

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF PRAMIPEXOLE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

Vilas Chaudhary¹ and Milind Ubale²

¹Department of Chemistry, Yogeshwari Mahavidyalaya, Ambejogai, Maharashtra, India.

²PG Department of Chemistry, Vasantao Naik Mahavidyalaya, Aurangabad, Maharashtra, India.

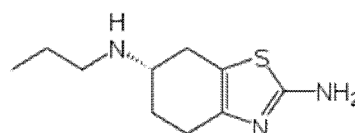
ABSTRACT

An isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Pramipexole hydrochloride in bulk drugs. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using a Phenomenex Luna C18 (250 x 4.6) mm, 5 μ m column and the mobile phase containing 0.1% Orthophosphoric acid pH 3.5 with Triethylamine and mixed. Prepare a homogenous mixture of buffer and Acetonitrile (65:35, v/v). The detection was carried out at wavelength 265 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

Keywords: Pramipexole hydrochloride, System Suitability, HPLC, Accuracy.

INTRODUCTION

Pramipexole (Mirapex, Mirapexin, Sifrol) (*S*)-*N*-propyl-4, 5,6,7-tetrahydro-1,3-Benzothiazole-2,6-diamine C₁₀H₁₇N₃S is a non-ergoline dopamine agonist indicated for treating early-stage Parkinson's disease (PD) and restless legs syndrome (RLS).¹ 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.² Pramipexole has shown robust effects on pilot studies in a placebo-controlled proof of concept study in bipolar disorder.³ It is also being investigated for the treatment of clinical depression and fibromyalgia.⁴⁻⁶



Chemical Structure of Pramipexole

Literature Survey

Pramipexole is used alone or with other medications to treat Parkinson's disease. It can improve your ability to move and decrease shakiness (tremor), stiffness, slowed movement, and unsteadiness. It may also decrease the number of episodes of not being able to move ("on-off syndrome"). This medication is also used to treat a certain medical condition (restless legs syndrome - RLS) that causes an unusual urge to move the legs. Symptoms usually occur at night along with uncomfortable/unpleasant feelings in the legs. This medication can decrease these

symptoms and thereby improve sleep. Pramipexole is a dopamine agonist that works by helping to restore the balance of a certain natural substance (dopamine) in the brain. Literature survey reveals that there are many experimental design applications in analytical method development and validation, especially in separation sciences. Experimental design has been used for optimization of separation,⁷⁻¹² for validation in RP-HPLC¹³⁻¹⁶ and for robustness testing in liquid chromatography¹⁷⁻¹⁹ or in capillary electrophoresis.²⁰

EXPERIMENTAL

Material and reagents

Pramipexole hydrochloride bulk drug was made available from Merck Ltd. India (purity 99.8). Orthophosphoric acid, triethylamine from Qualigens fine chemicals, India Limited. Acetonitrile, were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades; Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions

A chromatographic system (Systronic) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and UV detector. The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane Phenomenox Luna C18 stationary phase with particle size 5 micron and pore size 100Å was used. The instrumental settings were a flow of 1 ml/min; the injection volume was 20 µl and wavelength 265 nm.

Mobile Phase

The mobile phase prepared was the mixture of 0.1% Orthophosphoric acid pH 3.5 with Triethylamine. Also homogenous mixture of buffer and Acetonitrile (65:35, v/v) were prepared.

Preparation of Standard stock solutions

Standard stock solutions of 1000 ppm of Pramipexole hydrochloride in mobile phase were prepared in volumetric flasks.

Sample solution

1000 ppm of Pramipexole hydrochloride in 100ml calibrated flask in mobile phase was prepared in volumetric flasks. (1:1). The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure²¹⁻²²

Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for Pramipexole hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for Pramipexole hydrochloride bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.5 N Hydrochloric acid), alkali (0.025N NaOH), hydrogen peroxide (30%), heat (60 °C) to evaluate the ability of the proposed method to separate Pramipexole hydrochloride from its degraded products. For heat study, study period was 7 days where as for acid, oxidation 48 hr and for base 2 hour. Assay studies were carried out for stress samples against Pramipexole hydrochloride reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degraded products) was calculated.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main target for the development of chromatographic method was to get the reliable method for the quantification of Pramipexole hydrochloride from bulk drug and which will be also applicable for the degradable products. Initially, we took the effort for the development of HPLC method quantification of standard Pramipexole from bulk. For this purpose, we have used Water nova pack C18(150X4.6)mm,5µ, Kromasil C18(150X4.6)mm,5µ, Inertsil ODS 3V C18(250X4.6)mm,5µ and Kromasil C18(250X4.6)mm,5µ, Star ODS-II C18 (250X4.6)mm,5µ and Grace Alpha C18 (250mm x 4.6)mm,5µ. Out of these used HPLC column, Phenomenox Luna C18 (250mm x 4.6)mm,5µ found to comparatively better and gave the graph with better Gaussian shape at retention time 8.07 min. To improve the shape and width of the graph, for the above columns different solvents and buffer taken for trials such as 0.1M KH₂PO₄ and Acetonitrile (60:40,v/v) in these trials peak shape is not good, another trials 0.01M Ammonium acetate P^H-5.9 and acetonitrile(20:80,v/v) peak shape not found well, trials Acetonitrile and water (80:20, v/v) column temperature 35 °C peak shape not found good, trials K₂HPO₄, Methanol and water

