

# Analytical methodology for authentication of Ropinirole using HPLC and FT-IR

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## Abstract:

Ropinirole hydrochloride is a modern selective non-ergoline dopamine D2-like receptor agonist with affinity for D2, D3 and D4 receptor subtypes, indicated for the treatment of the signs and symptoms of Parkinson's disease and moderate-to-severe primary RLS (Restless Legs Syndrome). It has moderate in vitro affinity for the opioid receptors. Ropinirole HCl is weakly active at the 5-HT<sub>2</sub> and  $\alpha$ <sub>2</sub> receptors and is said to have virtually no affinity for the 5-HT<sub>1</sub>, benzodiazepine, GABA, muscarinic,  $\alpha$ <sub>1</sub> and  $\beta$ -adrenoreceptors. Ropinirole is metabolized primarily by cytochrome P450 CYP1A2, and at doses higher than clinical, is also metabolized by CYP3A4. At doses greater than 24 mg, CYP2D6 may be inhibited, although this has only been tested in vitro. Ropinirole HCl is a highly water soluble drug. UV spectroscopic studies show that ropinirole HCl gives maximum absorbance at  $\lambda$ <sub>max</sub> 250nm. Ropinirole HCl has been characterized by HPLC method which was achieved on a C<sub>18</sub>(250 x 4.6mm) 5 – micron Hypersil BDS using a mobile phase consisting of a degassed mixture of 0.05M glacial acetic acid (2.85 ml of glacial acetic acid in 1000 mL of water) and acetonitrile (50:50) with a flow rate of 1.0 mL/min. The mobile phase showed most favorable chromatographic parameter for analysis. The detection of the constituent was done using UV detector at 250nm. The retention time of ropinirole HCl was found to be 3.566 minutes. Ropinirole HCl was further characterized on the basis of FTIR studies.

**Keywords:** Ropinirole Hydrochloride, UV analysis, FTIR (Fourier Transform Infrared Spectroscopy), HPLC (High Performance Liquid Chromatography), Mobile Phase.

## Introduction

Ropinirole hydrochloride (RPR) is a potent, non-ergoline D2/D3 dopamine agonist. Chemically it is hydrochloride salt of 4-[2-(dipropylamino) ethyl] -1, 3-dihydro-2H-indol-2-one with empirical formula of C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O.HCl and molecular weight of 296.84. It is a white to pale greenish-yellow powder with a melting range of 243° to 250°C and a solubility of 133 mg/ml in water<sup>1</sup>. It 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<sup>2</sup>.

It has negligible affinity for the dopamine D1 receptor or other common neurotransmitter receptors, and is about 20 times more active at the central D3 than at the D2 receptor. Ropinirole

binds to both central and peripheral dopamine receptors. In the central nervous system, ropinirole binds to presynaptic dopamine D2 receptors, acting as a replacement stimulus for the dopaminergic neurotransmission. The action of ropinirole at presynaptic central dopamine receptors can reduce the turnover and release of dopamine from presynaptic terminals producing a neuroprotective effect by decreasing the amount of toxic metabolites and therefore oxidative stress. In the periphery, the action of ropinirole at dopamine D2 receptors increases the sympathetic activity tone. Activity at peripheral presynaptic dopamine receptors results primarily in hypotension and nausea, effects that are reduced by the slow titration of the dosage<sup>3</sup>.

Most of the absorbed dose of ropinirole is cleared by metabolism in the liver, with only 10% of the administered dose being excreted as unchanged ropinirole. The main enzyme responsible for ropinirole metabolism is the cytochrome P450 (CYP) enzyme CYP1A2, with a low affinity component CYP3A, making only a minor contribution. The excretion of ropinirole-derived products is predominantly via the urine, with a mean terminal elimination half life of about 6 hours (range 2 to 10 hours) independent of dose<sup>4</sup>.

## 2. Materials and methods

### 2.1 Reagents and chemicals

The gift sample of Ropinirole hydrochloride was obtained from Jubilant chemsys Noida. All the chemicals used throughout this work were of analytical grade and the solvents were of HPLC grade.

### 2.2. Analytical method Development

#### 2.2.1. Melting point

It is one of the parameters to judge the purity of crude drugs. In case of pure chemicals phytochemicals, melting points are very sharp and constant. Since the crude drugs contain the mixed chemicals, they are described with certain range of melting point.

