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Validated chiral liquid chromatographic method for the enantiomeric separation of Pramipexole dihydrochloride monohydrate

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Abstract

A chiral liquid chromatographic method was developed for the enantiomeric resolution of Pramipexole dihydrochloride monohydrate, (*S*)-2-amino-4,5,6,7-tetra-hydro-6-(propylamino) benzothiazole dihydrochloride monohydrate, a dopamine agonist in bulk drugs. The enantiomers of Pramipexole dihydrochloride monohydrate were resolved on a Chiralpak AD (250 mm × 4.6 mm, 10 μm) column using a mobile phase system containing *n*-hexane:ethanol:diethylamine (70:30:0.1, v/v/v). The resolution between the enantiomers was found not less than eight. The presence of diethylamine in the mobile phase has played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The developed method was extensively validated and proved to be robust. The limit of detection and limit of quantification of (*R*)-enantiomer were found to be 300 and 900 ng/ml, respectively for 20 μl injection volume. The percentage recovery of (*R*)-enantiomer was ranged from 97.3 to 102.0 in bulk drug samples of Pramipexole dihydrochloride monohydrate. Pramipexole dihydrochloride monohydrate sample solution and mobile phase were found to be stable for at least 48 h. The proposed method was found to be suitable and accurate for the quantitative determination of (*R*)-enantiomer in bulk drugs.

Keywords: Pramipexole dihydrochloride monohydrate; Dopamine agonist; Chiral HPLC; Validation and active pharmaceutical ingredient

1. Introduction

Parkinson's disease (PD) is a chromic neurodegenerative disease characterized by bradykinesia, predominantly affecting the elderly, for which only symptomatic treatments are currently available. In the clinic, PD is viewed primarily as a disorder of the nigrostriatal dopaminergic pathway because presenting symptoms typically involve motor disturbances that can be modulated with dopamine agonists. It occurs when certain nerve cells (neurons) in a part of the brain called the substantia nigra die or become impaired. Normally, these neurons produce a vital chemical known as dopamine. Dopamine allows smooth, coordinated function of the body's muscles and movement [1,2].

Pramipexole, an amino-benzathiazole [(*S*)-4,5,6,7-tetrahydro-*N*-6-propyl-2,6-benzothiazolediamine dihydrochloride monohydrate] direct-acting dopamine receptor agonist effective in treating Parkinson's disease, bound selectively and with high affinity to dopamine D_2-like receptors, with highest affinity at dopamine D_3 receptors [3].

Few HPLC methods were reported in the literature for the quantitative determination of Pramipexole in human plasma with atmospheric pressure chemical ionization tandem mass spectroscopy [4] and with electrochemical and ultraviolet detection in human plasma and urine [5].

Pramipexole is produced as a single isomer and that the (*R*)-isomer could be present as chiral impurity. In the literature, there is no reference for the enantiomeric separation of Pramipexole dihydrochloride monohydrate in bulk drugs using high performance liquid chromatography.

Enantiomers of racemic drugs often differ in pharmacokinetic behaviour or pharmacological action [6].

In recent years, research has been intensified to understand the aspects of the molecular mechanism for stereoselective biological activities of the chiral molecules. The development of analytical methods for the quantitative analysis of chiral materials and for the assessment of enantiomeric purity is extremely challenging due to the fact that enantiomers possess virtually identical properties [7]. Recently, much work has been reported

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describing the use of chiral stationary phases, in conjunction with HPLC, as a way to separate and thereby individually quantitate the enantiomers of an enantiomeric pair [8–10].

This article describes a chiral LC method for the enantiomeric separation of Pramipexole dihydrochloride monohydrate using an amylose based chiral stationary phase, Chiralpak AD. The developed HPLC method was validated for determination of (*R*)-enantiomer in Pramipexole dihydrochloride monohydrate.

2. Experimental

2.1. Chemicals

Pramipexole dihydrochloride monohydrate and (*R*)-enantiomer were kindly supplied by Process Research Department of Wockhardt Limited, Mumbai, India and the chemical structures were given in Fig. 1. HPLC grade *n*-hexane and ethanol were purchased from Merck, Germany. Laboratory reagent grade diethyl amine was purchased from Merck, Germany.

2.2. Equipment

A shimadzu 2010 series LC system with photo diode array detector and inbuilt auto injector (Shimadzu Crop., Kyoto, Japan) was utilized for method development and validation. LC Solution software (Shimadzu Crop., Kyoto, Japan) was used for data acquisition and system suitability calculations.

2.3. Sample preparation

Stock solutions of (*R*)-enantiomer (100 μg/ml) and Pramipexole dihydrochloride monohydrate (5 mg/ml) were prepared by dissolving the appropriate amount of the substances in ethanol. The analyte concentration of Pramipexole dihydrochloride monohydrate was fixed as 1.0 mg/ml. Working solutions of Pramipexole dihydrochloride monohydrate and (*R*)-enantiomer was prepared in mobile phase.

