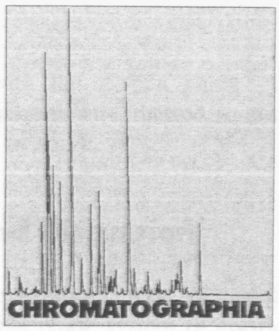
Method Development and Validation of a Rapid Determination of Ropinirole in Tablets by LC-UV



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Abstract

A reversed-phase high-performance liquid chromatographic (HPLC) method with UV detection was developed and validated for the determination of ropinirole (ROP) in tablets. The assay utilized UV detection at 250 nm and a Luna CN column (250 × 4.6 mm I.D, 5 µm). The mobile phases were comprised of acetonitrile: 10 mM nitric acid (pH 3.0) (75:25, *v/v*). Validation experiments were performed to demonstrate linearity, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), and robustness. The method was linear over the concentration range of 0.5–10.0 µg mL^{-1}. The method showed good recoveries (99.75–100.20%) and the relative standard deviations of intra and inter-day assays were 0.38–1.69 and 0.45–1.95%, respectively. The method can be used for quality control assay of ropinirole.

Keywords

Column liquid chromatography
Tablet analysis
Quality control
Ropinirole

Introduction

Ropinirole (ROP), 4-[2-(dipropylamino) ethyl]-l,3-dihydro-2H-indol-2-one hydrochloride, is a specific D2 and D3 receptor non-ergoline dopamine agonist that is probably equally effective as L-dopa in mild, early Parkinson's disease [1]. Very few high-performance liquid chromatographic (HPLC) methods in plasma were developed using ultraviolet [2, 3], electrochemical [4] or mass spectrometric [5] detection. Capillary zone electrophoresis was used for the determination of the dissociation constants of ROP and five structurally related impurities, potentially formed during its synthesis and for separation and quantification of these substances [6].

As to our best knowledge, an official monograph of ROP does not exist in any pharmacopoeia and determination of ROP in tablets has not been yet described. In the present study, an HPLC method with UV detection is described for the determination of ropinirole in tablets.

Experimental

Apparatus

The analyses were performed on a Thermo Separation Products Liquid Chromatograph (TX, USA) which consisted of a P4000 solvent delivery system equipped with a Rheodyne injection valve with a 20 µL loop, a UV3000 detector and an SN4000 automation software system. Separations were carried out at room temperature on a Luna CN column (250 × 4.6 mm I.D, 5 µm; Phenomenex, Texas, USA), with a guard column (4 × 3 mm I.D, Phenomenex, Texas, USA) packed with the same material. The mobile phase consists of acetonitrile: 10 mM nitric acid (pH 3) (75:25, *v/v*) at a flow rate of 1.0 mL min^{-1}. In these conditions, ROP retention time (t_R) was roughly 4 min. The injection volume was 20 µL and ultraviolet detection was at 250 nm.

Reagents and Solutions

Ropinirole hydrochloride and its pharmaceutical preparation (Requip®) containing 2 mg of ropinirole per tablet were kindly supplied by GlaxoSmithKline (Istanbul, Turkey). All solvents and reagents were of analytical or HPLC grade. HPLC-grade water was prepared by using AquaMAX-ultra water purification system from Young Lin Inst (Korea).

