Development and Validation of Stability-Indicating TLC-Densitometric Determination of Ropinirole Hydrochloride in Bulk and Pharmaceutical Dosage Form

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Abstract

A TLC/densitometry of ropinirole hydrochloride as a bulk drug was developed and validated. The separation was achieved on TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase using chloroform: acetone: triethylamine (3.5:1.5:0.2 v/v) as mobile phase and densitometry analysis at 250 nm. The system showed compact spot for ropinirole hydrochloride (Rf = 0.52 ± 0.02). The drug follows linearity in the concentration range 300 - 1800 ng per band (r2 = 0.9983 ± 0.0008). Drug was subjected to hydrolysis, oxidation and thermal degradation which indicate the drug is susceptible to hydrolysis, oxidation and heat and degraded product did not interfere with detection and assay of ropinirole hydrochloride. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of ropinirole hydrochloride.

Keywords: Ropinirole hydrochloride; TLC/densitometry; Validation; Stability

Introduction

Ropinirole Hydrochloride is chemically 4-[2-[(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one Hydrochloride, and is used as an antiparkinson drug [1,2]. Ropinirole acts as an agonist at the D2 and D3 dopamine receptor subtypes, binding with higher affinity to D3 than to D2 or D4 and it has no affinity for D1 receptors. Ropinirole 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 [3]. In literature, various liquid chromatographic methods have been reported for the estimation of ropinirole hydrochloride from pharmaceutical dosage form [4] and biological fluids [5] with different impurity studies [6-8]. Stability of drug by different chromatographic method has also been reported [9,10]. More over LC/ESI/MS method has been described [11], and spectrourimetric method was also given [12]. However, to our knowledge, no article related to the TLC/densitometry determination of ropinirole hydrochloride has ever been mentioned in literature.

The aim of this work is to develop an accurate, specific and repeatable method for the determination of ropinirole hydrochloride and validation of method as per ICH guidelines.

Experimental

Torrent Pharmaceuticals, Ahmadabad (Gujarat) India has kindly supplied a pure drug sample of ropinirole hydrochloride and drug was used without further purification. All chemicals and reagents used were of analytical grade and purchased from Merck fine chemicals, Mumbai, India.

HPTLC instrumentation

Chromatography was performed on silica gel precoated aluminum plate 60F-254 plates, (10 cm × 10 cm with 250 μm thickness. The previously developed plates with methanol were dried and activated at 110°C for 5 min. prior to chromatography. The samples were spotted in the form of bands of width 6 mm with a Camag 100 microliter sample syringe (Hamilton, Bonaduz, Switzerland) using Camag Linomat 5 (Switzerland) sample applicator. The mobile phase consisted of chloroform: acetone: triethylamine (3.5:1.5:0.2 v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was approximately 8.0 cm. Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometry scanning was performed using Camag TLC scanner 3 in the absorbance mode at 250 nm (Figure 1). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190 - 400 nm [13,14].

Preparation of stock standard solution and study of calibration curve

A standard stock of ropinirole hydrochloride (1000 µg/ml) was prepared in methanol. Standard solution was further prepared by dissolving 3ml of stock solution in volumetric flask and made up to mark with methanol. Different volumes of standard solution were spotted in six replicates on TLC plates from 1.0 to 6.0 µl to obtain concentration of 300 - 1800 ng per bands of ropinirole hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

Preparation of sample

To determine the concentration of ropinirole hydrochloride in tablets (label claim: 2 mg per tablet), twenty tablets (Ropitor 2 tablets) were weighed, mean weight was determined and grinded to fine powder. The quantity of powder equivalent to 10 mg of ropinirole hydrochloride was used without further purification. All chemicals and reagents used were of analytical grade and purchased from Merck fine chemicals, Mumbai, India.

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Figure 1: A typical HPTLC chromatogram of ropinirole hydrochloride ($R_f = 0.52$) in chloroform: acetone: triethylamine (3.5:1.5:0.2, v/v/v) at 250 nm.

Figure 2: HPTLC chromatogram of ropinirole hydrochloride (2) after HCl degradation (1) for “8h” at room temperature.
Figure 3: HPTLC chromatogram of ropinirole hydrochloride (3) after NaOH degradation (1, 2) for “8 h” at room temperature.

Figure 4: HPTLC chromatogram of ropinirole hydrochloride(2) after treatment with 30% hydrogen peroxide (1) for “8h”.
hydrochloride was weighed. The drug from the powder was extracted by methanol. To ensure the complete extraction of the drug, it was sonicated for 20 min and the volume was made up to the 10 ml. The 3 ml of above solution (1000 µg/ml) was taken in volumetric flask and volume was made up to mark with methanol. From this 2ml of solution was applied on TLC plate (600 ng per spot) followed by development and scanning as described in section 2.1. The analysis was repeated in six times. The possibility of excipients interferences in the analysis was studied.

