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Development and validation of new RP-HPLC method for estimation of pramipexole dihydrochloride in bulk and pharmaceutical formulation

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Abstract

A novel high-performance liquid chromatographic assay method was developed and validated for the quantitative determination of the anti-Parkinson agent pramipexole dihydrochloride monohydrate in bulk and its tablet dosage form. In this perspective, the chromatographic separation was accomplished on Eclipse XDB-12 C18 (150 mm x 4.6 mm, 5 µm particle size) column using UV detection at 263 nm. The mobile phase consisted of distilled water: acetonitrile (10: 90 v/v), run at a flow rate of 1.0 mL/min with isocratic elution. The method was validated in accordance with ICH guidelines by evaluating the system suitability, linearity, limits of detection (LOD) and quantitation (LOQ), precision, accuracy, specificity, selectivity and short-term stability. Our findings revealed that retention time for pramipexole dihydrochloride was found to be 5.2 minutes. The linearity range was established between 6.25-225.0 µg/mL with a mean recovery of 101.26 % ± 0.56. The limits of detection and quantification were determined to be 4.18 µg/mL and 12.66 µg/mL, respectively, indicating that the method is very sensitive. Intra and inter-day precision were within acceptable limits (RSD<2, n=6) and the typical excipients included in the pharmaceutical product did not interfere with the selectivity of the method. The proposed method was found to be simple, specific, accurate, precise and could be applied to the quantitative analysis of pramipexole dihydrochloride monohydrate in a bulk and in a its tablet dosage form.

Keywords

HPLC method development, pramipexole dihydrochloride, recovery, ICH.

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INTRODUCTION

Pramipexole (PRA) is a non-ergot dopamine agonist with high relative in vitro selectivity and full intrinsic activity at the D2 subfamily of dopamine (Dooley and Markham, 1998). The molecular weight of PRA is 302.3 g/mol, and its chemical name is (S)-2-amino-4,5,6,7-tetrahydro-6-(propyl amino) benzothiazole dihydrochloride monohydrate (Rambhade *et al.*, 2010*)* . The melting point of PRA is 296-305°C, while its solubility in water is 61 mg/mL, in DMSO is 41 mg/mL, and in ethanol is 1 mg/mL.

Its elimination half-life is around 8-12 hours (Benbir and Guilleminault, 2006). PRA is a drug used to treat the symptoms of Parkinson's disease (PD), a neurological disorder that causes difficulties with movement, muscle control, and balance, including body shaking, stiffness, slower motions, and balance deficits (Goldenberg, 2008).

Recently new therapeutic potential of PRA has been associated with restless legs syndrome (RLS; Willis–Ekbom illness) a sensory motor disorder characterized by strong need to move the leg, which is generally accompanied by unpleasant sensations. RLS symptoms are present during rest, subside with movement, and are usually at their worst in the evening or night. RLS is a prevalent disorder that affects about 5% and 15% of the population, and its frequency has been shown to increase with age (Deleu *et al.*, 2002; Lipford and Silber, 2012). RLS responds well to treatment, particularly to drugs that boost dopamine (DA) neurotransmission. PRA works by replacing dopamine, a natural substance found in the brain that governs movement, confirming that it belongs to the dopamine agonist drug class, despite this, the US Food and Drug Administration has only licensed one agonist, ropinirole, for use in the treatment of RLS (MacKie and Winkelman, 2015; Silber *et al.*, 2004).

The development and validation of methods for quantifying and identifying pharmaceutical active ingredients are key components of drug quality control (QC). Because of its relevance, the development of novel testing procedures for drug determination has gained substantial attention in recent years, particularly in assessing the potency of active ingredients.

Today, the literature reports a wide number of analytical procedures for assessing of PRA, ranging from spectrophotometric approaches (Gurupadayya *et al.*, 2009; Dey *et al.*, 2012; Muthu *et al.*, 2013; Thangabalan *et al.*, 2011), to HPLC methods (Pawar *et al.*, 2013; Sevim and Erk, 2015; Panditrao *et al.*, 2011; Pathare *et al.*, 2006), and GC/MC (Panchal *et al.*, 2011).

For routine QC testing of drugs, utilizing analytical methods that are not difficult, time consuming, and can be done with a lower cost make the analytical method more favorable and useful. The primary goal of this work was to validate and extend a new simple, effective, accurate, adaptable, and repeatable method for obtaining consistent results with similar input data for regular QC testing of PRA and its tablet dosage form. HPLC was utilized because of its precision, sensitivity, repeatability, and accuracy.