(10:70:20,v/v/v)column temperature 35 °C, trials 1.0gm KH₂PO₄ and 0.45gm 1-Hexa sulphonic acid sodium salt make P^H-3.5 Ortho phosphoric acid and methanol(25:75, v/v) peak shape obtained but retention is not good, finally try for 0.1 % Orthophosphoric acid pH 3.5 with triethylamine. Homogenous mixture of buffer and acetonitrile (65:35, v/v) was prepared.

Result of forced degradation experiments

Considerable degradation was not observed in Pramipexole hydrochloride bulk samples, under

stress conditions such acid, thermal stress. Considerable degradation of Pramipexole hydrochloride was observed under stress condition such as base and oxidative hydrolysis leads to the formation of some unknown degradation peaks. The mass balance of Pramipexole hydrochloride in stress samples was close to 100% and moreover, the unaffected assay of Pramipexole hydrochloride in the Tablets confirms the stability indicating power of the method. The summary of forced degradation studies is given in Table I.

Table I: Summary of Forced degradation results

Stress condition	Time	Assay of active Substance %	Remarks
Acid Hydrolysis (0.5 N HCl)	48 Hrs	98.77	negligible
Base Hydrolysis (0.025 N NaOH)	2 Hrs	79.42	Degradation
Oxidation (30% H ₂ O ₂)	48 Hrs	99.12	No Degradation
Thermal (80°C)	7 days	99.46	No Degradation
Photolytic degradation	1.2 Lux million Hrs	98.59	negligible degradation

Method Validation

System suitability

For system suitability studies, five replicate injections of acid, base and oxidative degraded solutions were used and the RSD of peak area

ratio, resolutions, tailing factor and number of theoretical plates of the peak were calculated. The system suitability results are shown in Table II.

Table II: System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Pramipexole HCl	8.07	1.11	0.88	8970

Precision

The precision of the method was studied by determining the concentrations of the drug Pramipexole hydrochloride in the tablet for six

times²³. Results of the precision study (Table IV) indicate the reliability of the method (RSD % < 2).

Table IV. Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Pramipexole HCl	0.91	80-120	3745.12	0.9998

*Standard deviation shown in parentheses

Accuracy (Recovery test)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was

performed at three levels, 80%, 100% and 120%. The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for Pramipexole hydrochloride ranged from 99.12% to 100.17% (Table V). The average recoveries of three levels nine determinations for Pramipexole hydrochloride were 99.58- 100.44%.

Table V: Results of the Recovery Tests for the Pramipexole HCl

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery^
80	500	99.12	99.11
100	1000	99.79	100.45
120	1500	100.62	99.87

* RSD shown in parenthesis.

^ Average recovery = the average of three levels, nine determinations

Calibration and linearity

Linearity test solutions for the method were prepared from Pramipexole hydrochloride stock solutions at six concentrations levels from tested from 80% to 120% of the targeted level of the assay concentration Pramipexole hydrochloride. Standard solutions containing 80-120 µg/ml of Pramipexole hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis, the calibration graphs were found to be linear in the mentioned concentrations.

Robustness

To determine the robustness of the developed method experimental condition were purposely

altered and the resolution between Pramipexole hydrochloride and acid degraded product were evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.2 unit from 0.8 to 1.2ml/min while the other mobile phase component were held as stated in chromatographic conditions. The effect of percent organic strength on resolution was studied by varying acetonitrile from -10 to +10 % while other mobile phase components were held constant as stated in chromatographic condition. The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C while the other mobile phase components were held constant stated in chromatographic condition. The results are shown in table-VI.

Table VI: Results of robustness study

Sr. No.	Parameters	Variations	Resolutions between Pramipexole HCl and base degraded product
1	Temperature	25 °C	8.21
		35 °C	7.68
2	Flow rate	0.8 ml/min	8.02
		1.2 ml/min	8.94
3	Mobile phase	40.5 ml	3.7
		49.5 ml	3.3

LOD and LOQ (Sensitivity)

A series of solutions in the range 0.2–1.0% of the assay concentration (40 µg mL⁻¹) were prepared by dilution of the standard solutions. Each solution (20 µL) was injected five times, the areas were measured for the drug peak, and

the standard deviation for the five injections was calculated for each concentration. On the basis of data obtained, the standard deviation at concentration was calculated and this value was used for calculation of the LOD and LOQ. The results are shown in table-VII.