Method validation

Precision: The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (600, 900, 1200 ng per band) of ropinirole hydrochloride standard solution in six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Robustness of the method: The robustness of the method was investigated by making small deliberate changes. Seven different parameters such as mobile phase composition, mobile phase volume, development distance, relative humidity, duration of saturation, activation of previously developed TLC plates, time from spotting to chromatography and time from chromatography to scanning. Mobile phase having different composition i.e. chloroform: acetone: triethylamine (3.5:1.5:0.2 v/v) and (3.2:1.8:0.2 v/v); the mobile phase volume was changed from 5 ml to 10 ml; the development distance was changed (7.5 cm, 8.0 cm and 8.5 cm); experiment was performed at relative humidity (55 % and 65 %). The duration of saturation was changed from 20 min, 25 min and 30 min. The plates were activated at 60°C temperature and time of activation was varied from 8, 10 and 12 min. The time from spotting to chromatography and from chromatography to scanning was also varied from 10 to 15 min. The effect of these variations were studied by applying the 600 ng per band of ropinirole hydrochloride standard solution on TLC plates, developed and scanned as per section 2.1.

Limit of detection (LOD) and Limit of quantification (LOQ): In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. Standard solution of ropinirole hydrochloride (0.01 mg/ml) was prepared and different volumes of stock solution were spotted in triplicate. The amount of ropinirole hydrochloride by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3×A.S.D.)/b and quantification limit was calculated by (10×A.S.D.)/b, where “b” corresponds to the slope obtained in the linearity study of method.

Specificity: The specificity of the method was ascertained by analyzing drug standard and sample. The spot for ropinirole hydrochloride in sample was confirmed by comparing the Rf values and spectra of the spot with that of standard (Figure 7). The peak purity of ropinirole hydrochloride was accessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Recovery studies: The pre-analyzed sample was spiked with extra 80%, 100% and 120 % of the ropinirole hydrochloride standard and it was analyzed by the proposed method. At each level of the amount, three determinations were performed. This was done to check the recovery of the drug at different level in the formulation.

Ruggedness studies: The ruggedness of the method was studied by two analysts using same operational and environmental conditions. The ropinirole hydrochloride standard (600 ng per band) was applied on the TLC plates. The plate was developed and scanned as per section 2.1.

Forced degradation of ropinirole hydrochloride

Acid and Base induced degradation: Ropinirole hydrochloride standard (10 mg) was separately dissolved in 10 ml methanolic solution of 1 M HCl and 1 M NaOH. These solutions were kept for “8h” at room temperature in the dark in order to avoid the possible degradative effect of light. The 3 ml of above solution was taken and neutralized, then diluted up to 10 ml with methanol. The resultant solution were applied on TLC plates in triplicates (3 µl each, i.e. 900 ng per band). The chromatograms were run as described in section 2.1 (Figure 2-3).

Hydrogen peroxide – induced degradation: Ropinirole hydrochloride standard (10 mg) was separately dissolved in 10 ml of methanolic solution of hydrogen peroxide (30.0 %, v/v). The solution was kept for “8h” at room temperature in the dark in order to exclude the possible degradative effect of light. The 3 ml of above solution was taken and diluted up to 10 ml with methanol. The resultant solution were applied on TLC plate in triplicate (3 µl each, i.e. 900 ng per spot). The chromatogram was run as described in section 2.1 (Figure 4).

Dry heat degradation products: Ropinirole hydrochloride standard (10 mg) was kept at 55°C for “3h” in oven. It was transferred to 10 ml volumetric flask containing methanol and volume was made up to the mark. From it 3 ml was diluted to 10 ml mark with methanol. An appropriate volume 3 µl each i.e. 900 ng per band was applied on TLC plate in triplicate and chromatogram was run as described in section 2.1 (Figure 5).

Light heat degradation products: Ropinirole hydrochloride standard (10 mg) was dissolved in 10 ml of methanol. The solution was kept in the sun light for “8h”. The 3 ml of above solution was taken and diluted up to 10 ml with methanol. The resultant solution was applied on TLC plate in triplicate (3 µl each, i.e. 900 ng per band). The chromatogram was run as described in section 2.1 (Figure 6).