MATERIALS AND METHODS

PRA was obtained from Deva (Turkey). F-Melt® (Fuji Chem, Japan), Pearlitol® Flash (Roquette, Lestrem, France), Pharmaburst® 500 (SPI Pharma, New Castle, USA), Prosolv® Easytab SP (JRS Pharma, Rosenberg, Germany), Ludiflash® (BASF, Ludwigshafen, Germany), and Parteck® ODT (Merck, Darmstadt, Germany) ready-to-use ODT (Orally Disintegrating Tablet) excipients were used as received. Acetonitrile (ACN) was HPLC grade and purchased from Merck (Darmstadt, Germany). Double distilled water has been used for all experiments.

Instrumentation and chromatographic conditions

The Agilent 1260 Infinity HPLC system (Wilmington, DE, USA) was used for this study, which was outfitted with a solvent degasser, quaternary pump, auto sampler, column oven, and diode array detector. Agilent Chem Station software was used to process the data. The chromatographic separation in this item was achieved using an Eclipse XDB-12 C18 (150 mm x 4.6 m particle size) column with UV-detection at 263 nm wavelengths (λmax). The mobile phase consisted of distilled water: ACN (10: 90 v/v), run at a flow rate of 1.0 mL/min with 10 µL injection volume and isocratic elution.

Standard solutions and preparation of the samples

A standard stock solution was prepared by dissolving 10 mg of PRA in 10 mL of distilled water: ACN (10:90 v/v) mobile phase mixture. The solution was immersed in an ultrasonic bath (Selecta Ultrasound HD, Spain) for 30 minutes to achieve total dissolution.

Analytical method validation

The method has been validated in terms of linearity, limits of detection-LOD and quantitation-LOQ, precision, accuracy, specificity, and selectivity in accordance with ICH guidelines (The International Conference on Harmonization of Technical Requirements for Registration of

Pharmaceuticals for Human Use) (ICH, 2005). Linear calibration curve of the proposed method was evaluated by fitting least-squares regression analysis obtained by diluting stock solution with (10:90 v/v) mobile phase mixture and concentrations were 0.00 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 75 µg/mL, 125 µg/mL and 225 µg/mL.

The specificity of the method was determined by analyzing chromatograms of excipient(s) interfering with PRA determination. To achieve this, drug-free excipients solution, PRA bulk solution, and mobile phase chromatograms were injected into the chromatographic process.

By comparing theoretical and experimental data of three PRA concentration levels with concentrations of 10 µg/mL, 100 µg/mL, and 200 µg/mL, the accuracy of the analytical technique was determined.

Intermediate precision was tested by two consecutive days and by two different analysts preparing six solutions of the 10 µg/mL same concentration and injected to HPLC system. All results were evaluated in terms of standard deviation (SD) and relative standard deviation (RSD).

The limits of detection and quantification value was determined based on the standard deviation (SD) of the responses and the slope (S). Equations (1) and (2) were used to calculate LOD and LOQ values.

$$LOD = 3.3\ SD/S \quad (1)$$

$$LOQ = 10\ SD/S \quad (2)$$

Assay procedure for analysis in tablet dosage form

Drug contents of the PRA in tablet dosage form was determined by weighing of twenty tablets and then finely powdered them in the mortar. A powder containing 10 mg of PRA was precisely weighed and placed into a 10 mL volumetric flask. Appropriate dilutions were made with the mobile phase. To obtain full dissolving of PRA at yield concentrations of 50 µg/mL, the solution was sonicated for 20 minutes. The resultant solution was then passed through 0.45 µm membrane filters before being injected to HPLC analysis.

Short-term stability of PRA

A solution of 50 µg/mL concentration of PRA was prepared from the stock solution. The prepared solution was kept at 37 °C for 48 hours. Samples were taken at 0, 24, and 48 hours, and HPLC analyzes were performed (n=3).

RESULTS AND DISCUSSION

Preliminary experiments were undertaken to determine suitable and optimal conditions to design an effective and easy RP-HPLC method for the analysis of the drug in bulk and tablet dosage forms. HPLC variables such as detection wavelength, optimum mobile phase & proportions, and flow rate were thoroughly investigated. For the trials, a variety of solvent combinations were utilized, including: Methanol: Distilled water; 10:90 v/v (Thangabalan *et al.*, 2011), Methanol: ACN; 10:90 v/v, and Ammonium Acetate Buffer (pH 4.4): ACN; 35:65 v/v (Sevim and Erk 2015) showing unsatisfactory results. The combination of ACN and distilled water (50:50 v/v, 60:40 v/v, 70:30 v/v, 80:20 v/v, and 90:10 v/v) yielded the best results, notably when ACN: distilled water (90:10 v/v) was utilized, which generated a well-defined peak and retention duration (5.2 minutes) for PRA. Table 1 summarizes the HPLC conditions, retention time, and symmetry factor used for this study.

