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Gradient Ultra Fast Liquid Chromatographic Analysis of Pramipexole and its Application to Drug Quality control

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ABSTRACT

A rapid, sensitive and selective method for the determination of Pramipexole in pure drug and in tablets was developed using gradient Ultra Fast Liquid Chromatography (UFLC). The devised method involved separation of Pramipexole (PRM) on a Reversed Phase Waters Symmetry Column and determination with UV detection at 260 nm. The standard curve was linear (R = 0.999) over the concentration range of 50–300µg $mL^{-1}$with a detection limit of 0.04 mg mL–1 and a quantification limit of 0.16 mg mL^{-1}. Intra-day and inter-day precision and accuracy of the method were established according to the current ICH guidelines. Intra-day and interlay of RSD values at three QC levels (100, 150 and 200 mg mL^{-1}) were 0.02–0.05%, based on the peak area. The intra-day relative error (e^r) was between 0.01 and 0.2%. The developed method was successfully applied to the determination of PRM in tablets and the results were statistically compared with those obtained by a literature method. Accuracy evaluated by means of the spike recovery method, was the excellent with percent recovery in the range 97.7–103.2 with precision in the range 1.6–2.2%. No interference was observed from the co-formulated substances. The method was economical in terms of the time taken and the amount of solvent used.

Keywords: pramipexole, gradient UFLC, pharmaceuticals

INTRODUCTION

Pramipexole (Fig 1) (1) is a new drug used in therapy of Parkinson's disease. Chemically it is (S)-2-amino-4,5,6,7-tetrahydro-6-(propylamino)benzothiazole-2,6-diamine, a non-ergoline dopamine agonist, initially introduced for the treatment of early and advanced Parkinson's disease and recently approved in US and Europe also for the treatment of idiopathic restless legs syndrome in adults(2).

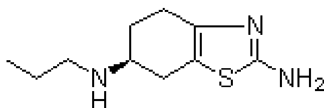


Fig.1

Parkinson's disease (PD) is chronic neurodegenerative disease characterized by bradykinesia, predominantly affecting the elderly, for which only symptomatic treatments are currently available. In the clinic, Parkinson's disease is viewed primarily as a disorder of the nigrostriatal dopaminergic pathway presenting symptoms involves motor disturbances that can be modulated with dopamine agonists. It occurs when certain nerve cells (neurons) in a part of brain called substantia niagra die or become impaired. Normally, these neurons produce a vital chemical known as dopamine which allows smooth,coordinated function of the body's muscles and movement(3).

Few HPLC methods were reported in the literature for the quantitative determination of PRM and its related substances in bulk drug and pharmaceuticals (4,5) ,in human plasma with atmospheric pressure chemical ionization tandem Mass spectroscopy(6) with Electrochemical and Ultraviolet detection in human plasma and urine(7),a chiral liquid chromatographic method for enantiomeric separation in bulk drugs (8) and a Capillary electrophoresis' method with laser induced fluorescence detection (9). A simple UV spectrophotometric method for the determination of PRM in Pharmaceuticals is also reported in the literature (10). Further, a Chromatographic method, for determination of dissociation constants of PRM and its impurities (11), for determination of PRM and its two impurities is also reported(12).Further more HPLC-MS/MS method are also reported in the literature(13-14). Some of the reported methods however suffer from such disadvantages as poor selectivity, sensitivity, accuracy and precision (Table I).

This paper deals with the Development and Validation of a sensitive gradient UFLC method for the assay of PRM in pharmaceuticals. Separation and determination were done on a Reverse Phase Waters Symmetry C_{18} column and UV detection at 260 nm. .

MATERIALS AND METHODS

Apparatus

The separation was carried out on gradient UFLC system(Shimadzu Prominence) with Shimadzu quaternary LC20AD pump, Shimadzu PDA(SPD-M20A) UV-Visible absorbance detector,Spinchrom software and Rp-C_{18} ,Waters Symmetry column (150mmX 4. 6mm I.D; particle size 5μ)

Reagents and standards. – All chemicals used were of analytical reagent grade. Trifluoroacetic acid (from S.D Fine Chemicals. Ltd, India) and HPLC grade methanol (from Merck. Ltd, India) were used. Distilled water filtered through a 0.45 mm filter (Millipore, India) was used to prepare solutions. The mobile phase consisting of 0.1% Trifluoroacetic acid in water (mobile phase A) and methanol (mobile phase B) (50:50) was prepared and used, the same was used as diluent .Pharmaceutical grade PRM, certified to be 99.8% pure, was supplied as gift sample by Aurobindo Pharma,Hyd, India, and was used as received. For the study, an accurately weighed 50 mg of PRM was dissolved and diluted to the volume with the diluent solution in a 50 mL calibrated flask to obtain a concentration of 1000**µ**g mL^{-1} PRM.