**Procedure:** A small quantity of previously dried and finely powdered sample was placed into the melting point capillary tube to form a compact column (4 to 6 mm height). The temperature of the bath was raised to about 10°C below the expected melting point of the substance and then the rate of heating was adjusted to about 1 ±0.5°C per minute. When the temperature is about 5°C than the expected melting point, the capillary tube was placed into the bath. The heating was continued at 1°C per minute. The temperature at

which the column of the substance under test was observed to collapse definitely against the side of the tube at any point was defined as the beginning of melting, and the temperature at which the substance becomes liquid throughout was defined as the end of melting or the "melting point" and the two temperatures fall within the limits of melting range<sup>4</sup>.

### 2.2.2. Standard curve of ropinirole HCl

#### Calibration curve in 0.1N HCl

100mg of Ropinirole HCl was accurately weighed and transferred into 100ml volumetric flask. It was dissolved and diluted to volume with 0.1N HCl to give stock solution containing 1000mcg/ml. The standard stock solution was then serially diluted with 0.1N HCl to get 1, 2, 4, 6, 8,10, 20 mcg/ml and the absorbance of the solution was measured against 0.1N HCl as the blank at 250 nm using with UV spectrophotometer (Analytical). The absorbance was plotted against concentration (mcg/ml) to obtain the standard calibration curve<sup>5,9</sup>.

#### Calibration curve in pH 7.4 buffer

100mg of Ropinirole HCl was accurately weighed and transferred into 100ml volumetric flask. It was dissolved and diluted to volume with pH 7.4 buffer to give stock solution containing 1000mcg/ml. The standard stock solution was then serially diluted with pH 7.4 buffer to get 1, 2, 4, 6, 8,10, 20 mcg/ml and the absorbance of the solution was measured against pH 7.4 buffer as the blank at 250 nm using with UV spectrophotometer (Analytical). The absorbance was plotted against concentration (mcg/ml) to obtain the standard calibration curve<sup>5,9</sup>.

#### Calibration curve in methanol

100mg of Ropinirole HCl was accurately weighed and transferred into 100ml volumetric flask. It was dissolved and diluted to volume with methanol to

give stock solution containing 1000mcg/ml. The standard stock solution was then serially diluted with methanol to get 1, 2, 4, 6, 8,10, 20 mcg/ml and the absorbance of the solution was measured against methanol as the blank at 250 nm using with UV spectrophotometer (Analytical). The absorbance was plotted against concentration (mcg/ml) to obtain the standard calibration curve<sup>5,9</sup>.

### 2.2.3. FTIR Spectroscopy

The FTIR spectrum of Ropinirole was recorded using FTIR spectrophotometer. The drug: KBr in a ratio of 1: 99 was taken & pellet was prepared using KBr press. This pellet was analyzed under IR spectrophotometer<sup>6</sup>.

### 2.2.4. High Performance Liquid Chromatography (HPLC)

#### Method Development

A simple and precise and accurate High performance liquid chromatography (HPLC) method has been developed for the estimation of Ropinirole HCL. The separation was achieved on a C18 (250 x 4.6 mm) 5 - micron Hypersil BDS using a mobile phase consisting of a degassed mixture of 0.05 M glacial acetic acid (2.85 mL of glacial acetic acid in 1000 mL of water) with different ratios of mobile phase (Acetonitrile: 0.05M Glacial Acetic Acid) that is 60:40, 40:60, 50:50. It was found 50:50 ratio of mobile phase offered more advantageous than others<sup>7</sup>. Because with this combination the peak shape of Ropinirole was found to be good and has optimum plate count and tailing. Individual drug solution of 20  $\mu$ L was injected into the column at ambient temperature at a concentration of 20 ppm and it was chromatographed for 10 min using mobile phase at a flow rate of 1.0 mL/min listed in table.1 and the UV spectra of ropinirole was recorded at a wavelength of 250 nm<sup>8</sup>.

**Table 1:** HPLC Parameters

Concentration of Ropinirole HCl (in $\mu$ g/ml)	Mobile Phase	Run time	Flow rate	Retention time
20	0.05M Glacial acetic acid: Acetonitrile (50:50)	10 minute	1.0mL / min	3.566 minute

#### Procedure

*Preparation of 0.05 M Glacial Acetic Acid:* Accurately measured volume of 2.85 mL of glacial acetic acid (0.05 M) was transferred to a 1000 mL volumetric flask containing 500 mL of distilled water and put on sonication for 5 min and final volume was made with distilled water. The solution was filtered through 0.45  $\mu$ m membrane filter.