Table 1: Data for optimized RP-HPLC method.

Parameters	
Mobile phase	Acetonitrile : Distilled water (90:10, v/v)
Flow rate	1.0 mL/min
Injection volume	10 µL
Wavelength	263 nm
Dilution solvent	Mobile phase
Retention time for PRA	5.2 min
Symmetry factor for PRA	0.23

By graphing the Area Under Curve (AUC) of PRA, a calibration curve was produced using the least squares approach. In the concentration range of 6.25-225.00 µg/mL, the calibration curves for PRA developed high linearity with an excellent regression coefficient (R^2=0.99). Figure 1 depicts the linearity findings.

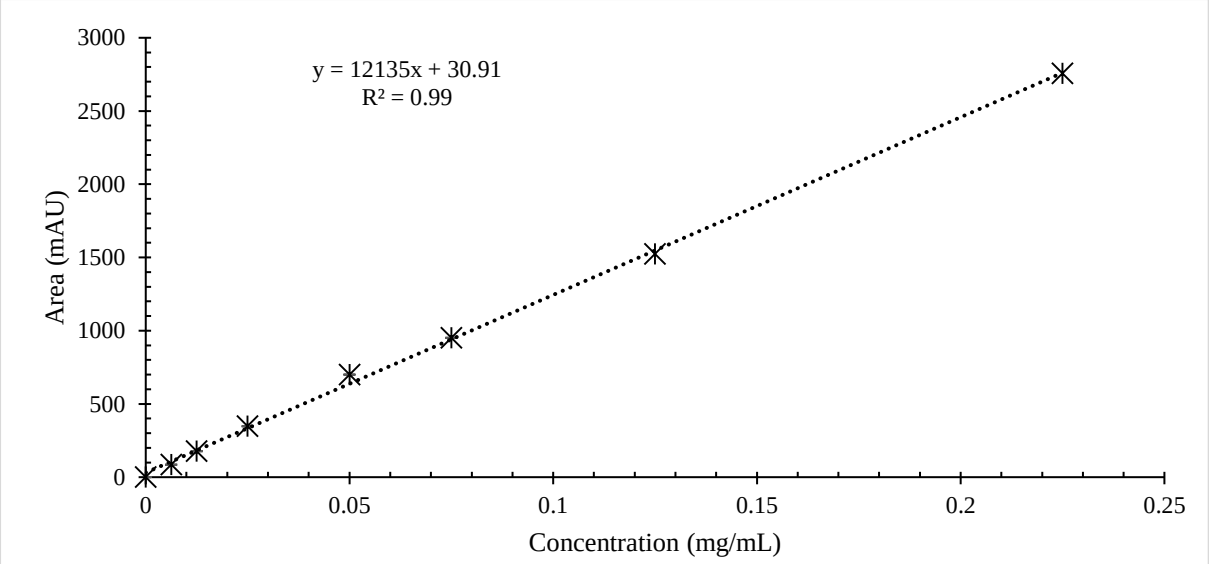

Figure 1: Calibration curve for PRA.

Specificity

Based on the comparation of the chromatograms of placebo (drug-free mixture of excipients), PRA solution and constituents of mobile phase, the methodology for specificity was determined to be unique. Figure 2 illustrates that no interference from excipients was found in the resulting derivative spectra and no other peak was observed other than the standard solution.