Table VII: Results of the LOD and LOQ

Name	%LOD	%LOQ
Pramipexole HCl	0.29	0.61

Stability of analytical solution

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of

the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for Pramipexole hydrochloride was 0.35 %. The assay values were within ± 2 % after 72 h. The

results indicate that the solutions were stable for 72 h at ambient temperature.

CONCLUSION

The method developed for quantitative determination of Pramipexole hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability indicating and can be used for assessing the stability of Pramipexole hydrochloride as bulk drugs. The developed method can be conveniently used for the assay determination of Pramipexole hydrochloride in bulk drugs and pharmaceutical dosage form.

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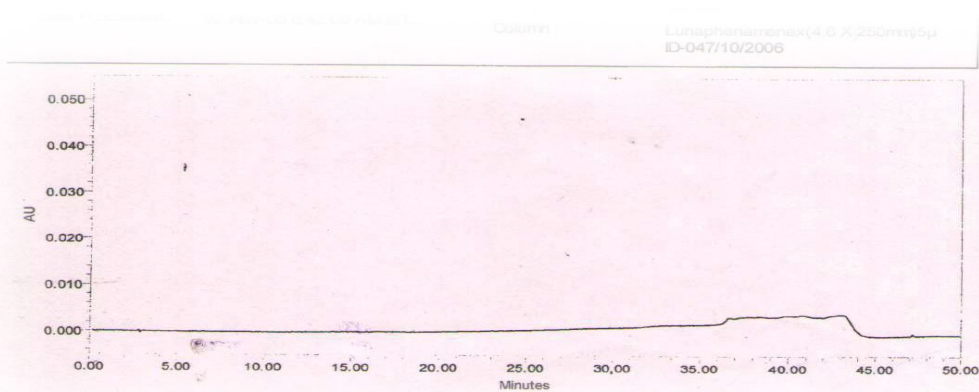


Fig. 1: A Typical Chromatogram of Pramipexole HCl Blank

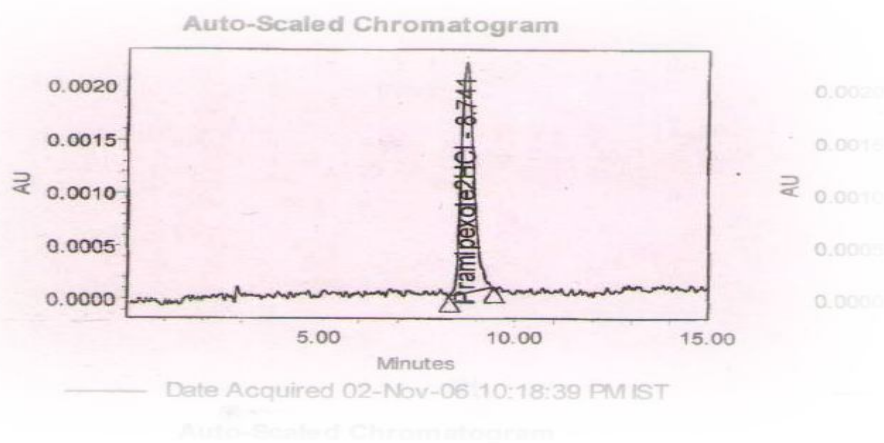


Fig. 2: A Typical Chromatogram of Pramipexole HCl Standard Preparation

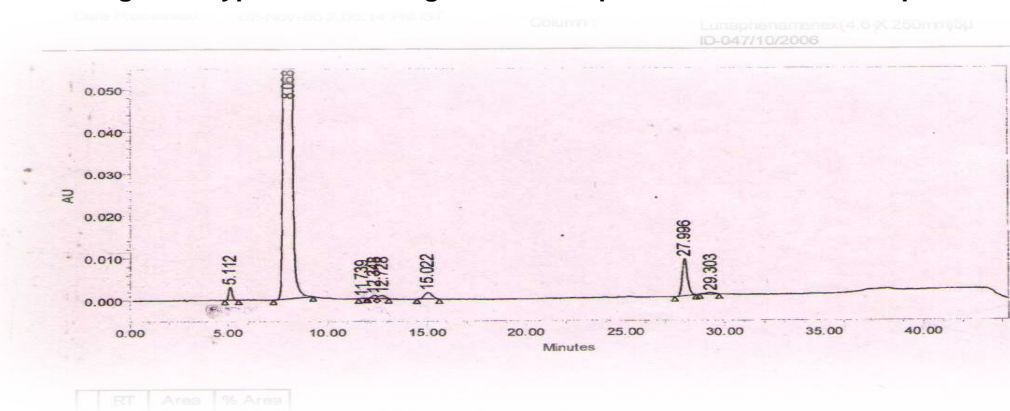


Fig. 3: A Typical Chromatogram of Pramipexole HCl Sample Preparation