*Preparation of Mobile Phase:* A mixture of Acetonitrile: 0.05M Glacial Acetic Acid (50:50) previously filtered through 0.45 $\mu$ m membrane filter was used as a mobile phase.

*Preparation of Standard Stock Solution:* 50 mg of Standard Ropinirole was accurately weighed and transferred into a 50 mL of clean and dry volumetric flask. Dissolve and dilute to volume with mobile phase to give a solution containing 1 mg/ml.

*Preparation of Working Standard Solution:* From the prepared stock solution further dilution were made in order to get concentrations of 4, 6, 8, 10, 20 ppm for the construction of calibration curve.

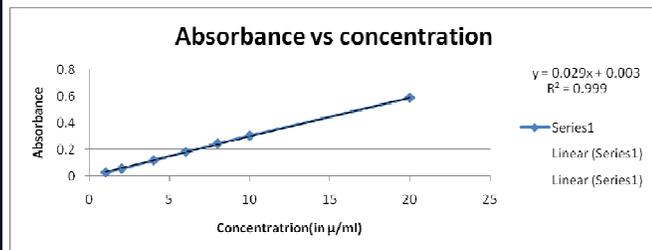
## 3. Result and Discussion

### 3.1 Melting point

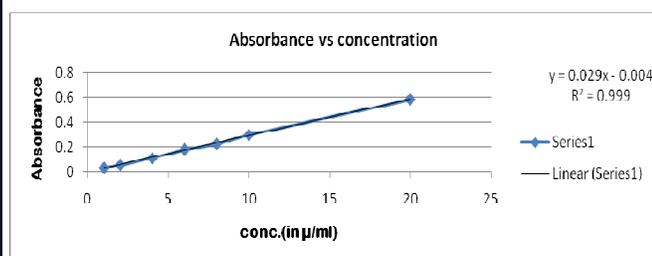
Melting point of Ropinirole HCl was determined by capillary method found to be 245.9°C which complied with USP standards that is in the range of 243°C to 250°C, indicating purity of the drug sample.

### 3.2 Standard curve of Ropinirole HCl

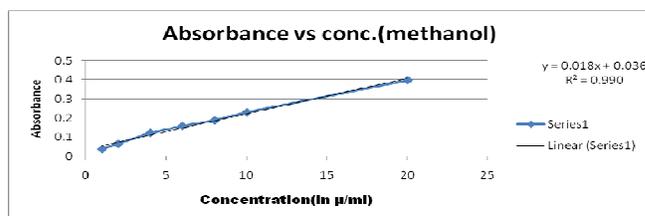
Standard curve of Ropinirole HCl was plotted in 0.1N HCl, pH 7.4 buffer and methanol, by plotting absorbance against concentration at 250 nm. Results were shown in Fig.1-3. below.



**Fig. 1:** Standard curve of Ropinirole HCl in 0.1N HCl at 250nm.



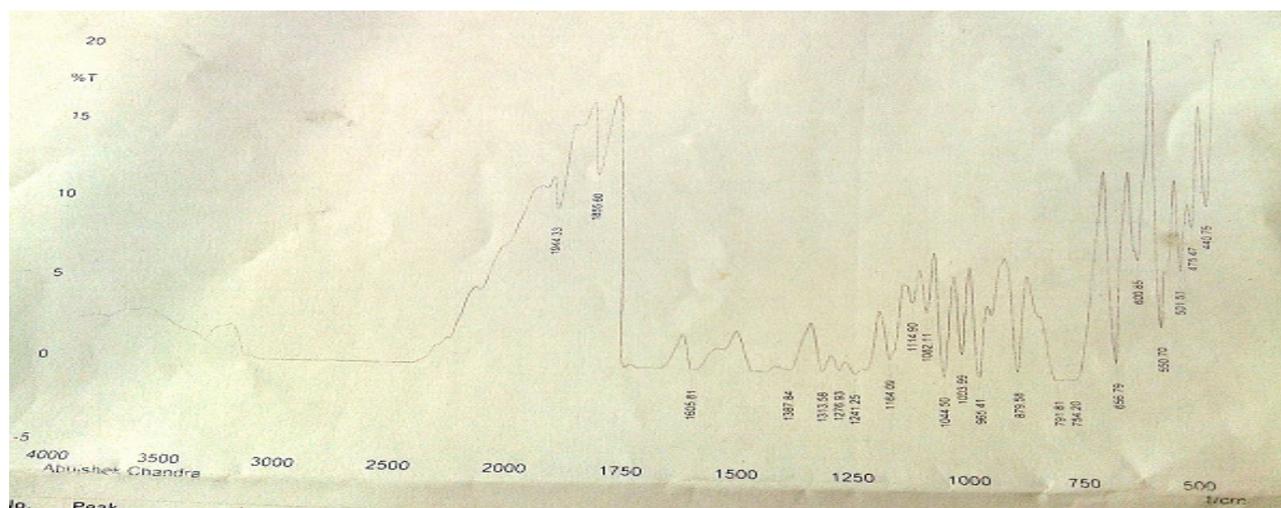
**Fig. 2:** Standard curve of Ropinirole HCl in pH 7.4 Phosphate buffer at 250nm.