Results and Discussion

Development of optimum mobile phase

The mobile phase consisting of chloroform: acetone (3.5:1.5 v/v) gave good resolution for ropinirole but a typical peak nature was missing and tailing was observed. To overcome the problem, few drops of triethylamine were added to the mobile phase. Finally, the mobile phase consisting of chloroform: acetone: triethylamine (3.5:1:0.2 v/v) showed compact spot for drug with Rf value of 0.52 ± 0.02. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

Calibration curve

The calibration plot was linear over the concentration range of 300 - 1800 ng per band (n = 6). The correlation coefficient r² = 0.9983 ± 0.008 with % RSD value 0.95 across the concentration range studied, were obtained by linear regression analysis. The regression equation was y = 2.975 x ± 1263.4 and the mean value of slope and intercept were 2.957 and 1263.36, respectively. The standard error (SE), the chi square, multiple correlation coefficient and residual standard deviation were 37.97, 5.99001, 0.99864 and 61.3630 respectively.
Figure 5: HPTLC chromatogram of ropinirole hydrochloride (2) after “3h” dry heat degradation(1,3) at 55°C.

Figure 6: HPTLC chromatogram of ropinirole hydrochloride (3) after photo degradation (1, 2) at “8h” in sun light.
Validation of the method

Precision: The repeatability of ropinirole standard application was studied by measuring peak area in the terms of % RSD and results are depicted in Table 1, which revealed intra-day and inter-day variation of ropinirole at three different concentration levels 600, 900 and 1200 ng per band.

Robustness of the method: The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2%; which indicates robustness of the method and results are depicted in Table 2.

LOD and LOQ: Detection limit and quantification limit was calculated by the method as described in section 2.4.3 and found to be 17.21 and 52.18 ng, respectively. This indicates that adequate sensitivity of the method.

Specificity: The peak purity of the ropinirole was \( r^2 = 0.9996 \) assessed by comparing the spectra at peak start, peak apex and peak end positions of the band.

Recovery studies: The proposed method when used for extraction and subsequent estimation of ropinirole from pharmaceutical dosage form after spiking with 80%, 100% and 120% of additional drug afforded recovery of 100–102% as listed in Table 3.

Ruggedness studies: The results from the ruggedness studies were calculated in the term of % RSD. The low values of % RSD (less than 2%) are the indication of the ruggedness of the proposed method.

Preparation of sample

A single spot of R 0.52 ± 0.02 was observed in chromatogram of the ropinirole samples extracted from tablets. There was no interference from the excipients commonly present in the tablets. Ropinirole content was found to be 100.65% with % RSD of 1.016. It may therefore be inferred that degradation of ropinirole had not occurred in the formulation that was analyzed by this method. The low % RSD value indicated the suitability of this method for routine analysis of ropinirole in pharmaceutical dosage form.

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### Table 1: Intra-day and Inter-day Precision

<table>
<thead>
<tr>
<th>Amount ng per spot</th>
<th>For ropinirole standard</th>
<th>SD</th>
<th>%RSD</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Intra-day precision</td>
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<tr>
<td>600</td>
<td>15.91</td>
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<tr>
<td>900</td>
<td>7.37</td>
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<tr>
<td>1200</td>
<td>19.28</td>
<td>0.40</td>
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<tr>
<td>Inter-day precision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>19.18</td>
<td>20.06</td>
<td></td>
</tr>
<tr>
<td>900</td>
<td>25.44</td>
<td>0.06</td>
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</tr>
<tr>
<td>1200</td>
<td>20.26</td>
<td>0.04</td>
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</tr>
</tbody>
</table>

*mean of six estimations at each level

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### Table 2: Robustness of the method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SD of peak area for ropinirole standard</th>
<th>% RSD of peak area for ropinirole standard</th>
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</thead>
<tbody>
<tr>
<td>Mobile phase composition</td>
<td>16.12</td>
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<tr>
<td>Mobile phase volume</td>
<td>17.23</td>
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</tr>
<tr>
<td>Development distance</td>
<td>30.40</td>
<td>1.01</td>
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<tr>
<td>Relative humidity</td>
<td>31.84</td>
<td>1.05</td>
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<tr>
<td>Duration of saturation</td>
<td>22.14</td>
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<tr>
<td>Activation of prewashed TLC plates</td>
<td>18.36</td>
<td>0.60</td>
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<tr>
<td>Time from spotting to chromatography</td>
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<tr>
<td>Time from chromatography to scanning</td>
<td>41.29</td>
<td>1.37</td>
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</table>

*each parameter was repeated for six times
### Stability-indicating property

The chromatogram of samples degraded with acid, base, hydrogen peroxide, heat and light showed well separated spots of pure ropinirole as well as some additional peaks at different Rf values. The spots of degraded product were well resolved from the drug spot. The results are shown in Table 5.

### Conclusion

Introducing TLC/densitometry into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC/densitometry technique is precise, specific and accurate. The developed method was validated as per the ICH guidelines [14, 15]. Statistical analysis proves that the method is suitable for the analysis of ropinirole as bulk drug and in tablets without any interference from the excipients. The method can be used to determine the purity of drug available from various sources by detecting any related impurities.

### References