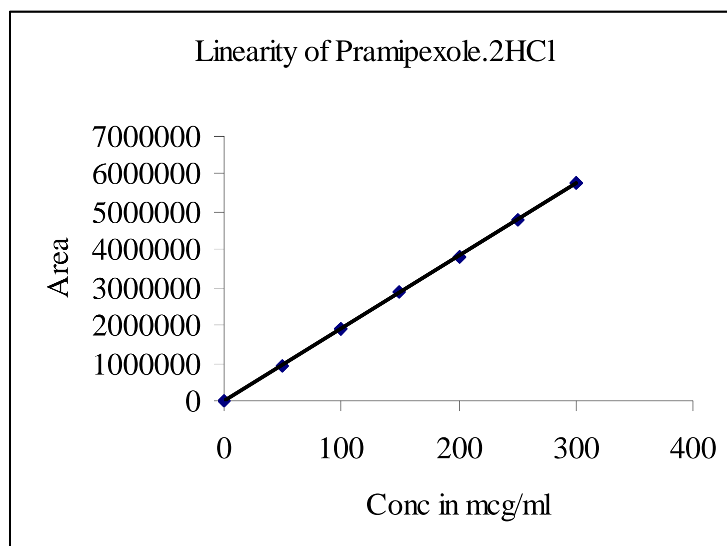
Procedures

Chromatographic conditions. – Separation was achieved at ambient temperature on the column using the mobile phase at a flow rate of 0.8 mL min^{-1}. The detector wavelength was set at 260 nm with sensitivity of 0.2 a.u.f.s. Gradient composition was as follows:

Time(min)	**0.01**	**5.00**	**9.00**	**12.00**	**16.00**	**22.00**
A (%)	90	90	30	30	90	90
B (%)	10	10	70	70	10	10

Calibration.–Working standard solutions equivalent to 50 to 300 µg mL^{-1} PRM were prepared by appropriate dilution of the stock standard solution (1000 µg mL^{-1}) with the diluent solution. 10µL aliquot of each solution was injected automatically onto the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area *vs.* PRM concentration.The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the mean peak area-concentration data.

Conc in µg/ml	**Area**
50	948760
100	1905432
150	2868062
200	3825379
250	4787678
300	5749591



Assay in dosage forms. – The following formulations containing PRM were purchased from local commercial sources and used in the investigation: One brand of Movexol tablets (Lupin Pharma, India) containing 0.5 mg of PRM, and 1.0 mg of PRM. A quantity of tablet powder equivalent to 50 mg of PRM was accurately weighed into a 50 mL calibrated flask, 30 mL of diluent solution was added and the content was shaken for 20 min; the volume was then diluted to the mark and mixed well. A small portion of the extract (say, 10 mL) was withdrawn and filtered through a 0.2-mm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as already mentioned..

Recovery experiment. – To a fixed and known amount of the drug in tablet powder (pre-analyzed), pure PRM was added at three different levels, and the total was found by the proposed methods from which the percent recovery of pure drug added was calculated.

Selectivity testing. – A separate selectivity test was performed by applying the proposed methods to the determination of PRM in a synthetic mixture consisting of PRM, talc, starch, lactose, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate, in the mass ratio of 1: 2.5: 3.0: 0.3: 0.5: 0.2: 0.7: 1 PRM was extracted with three 20-mL portions of diluent and filtered. The filter was washed with diluent; the filtrate and washings were collected in a 100-mL calibrated flask and diluted to the volume with diluent and mixed well. An appropriate aliquot of the extract was subjected to analysis as stated earlier.