**Fig. 3:** Standard curve of Ropinirole HCl in Methanol at 250nm.

### 3.3 FTIR Spectroscopy

Pure drug Ropinirole Hydrochloride spectra showed sharp characteristic peaks at 1350 cm<sup>-1</sup> (CH<sub>3</sub> bending), 1456cm<sup>-1</sup>(C=C stretching), 1700 cm<sup>-1</sup> (C=O stretching) and 3150 cm<sup>-1</sup>(N-H stretching) which is shown in Fig.4. and is similar that mentioned in the monograph.

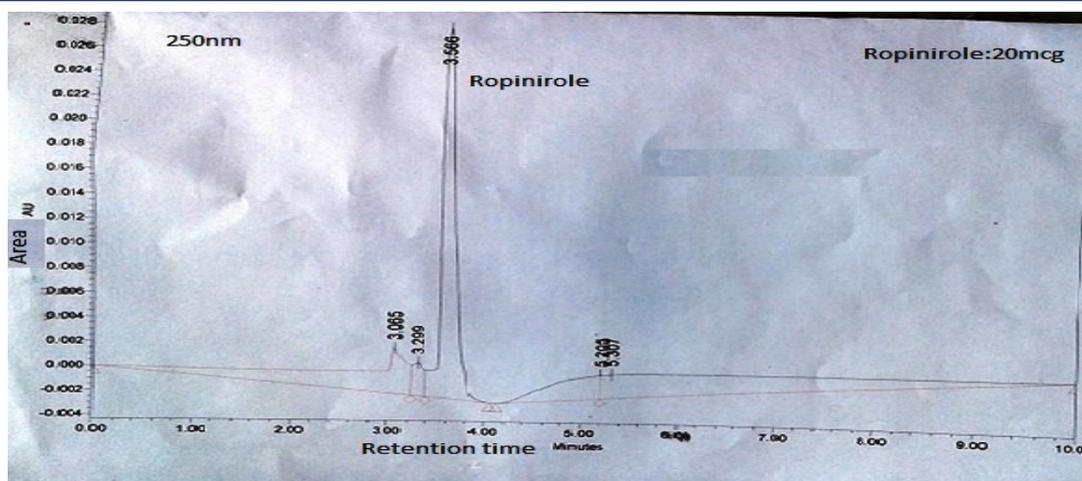


**Fig. 4:** FTIR Spectra of Ropinirole HCl

### 3.4 HPLC (High Performance Liquid Chromatography):

The retention time of ropinirole was found to be 3.566 min as shown in Fig. 5 using C<sub>18</sub> Hyper sil BDS(250 x 4.6mm) column and mobile phase of

0.05 M Glacial acetic acid: Acetonitrile (50:50) with a flow rate of 1.0mL/min and detection using a UV detector at 250 nm. The retention time, area, % area height of ropinirole chromatogram is shown in Table 2.



**Fig. 5:** HPLC Chromatogram of Ropinirole HCl

**Table 2:** Observation of Ropinirole chromatogram

S. No.	Retention Time (min)	Area ( $\mu\text{V}\cdot\text{sec}$ )	% Area	Height ( $\mu\text{V}$ )
1	3.065	1927653	21.96	3780
2	3.299	24454	2.79	2756
3	3.566	286531	32.64	30297
4	5.205	73491	8.37	1959
5	5.307	300586	34.24	1974

### 3. Conclusion

The drug ropinirole HCl undergoes preformulation studies i.e. solubility, melting point and was further characterized and authenticated on the basis of UV spectroscopy, FT-IR and HPLC study and the result shows that the drug is active and is ready for the formulation studies. The given method can be used for authentication of Ropinirole HCl.

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