2.4. Chromatographic conditions

The chromatographic conditions were optimized using an amylose based chiral stationary phase Chiralpak AD (250 mm × 4.6 mm, 10 μm, Daicel make) which was safeguarded with a 1 cm long guard column. The mobile phase was *n*-hexane:ethanol:diethylamine (70:30:0.1, v/v/v). The flow rate was set at 1.0 ml/min. The column was maintained at 25 °C and the detection was carried out at a wavelength of 260 nm. The injection volume was 20 μl. Protein based chiral stationary phase Chiral AGP (ChromTech make), Cellulose based chiral stationary phase Chiralcel OJ-H (Diacel make) and pirkle based chiral stationary phase Whelk-O-1 (Merck make) were also employed during method development.

2.5. Validation of the method

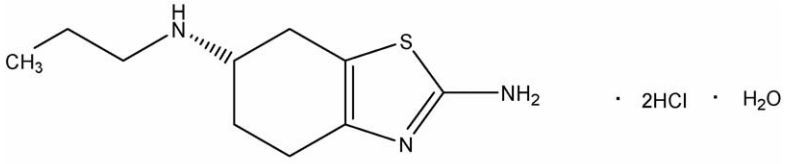
2.5.1. Method reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between-day precision) of retention times and peak areas for each enantiomer.

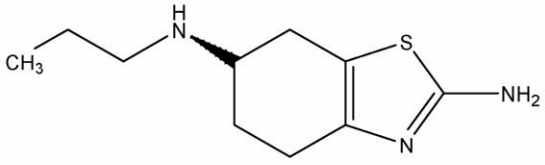
In order to determine the repeatability of the method, replicate injections ($n = 6$) of a 1.0 mg/ml solution containing Pramipexole dihydrochloride monohydrate spiked with (*R*)-enantiomer (0.5%) was carried out. The intermediate precision was also evaluated over 3 days by performing six successive injections each day.

2.5.2. Limit of detection and limit of quantification of (R)-enantiomer

The limit of detection, defined as lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal to noise ratio [11]. The



(S) enantiomer of Pramipexole



(R) enantiomer of Pramipexole

Fig. 1. Chemical structures of (*S*)-enantiomer of Pramipexole and (*R*)-enantiomer of Pramipexole.

limit of quantitation, defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as 10 times the signal to noise ratio [11]. LOD and LOQ were achieved by injecting a series of dilute solutions of (*R*)-enantiomer.

The precision of the developed chiral method for (*R*)-enantiomer at limit of quantification was checked by analyzing six test solutions of (*R*)-enantiomer prepared at LOQ level and calculating the percentage relative standard deviation of area.

2.5.3. Linearity of (R)-enantiomer

Detector response linearity was assessed by preparing six calibration sample solutions of (*R*)-enantiomer covering from 900 ng/ml (LOQ) to 6000 ng/ml (900, 1800, 2700, 3600, 4500 and 6000 ng/ml), in mobile phase from (*R*)-enantiomer stock solution.

Regression curve was obtained by plotting peak area versus concentration, using the least squares method. Linearity was checked for three consecutive days in the same concentration range from the same stock solution. The percentage relative standard deviation of the slope and *Y*-intercept of the calibration curve was calculated.

2.5.4. Quantification of (R)-enantiomer in bulk sample

The Pramipexole dihydrochloride monohydrate bulk sample, provided by Process Research Department of Wockhardt Limited, showed the absence of (*R*)-enantiomer. Standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of (*R*)-enantiomer in bulk drug samples.

The study was carried out in triplicate at 0.4, 0.5 and 0.6% of the Pramipexole dihydrochloride monohydrate target analyte concentration. The recovery of (*R*)-enantiomer was calculated from the slope and *Y*-intercept of the calibration curve obtained.

2.5.5. Robustness

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, mobile phase composition and column temperature. To determine robustness of the method, experimental conditions were purposely altered and chromatographic resolution between Pramipexole and (*R*)-enantiomer was evaluated.

The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on the resolution of enantiomers, 0.2 units changed it from 0.8 to 1.2 ml/min. The effect of change in percent ethanol on resolution was studied by varying from −1 to +1% while the other mobile phase components were held constant as stated in Section 2.4. The effect of column temperature on resolution was studied at 20 and 30 °C instead of 25 °C while the other mobile phase components were held constant as stated in Section 2.4.

2.5.6. Solution stability and mobile phase stability

Stability of Pramipexole dihydrochloride monohydrate in solution at analyte concentration was studied by keeping the solution in tightly capped volumetric flask at room temperature on a laboratory bench for 2 days. Content of (*R*)-enantiomer was checked for 6 h interval up to the study period.