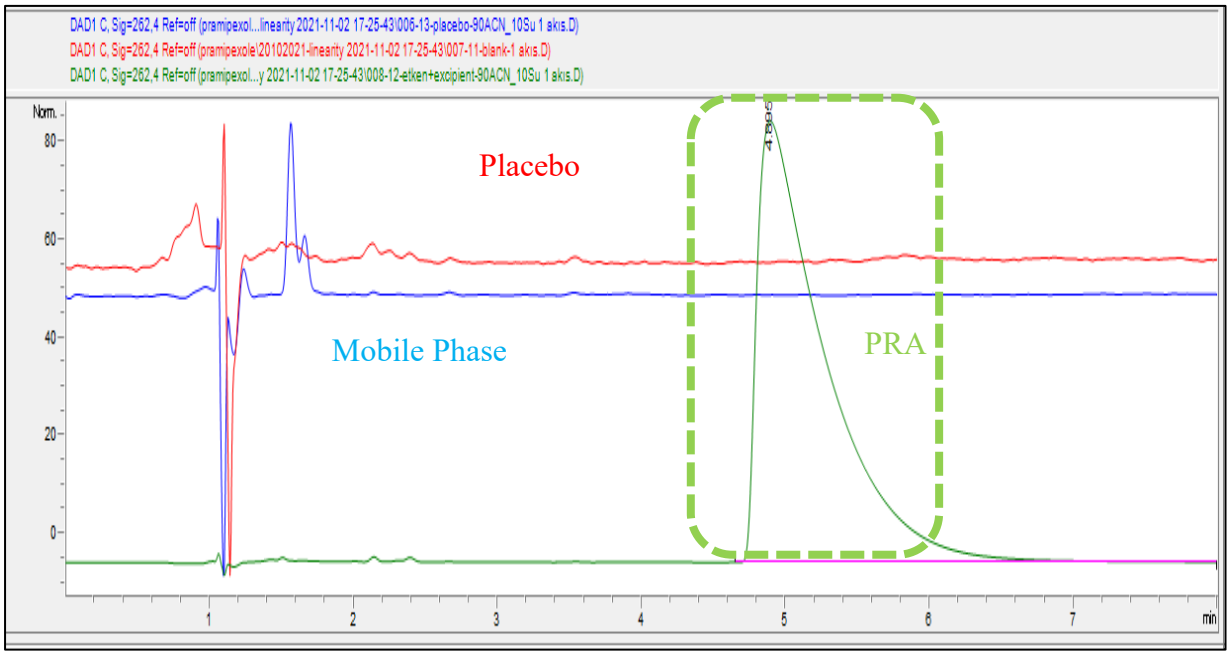

Figure 2: Specificity of the developed HPLC for PRA.

Accuracy and recovery

Using a stock solution containing PRA, three concentration sets (high, medium, and low) were prepared to test the accuracy of the analytical process. Using HPLC and first derivative spectroscopy techniques, the accuracy of the HPLC technique was determined and expressed as percent recovery. According to Table 2, percentage of total recovery values measured for PRA is below 2%, showing the accuracy of the process. The mean recovery and RSD data for the HPLC method were 100.50% and 1.10%, respectively.

Table 2: Recovery results for PRA convert

Drug	n	Theoretical concentration of the PRA (µg/ml)	Practical concentration of the PRA (µg/ml)	Recovery (%)	RSD (%)
	6	10.00	9.00	92.86	0.31
PRA	6	100.00	109.00	109.36	0.57
	6	200.00	203.00	101.55	0.79

Intermediate precision

There was no difference in peak area higher than 2% between the two successive days, showing that the procedure was very reproducible. The results (RSD values less than 2%) for intermediate precision reviewed by two analysts over two consecutive days met the precision criterion (Venkata Rajesh *et al.*, 2013). The intermediate precision results are presented in Table 3.

Table 3: Intermediate precision checked by two analysts and two different days.

Drug		1. Analyst	2. Analyst	1. Day	2. Day
PRA	Theoretical concentration: 100 µg/mL (n=6)	90.00	90.00	100.00	98.00
	RSD (%)	0.91	0.41	0.52	0.28

RSD: Relative standard deviation.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ values were determined using the above-mentioned equation to evaluate the method's sensitivity. Table 4 shows that the approach was found to be sensitive enough to evaluate PRA in low concentrations level.

Table 4: Limits of detection (LOD) and quantitation (LOQ) for PRA

	PRA (µg/mL)
Limits of detection - LOD	4.18
Limits of quantitation - LOQ	12.66

Assay procedure for analysis in tablet dosage form

A significant level of agreement with the labeled quantity was demonstrated. Table 5 presents the PRA analysis findings for Pexola® Tablet 1.0 mg using the established HPLC method.

Table 5: Assay of PRA for its tablet form

Tablet form of PRA (Pexola®)	n	Recovery for PRA (%) ± RSD (%)
	6	94.00 ±2.10

RSD: Relative standard deviation

Short-term stability of PRA

The short-term stability test results revealed no change in retention time or deterioration in the peak characteristics of the observed HPLC peaks. Table 6 reveals that the drug remained stable at 37 °C for 48 hours with an RSD value less than 2%.

Table 6: Short-term stability results for PRA

Time	0. Hour	48. Hour	Average	RSD (%)
PRA (µg/mL)	51.20	50.01	50.60	1.23

CONCLUSION

Validation is widely acknowledged as a vital step in the development of an analytical method. Following the development of the method, it was tested in accordance with the ICH guidelines. Validations of the suggested method demonstrated to be simple, specific, accurate, and precise and as a result, it might be a reliable HPLC approach for regular PRA analysis in bulk and tablet dose form.

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