Table 1.: Comparison of the proposed method with existing HPLC and other methods

Method	Experimental details	Detection	Linear range(μg^{-1}ml)	QL	Remarks	Ref
HPLC	C_{18} column with mobile phases containing different ratios of acetonitrile and water phase (aqueous triethylamine/ orthophosphoric acid)	UV at 262 nm	NA	NA	Less precise	4
RP-HPLC	acetonitrile/phosphate buffer (60/40 ; v/v) with a flow rate of 0.8 mL min^{-1}	UV detection	NA	4.5 μgmL^{-1}	Less sensitive	5
HPLC-MS-MS	Zorbax SB-CN column with a mobile phase of (15:5:80) water-0.1M ammonium acetate-methanol	with Atmospheric pressure chemical ionization (APCI) tandem Mass spectrometry (MS-MS)	50 to 5000 pg/ml	NA	Sensitive and accurate, wide range of linearity	6
HPLC	ion-pair chromatography on a Zorbax Rx C_8 column	Electrochemical detection at 0.6 V for plasma and ultraviolet detection at 286 nm for urine	50 to 15,000 pg/ml (plasma) 10 to 10,000 ng/ml (urine	NA	Less precise,intra-day (n = 6) and overall (n = 18) mean values for the quality control samples being less than 6.4 and 5.8%	7
Chiral liquid chromatographi c method	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).	NA	NA	900 ngmL-1,	percentage recovery of (R)-enantiomer was ranged from 97.3 to 102.0. Less precise	8
Capillary electrophoresis	uncoated fused silica capillaries (75mm internal diameter, 75.0 and 60.0cm total and effective length, respectively),with a background electrolyte composed of borate buffer (50mM, pH	20 kV	25.0–1000 ngmL-1	25.0 ngmL-1	precision was =6.8 R.S.D.%, accuracy expressed as recovery% was >90.0 Less sensitive	9

	10.3),tetrabutylammonium bromide (30 mM), and acetone (15%, v/v)					
UV Spectrophotometry	UV method in methanol as solvent	UV 261 nm	4-60µg/ml		Simple and economical	10
HPLC	Stationery phase stable in a wide pH range Triethylamonium phosphoric buffer was selected as appropriate pH controlling solution because it can cover a wide pH range	UV,262 and 326 nm	NA	NA	Applied for the determination of dissociation constant of pramipexole and its impurities	11
HPLC	C18 column and the mobile phase containing 1-octanosufonic acid salt were chosen.	NA	NA	NA	applied in testing the stability of pramipexole under stress conditions like, the effect of oxidation on API and stability in the acidic and alkaline conditions	12
HPLC-MS/MS	NA	MS/MS in the multiple reaction monitoring mode using the respective [M + H]+ ions	200-8000 pg/mL	200 pg/mL	Application in pharmacokinetic, bioavailability or bioequivalence studies.	13
HPLC-MS/MS	0.01 m ammonium acetate buffer (pH 4.4):acetonitrile (30:70, v/v) on a Discovery CN column	Multiple-reaction Monitoring mode (MRM) using the electrospray ionization technique	20-3540 pg/mL	NA	Sensitive and accurate	14
UFLC (Gradient)	Waters, Symmetry, C18 column (150 ´ 4.6 mm i.d.)	UV-at 260 nm	50-300µgmL-1		Wide linear range, highly precise (intra-day and interday RSD <0.03%) and (er <0.2%)	This paper

RESULTS AND DISCUSSION

Method development

A solution of PRM was injected in duplicate onto the column and was monitored by UV-detection at 260 nm. A gradient method was selected rather than an isocratic one to get faster elution with less retention time. At a flow rate of 0.8 mL min^{-1}, the retention time was 3.625 min. Under the depict experimental conditions, the peak was well defined and free from tailing. PRM was determined by measuring the peak area. Plot of the mean peak area against concentration gave the linear relationship (R = 0.999, N = 5) , over the concentration range 50–300 µg mL^{-1}. Using the regression analysis, the linear equation, Y = –184.40 + 57.88 g was obtained, where Y is the mean peak area and g is concentration in mg mL^{-1}. The limits of detection and quantification calculated according to ICH guidelines were 0.06 and 0.18 µg mL^{-1}, respectively

Method validation

In order to determine the adequate resolution and reproducibility of the method,
Suitability parameters, including retention time, plate number and tailing factor, were investigated and were found to be 3.625 min, 5248 and 1.64, respectively, which amply demonstrates the method suitability. Retention time varied for 0.2%.