Mobile phase stability was carried out by evaluating the content of (*R*)-enantiomer in Pramipexole dihydrochloride monohydrate sample solutions prepared freshly at 6 h interval for 2 days. Same mobile phase was used during the study period.

3. Results and discussion

3.1. Optimization of chromatographic conditions

The aim of this work is to separate the enantiomers of Pramipexole dihydrochloride monohydrate and accurate quantification of (*R*)-enantiomer. Racemic mixture solution of 0.5 mg/ml prepared in mobile phase was used in the method development. To develop a rugged and suitable LC method for the separation of Pramipexole enantiomers, different mobile phases and stationary phases were employed. The main target of the chromatographic method is to separate the enantiomers of Pramipexole dihydrochloride monohydrate, various chiral columns namely Chiralcel AGP of ChromTech make, Chiralcel OJ-H, Chiralpak AD of Diacel and Whelk-O-1 of Merck make. Various experiments were conducted, to select the best stationary and mobile phases that would give optimum resolution and selectivity for the two enantiomers. No separation was found on Chiralcel OJ-H, Whelk-O-1 and Chiralcel AGP columns using different possible mobile phases. There is an indication of separation on Chiralpak AD column using a mobile phase consisting of *n*-hexane:ethanol (50:50, v/v) and the peak shapes were broad. Introduction of diethyl amine in the mobile phase enhanced the chromatographic efficiency and resolution between the enantiomers. Very good separation was achieved on Chiralpak AD column (resolution between enantiomers was found greater than eight) using the mobile phase system *n*-hexane:ethanol:diethyl amine (70:30:0.1, v/v/v). Reversal of the order of elution of the enantiomers on changing from ethanol to 1-propanol was observed on Chiralpak AD column. The enantioselectivity changes suggest that the ethanol affects the steric environment of the chiral cavities or channels of the stationary phase. Pramipexole is having only one chiral center and an amylose based chiral stationary phase is containing five chiral centers per unit. It is presumed that it could be due to high probability of interaction, better resolution was found on Chiralpak AD column. Due to the better chromatographic results obtained on the Chiralpak AD column, the method validation was carried out on the same.

In the optimized method, the typical retention times of (*R*)-enantiomer and Pramipexole were about 4.9 and 7.8 min, respectively. The enantiomeric separation of Pramipexole dihydrochloride monohydrate on Chiralpak AD column was shown in Fig. 2. The system suitability test results of the chiral LC method on Chiralpak AD are presented in Table 1.

3.2. Validation results of the method

In the repeatability study, the relative standard deviation (R.S.D.) was better than 0.5% for the retention times of both

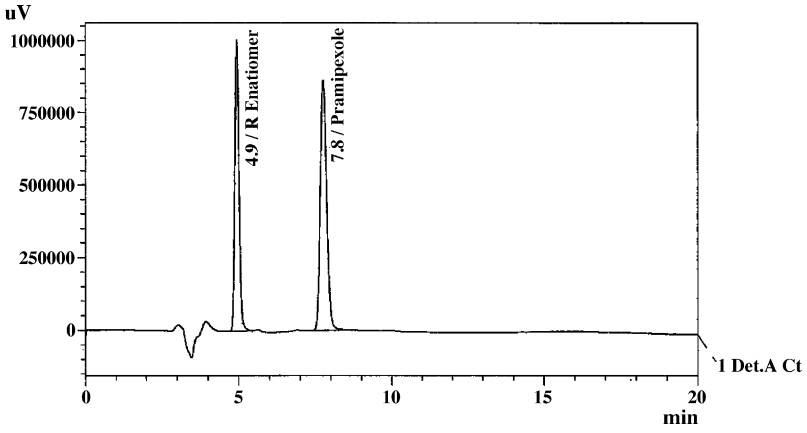


Fig. 2. Enantiomeric resolution of Pramipexole dihydrochloride monohydrate on Chiralpack AD column.

Table 1
System-suitability report

Compound ($n=3$)	R_t	R_s	N	T
(*R*)-enantiomer	4.9		6023	1.2
Pramipexole	7.8	8.8	6491	1.2

$n=3$ determinations. R_t: retention time, R_s: USP resolution, N: number of theoretical plates (USP tangent method), T: USP tailing factor.

