmacy Research, 4: 5.

5. Bharathi, D.V., K.K. Hotha, P.V. Sagar, S.S. Kumar, A. Naidu and R. Mullangi, 2009. Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of of Pramipexole dihydro chloride in human plasma: application to a clinical pharmacokinetic study. Biomed Chromatography, (2): 212-218.
6. Biljana, Jan, Mirjana Medenica, Darko Ivanovi and An|elija Malenovi, 2007. Experimental analysis in chromatographic analysis of pramipexole and its impurities. Acta Chim. Slov., 54: 49-54.
7. Panchal, J.G., R.V. Patel and S.K. Menon, 2011. Performed Development and validation of GC/MS method for the determination of Pramipexoledihydro chloride in rat plasma: Biomedical Chromotography, 25(4): 524-530.
8. Musenga, A., E. Kenndler, E. Morganti, F. Rasi and A. Maria, 2008. Developed analysis of the Anti Parkinson drug of pramipexole dihydrochloride in human urine by capillary electrophoresis with laser induced fluorescence detection, Analytica Chimica Acta, 626(1): 89-96.
9. ICH Q2A Guideline, 1995. Validation of analytical procedures, Definition and terminology (CPMPIII/5626/94), ICH, Geneva, Switzerland.
10. ICH ICH Q2B guideline, 1996. Validation of analytical procedures, Definition and terminology (CPMP/ICH/281/95), ICH, Geneva, Switzerland.
11. Gurupadayya, B.M., V. Vishwajith and N. Srujana, 2009. Spetrophotometric method for the estimation of pramipexole dihyrochloride in pharmaceutical formulation, World Journal of Chemistry, 4(2): 157-160.
12. Sharma, M.C. and S. Sharma, 2011. Development and validation of a dissolution method With Isocratic HPLC determination of nitazoxanide and oflaxacin in pharmaceutical dosage form. American-Eurasian Journal of Scientific Research ISSN 1818- 6785, IDOSI Publications, 6(4): 182-187.