Specificity. – Specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix (15–17). Specificity was evaluated by preparing an analytical placebo and it was confirmed that the signal measured was caused only by the analyte. A solution of analytical placebo (containing all the tablet excipients except PRM) was prepared according to the sample preparation procedure and injected**.**

The resulting chromatogram did not show any peak. To identify the interference by these excipients, the tablet extract after appropriate dilution was chromatographed. The resulting chromatogram did not show any peak other than that of PRM (Fig. 2a), which confirmed the specificity of the method. In addition, the slope of the calibration curve for standards was compared with that prepared from the tablet extract. It was found that there was no significant difference between the slopes, which indicated that excipients did not interfere with PRM.

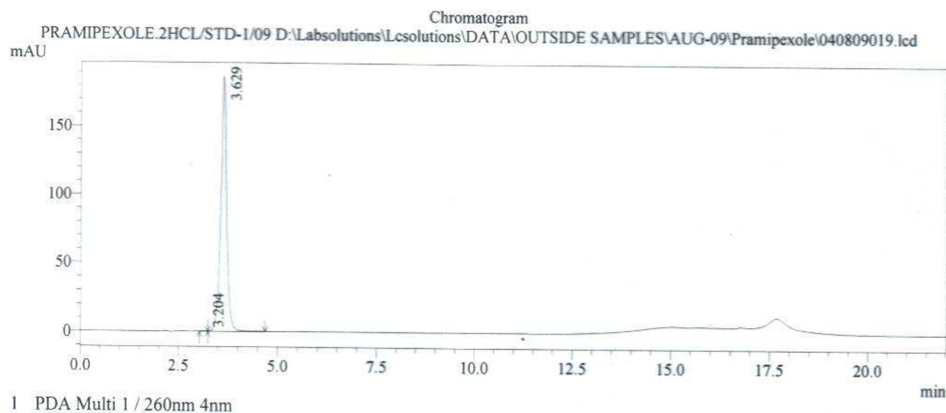


Fig: 2a—Chromatogram of PRM (Pure Drug)

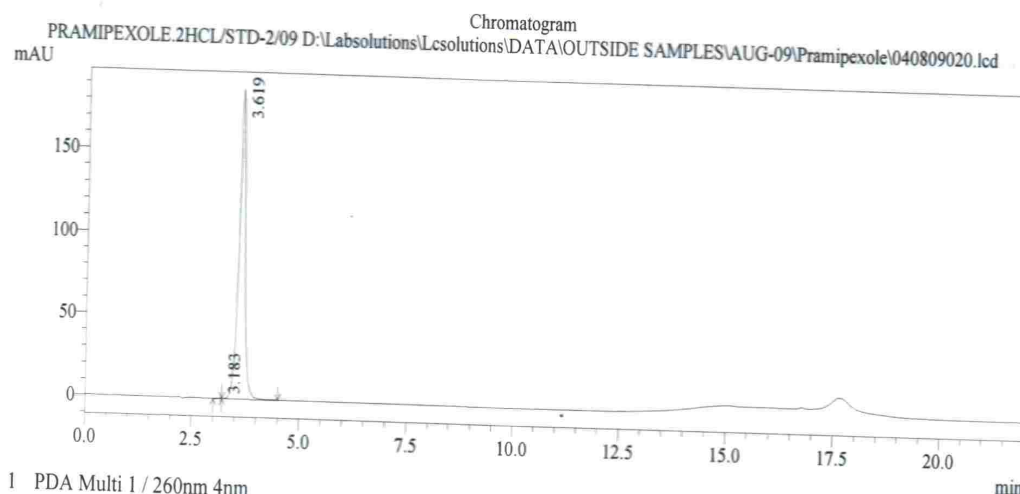


Fig: 2b—Chromatogram of PRM (Tablet Formulation)

Precision. – Precision of the method was evaluated in terms of intra-day and inter-day precision(15-17). Three different concentrations of PRM were analyzed in seven replicates on the same day (intra-day precision) and in five consecutive day (inter-day precision).Within each series, every solution was injected in triplicate. The peak-area based intra-day RSD values were 0.02–0.03%. The results of the study compiled in Table IIA are quite satisfactory. The inter-day precision showed somewhat higher RSD values of 0.01–0.04%.Intermediate precision was also evaluated by calculating the RSD values of six replicate determinations performed in standard PRM solutions by three different analysts with two different instruments. The inter-analyst RSD values were in the range 0.82–1.1% where the inter-instrument RSD values were 1.0 and 1.1%, respectively, for the two instruments used**.**

Accuracy. – Accuracy of an analytical method expresses the closeness between the reference value and the found value (15–17). The results obtained for *e*r at three concentrations (within the linear range) are shown in Table IIA IIB and were 0.01 to 0.2%. Accuracy was assessed by analyzing the synthetic mixture (prepared by adding PRM to the placebo) as described earlier.