Table 2
Validation results of the developed chiral LC method

Validation parameter	Results
Repeatability ($n=6$, %R.S.D.)	
Retention time (*R*-enantiomer)	0.4
Retention time (*S*-enantiomer)	0.2
Peak area (*R*-enantiomer)	1.8
Peak area (*S*-enantiomer)	0.8
Intermediate precision ($n=18$, % R.S.D.)	
Retention time (*R*-enantiomer)	0.5
Retention time (*S*-enantiomer)	0.4
Peak area (*R*-enantiomer)	2.7
Peak area (*S*-enantiomer)	1.0
LOD–LOQ (*R*-enantiomer)	
Limit of detection (ng/ml)	300
Limit of quantification (ng/ml)	900
Precision at LOQ (%R.S.D.)	2.9
Linearity (*R*-enantiomer)	
Calibration range (ng/ml)	900–6000
Calibration points	6
Correlation coefficient	0.9998
Slope (%R.S.D.)	1.7
Intercept (%R.S.D.)	9

the enantiomers, 0.8% for Pramipexole peak area and 1.8% for (*R*)-enantiomer peak area (Table 2). In the intermediate precision study, results show that R.S.D. values were in the same order of magnitude than those obtained for repeatability (Table 2).

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were estimated to be 300 and 900 ng/ml for (*R*)-enantiomer, when a signal-to-noise ratio of 3 and 10 were used as the criteria. The method precision for (*R*)-enantiomer at limit of quantification was less than 3% R.S.D. (Table 2).

Good linearity was observed for (*R*)-enantiomer over the concentration range of 900–6000 ng/ml, with the linear regression equation $y = 27.08173X - 386.46288$ (correlation coefficient $R = 0.99987$). Linearity was checked for (*R*)-enantiomer over the same concentration range for three consecutive days. The %R.S.D. of the slope and *Y*-intercept of the calibration curve were 1.7 and 9, respectively (Table 2).

The standard addition and recovery experiments were conducted for (*R*)-enantiomer in bulk samples in triplicate at 0.4, 0.5 and 0.6% of analyte concentration. Recovery was calculated from slope and *Y*-intercept of the calibration curve obtained in linearity study and percentage recovery was ranged from 97.3 to 102.0 (Table 3).

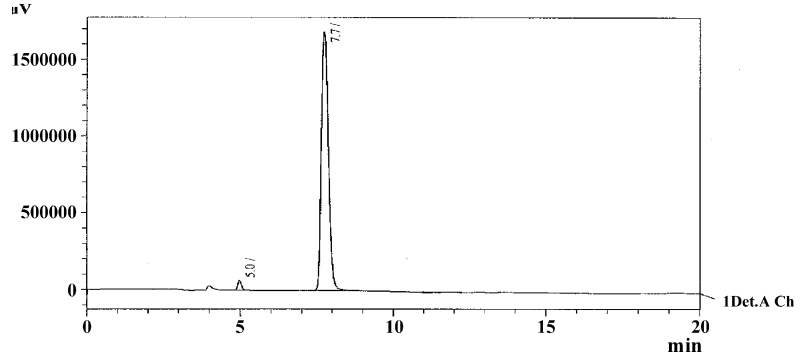


Fig. 3. Typical HPLC chromatogram of Pramipexole dihydrochloride monohydrate bulk sample (1.0 mg/ml) spiked with (*R*)-enantiomer (0.5%).

Table 3
Recovery results of (*R*)-enantiomer in bulk drugs

Added (ng) ($n = 3$)	Recovered (ng)	%Recovery	%R.S.D.
4001	3893	97.3	2.8
5003	5103	102.0	2.6
6007	5941	98.9	2.2

$n = 3$ determinations.

Table 4
Robustness of the chiral LC method

Parameter	USP resolution between Pramipexole and (*R*)-enantiomer
Flow rate (ml/min)	
0.8	9.1
1.0	8.8
1.2	8.6
Column temperature (°C)	
20	9.2
25	8.8
30	8.5
Ethanol percentage in mobile phase	
29	9.0
30	8.8
31	8.7

A HPLC chromatogram of spiked (*R*)-enantiomer at 0.5% level in Pramipexole dihydrochloride monohydrate sample was shown in Fig. 3.

The chromatographic resolution of Pramipexole and (*R*)-enantiomer peaks was used to evaluate the method robustness under modified conditions. The resolution between Pramipexole and (*R*)-enantiomer was greater than 8.0, under all separation conditions tested (Table 4), demonstrating sufficient robustness.

No significant change in the (*R*)-enantiomer content was observed in Pramipexole sample during solution stability and mobile phase stability experiments. Hence, Pramipexole sample solution and mobile phase are stable for at least 48 h.

4. Conclusion

A simple, rapid and accurate normal phase chiral LC method was described for the enantiomeric separation of Pramipexole dihydrochloride monohydrate. Amylose based chiral columns Chiralpak AD column found to be selective for the enantiomers of Pramipexole dihydrochloride monohydrate. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for the quantitative determination of chiral impurity ((*R*)-enantiomer) in bulk materials.

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