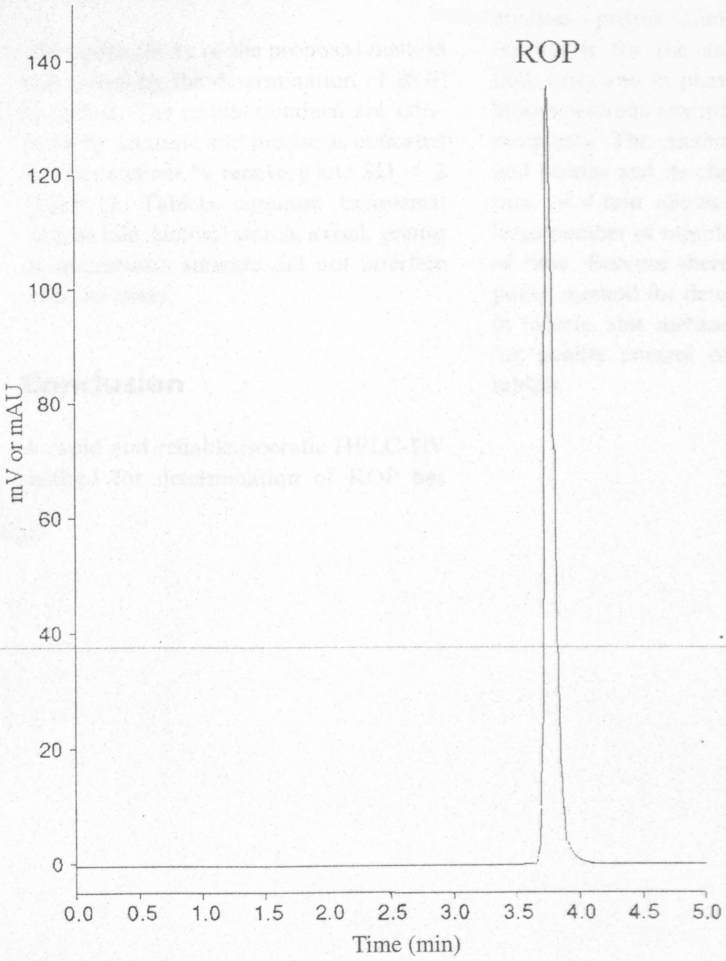

Fig. 1. A typical chromatogram of 5 μg mL^{-1} ROP standard, 20 μL injection

Table 1. Analysis of ROP in tablets

Formulation	Label claim (mg/per tablet)	Amount found (mg) ± SD (n = 5)	Recovery (%)	RSD (%)
Requip[a]	2	2.01 ± 0.04	100.50	0.77

SD standard deviation
[a]Marketed by GlaxoSmithKline

A stock solution of ropinirole hydrohloride (ROP) (1 mg mL^{-1}, calculated as free base) was prepared in water and this was further diluted with the mobile phase to give a working solution of 0.1 mg mL^{-1}.

Linearity

The calibration curve was obtained at six concentrations levels of ROP standard solutions (0.5–10 μg mL^{-1}). The solutions (20 μL) were injected to the HPLC system (n = 5) according to the chromatographic conditions previously given. The linearity was evaluated by the least square regression method.

Assay Procedure for Tablets

Twenty tablets were individually weighed to get the average weight of the tablets and powdered in the mortar. A sample of the powdered tablets, claimed to contain 10.0 mg of ROP was transferred to 10.0 mL volumetric flask. About 7.5 mL of water was added and then extraction was performed mechanically for 20 min and sonicated for 20 more minutes. The volume was brought to 10.0 mL with water. The content was centrifuged for 10 min at 3,000 × g, and then a 1 mL aliquot of the supernatant was diluted to 10 mL with the mobile phase. One milliliter of this solution was transferred into a 10 mL volumetric flask and diluted to the volume with the mobile phase. A 20 μL of its aliquot was injected to the HPLC system (n = 5).

Results and Discussion

Satisfactory resolution was obtained using the mobile phase system of acetonitrile: 10 mM nitric acid (pH 3) (75:25, v/v) at a flow rate of 1.0 mL min^{-1}. The UV-spectrum of the drug shows absorption band at 250 nm. Under the experimental conditions, the chromatogram of ROP (Fig. 1) showed a single peak of the drug around 4 min.

The calibration curve was prepared by plotting the peak area of ROP against drug concentration and was linear in the range of 0.5–10 μg mL^{-1}. Peak area and concentration were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression equation was found as $A = 125967\,C + 105211$ ($r = 0.9999, n = 5$) ($A = aC + b$, where A is the peak area of ROP, a is the slope, b is the intercept and C is the concentration of the measured solution in μg mL^{-1}).

Limit of detection (LOD) value was found as 0.1 μg mL^{-1} which is the concentration that yields a signal-to-noise ratio of 3:1. Limit of quantitation (LOQ) value under the described condition was 0.3 μg mL^{-1}, which is the signal-to-noise ratio of 10:1. The repeatability of sample application and measurement of peak area were expressed in terms of % RSD which revealed intra-day (n = 5) and inter-day (n = 5, at 5 different day) variation of ROP at three different concentration levels of 0.5, 5.0 and 10.0 μg mL^{-1}. The RSD values were found to be 0.38–1.69 and 0.45–1.95%, for intra- and inter-day variation, respectively, indicating good precision. To examine the accuracy of the method, recovery studies were carried out by standard addition method. The average percent recoveries obtained as 99.75–100.20% indicate good accuracy of the method. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method conditions. The composition of HPLC mobile phase, flow rates at 1 ± 0.05 mL min^{-1}, the effect of pH (varying ± 0.2 pH units) and two analytical columns were studied to evaluate for the robustness of the method. The low RSD values 0.56–1.57% were indicative of the robustness of the method.

Assay Procedure for Tablets

The applicability of the proposed method was tested by the determination of ROP in tablets. The results obtained are satisfactorily accurate and precise as indicated by the excellent % recovery and SD < 2 (Table 1). Tablets common excipients, such as talc, lactose, starch, avisel, gelatin or magnesium stearate did not interfere with the assay.

Conclusion

A rapid and reliable isocratic HPLC-UV method for determination of ROP has been developed and validated. Statistical analysis proves that the method is repeatable for the analysis of ROP as bulk drug and in pharmaceutical formulations without any interference from the excipients. The method was also linear and precise and its chromatographic run time of 4 min allows the analysis of a large number of samples in a short period of time. Because there is no pharmacopoeial method for determination of ROP in tablets, this method is recommended for quality control of drug content in tablets.

References

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