The calculated percent recovery of the active ingredient was found to be 99.6 ± 0.8 (n = 5) indicating that the co-formulated substances did not interfere with the assay.

Robustness. – Robustness of the method was checked by deliberately altering the flow rate from 0.8 mLmin^{-1} to 0.9 mL min^{-1} and 0.7 ml^{-1}. The differences in the retention time and peak area (for a given PRM concentration) caused by the above minor alterations were insignificant.

Application

The developed and validated method was applied to the determination of PRM in two brands of tablets, containing 2.5mg and 5 mg per tablet. Evaluation was performed using the calibration curve method, since no significant difference between the slopes of the calibration curves for standards and tablet extracts was observed. The results obtained by the proposed method were statistically compared with those of the literature (UV-Spectrophotometry) method (10) by applying Student's *t*-test for accuracy and *F*-test for precision. As shown by the results compiled in Table III, the calculated *t*- and *f*-values did not exceed the tabulated values at the 95% confidence level for four degrees of freedom, suggesting that the proposed method and the literature method did not differ significantly with respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analyzed tablet powder was spiked with pure PRM at three different levels and the total was found by the proposed methods. Each determination was repeated three times. Recovery of the pure drug added was in the range 97.7–103.2%, with the RSD values of 0.016–0.04%. The results of this study given in Table IV reveal that the common tablet excipients did not interfere with the determination. The main features of the method are its wide linear dynamic range, high sensitivity, as shown by the *LOQ* value, and high accuracy and precision, as revealed by the recovery study and intra-day and inter-day precision studies.

Table II.A. Relative error and intra-day precision

PRM taken(μg ml^{-1})	PRM found (μg ml^{-1})[a]	er(%)	RSD(%)[b]
100	100.02	0.02	0.022
150	149.99	0.01	0.028
200	199.81	0.19	0.026

[a] Mean value of seven determinations.
[b] Based on peak area.

Table II.B. Relative error and inter-day precision

PRM taken(μg ml^{-1})	PRM found (μg ml^{-1})[a]	er(%)	RSD(%)[b]
100	100.02	0.02	0.022
150	150.72	0.72	0.036
200	199.81	0.19	0.018

[a] Mean value of seven determinations.
[b] Based on peak area.

Table III. Determination of Pramipexole hydrochloride in tablets and comparison with the reference method

Formulation	Nominal amount(mg)	Found(%)			
		Literature method	Proposed method	t-value	F-value
T_1	0.5	100.04±0.6	100.2±1.3	0.82	2.67
T_2	1.0	98.75±1.2	101.2 ±1.2	1.64	1.95

[a]Mean ±SD, n=5
Tabulated t-value at 95% confidence level is 2.17
Tabulated f-value at 95% confidence level is 6.02
T_1 and T_2 are the tablets formulations of the same brand (Moverol)

Table IV. Recovery Study

Formulation Studied	PRM in Formulation (μgmL^{-1})	Pure PRM added (μgmL^{-1})	Total found (μgmL^{-1})	Pure PRM Recovered(%)[a]
A	50.2 50.6 50.5	50 100 150	100.02 149.98 200.02	99.52 99.55 100.02
B	55.3 55.6 55.7	50 100 150	105.02 154.55 204.99	99.65 99.34 99.86

[a]Mean ±SD, n=3

CONCLUSION

In conclusion, a gradient reversed-phase UFLC-UV assay was developed for the determination of Pramipexole and validated as per the current ICH guidelines. The method is simple, precise and accurate, selective and sufficiently sensitive compared to many similar methods reported earlier (Table I). Hence, it seems suitable for the determination of the drug either in bulk or in tablets without interference from commonly used excipients and could be used in a quality control laboratory.

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