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Study of Degradation Profile and Development of Stability Indicating Spectrophotometric Method for Ropinirole Hydrochloride under Acid/Base Hydrolytic and Oxidative Conditions

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ABSTRACT

The degradation behavior of Ropinirole Hydrochloride (ROPI) was investigated under various stress conditions of acid/base hydrolysis and oxidation using Spectrophotometry. Stability indicating spectrophotometric method was developed that could separate the drug from its degradation products formed under these stressed conditions. The UV spectral characteristics of the drug and degraded products were quite different and first order derivative UV spectrophotometric method was used to study the extent of degradation. ROPI was found to degrade extensively under experimental conditions. The method was validated with respect to linearity, precision, accuracy and specificity. The described method was found to be linear over the range of 5-50 µg mL-1 for ROPI. The mean recovery was found to be 99.03±1.11, %. The intermediate precision data were obtained under different experimental conditions and calculated value of the coefficient of variation (CV, %) was found to be less than critical value. The proposed method can be successfully useful to determine the degradation of drug during storage.

KEYWORDS

Stress conditions, Stability-indicating, Spectrophotometry, Ropinirole Hydrochloride

INTRODUCTION

The need to develop a stability indicating method using stress degradation has been recommended by International Conference on Harmonization (ICH)[1]. The stress conditions should include the effect of temperature, humidity, light, acid/base hydrolysis and oxidation. The aim of the current study was to study the degradation behavior of ropinirole hydrochloride (ROPI) under few ICH prescribed conditions and to develop validated stability indicating spectrophotometric assay method.

Ropinirole hydrochloride[2,3] is chemically known as 4-[2-(dipropylamino)ethyl]-1,3-dihydroindol-2-one monohydrochloride, is a non-ergot dopamine D_2 antagonist and is used as antiparkinson agent. ROPI is not official in any of pharmacopoeia. Literature survey revealed HPLC[4-6], HPTLC[7], UV spectrophotometric[8] and spectrofluorimetric[9] methods for estimation of ropinirole in tablet formulations while LC-MS-MS[10], LC-MS[11], LC-ESI-MS-MS[12] methods in plasma, HPLC-MS/MS[13], HPLC for ropinirole impuritie[14], chemometric evaluation of ropinirole and its impurities[15], capillary liquid chromatography[16],capilarizone electrophoresis[17] for determination of dissociation constant of ropinirole and stability indicating HPTLC[18]. No stability indicating method was reported for the estimation of ROPI, the objective of the present study was to develop inexpensive, simple and rapid stability indicating spectrophotometric

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method for ROPI which would be accurate and precise.

MATERIAL AND METHODS

A double beam UV-visible Spectrophotometer (Shimadzu model UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was employed. Silica gel 60F 254 precoated plates from Merck Ltd., TLC applicator (Camag Linomat V, Switzerland) and Camag TLC scanner III were used for TLC studies. Kindly gifted reference standard of ROPI (Torrent Research Centre, Gandhinagar) was used for the study. The tablet formulation containing 1 mg ROPI was procured from the local pharmacy. All other reagents used were of AR grade.

PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

Stock solution of ROPI (100 µg mL^{-1}) was prepared in methanol. Suitable aliquots ranging from 0.5 to 5.0 ml were taken and diluted to 10 ml with water to achieve concentration in the range of 1-50 µg mL^{-1}.

For sample solution, 20 tablets were weighed; their mean weight was determined, and grounded into fine powder in a mortar. An amount of powdered mass equivalent to 10 mg ROPI was accurately weighed, and transferred in to a 100 mL volumetric flask, 60 mL of methanol was added and sonicated for 20 min, the volume was diluted to mark with methanol and mixed well. The solution was filtered through Whatmann No.42 filter paper. Suitable aliquots of filtrate were diluted with water to analyze the ROPI by proposed method.

FORCED DEGRADATION STUDY

For all degradation studies, ROPI at a concentration of 2 mg mL^{-1} was used. The degradation was done under strong, moderate as well as mild condition. The sample were withdrawn initially and then after at regular time intervals. The degradation was checked using TLC. The aliquots withdrawn were suitably diluted with water to get the working solutions for spectrophotometric study. For acid degradation, drug solution was exposed to 0.1N, 1N, 2N, 5N HCL at 25° C, 50° C and 60° C for up to 6 hr. For basic degradation, drug solution was exposed to 0.1N, 1N, 3N, 5N NaOH at 25° C, 50° C and 80° C for up to 3 hr. For peroxide degradation, drug solution was exposed to 3%, 10%, 30% H_2O_2 at 25° C, 50° C and 60° C for up to 24 hr. For analysis of degraded samples, 1 mL of the degraded solution was withdrawn and diluted to 10 mL with water. The initial absorbance of the drug, at zero time was considered as 100% concentration and degradation was correlated with this concentration.

THIN LAYER CHROMATOGRAPHY STUDY

To check the decomposition of ROPI, TLC was performed on Silica gel 60F 254 precoated plates using Methanol:Acetonitrile (80:20, v/v) as the mobile phase and one drop of glacial acetic acid in TLC chamber to overcome tailing. After added required amount of mobile phase to the TLC chamber, it was kept at room temperature for 20 min to saturate the TLC chamber with mobile phase. For TLC analysis, 400 µg mL^{-1} solution of ROPI & its degraded solutions were spotted over TLC plate. Then mobile phase was run over the TLC plate. After sufficient runtime, TLC plate was dried at the room temperature for 20 min. Spots were detected in the UV chamber as well as in the HPTLC scanner at 250 nm wavelength.

DETERMINATION OF ZERO CROSSING POINT

The zero order UV spectrum of ROPI showed maximum absorbance at 250 nm, while 100% degraded samples of ROPI in acid/base hydrolytic and oxidative conditions showed interference at this wavelength. Though, the spectral characteristics of degraded solutions were quite different from the standard drug, the solution showed substantial absorption at 250 nm even after 100% degradation at various stress condition, so the further work was carried out in 1st order derivative spectrum of standard ROPI and its degraded samples. The first order

derivative overlain spectra of standard ROPI and its degraded solutions in various stressed conditions were obtained and zero crossing points for ROPI and degraded solution were determined (Figure: 1a-1c). In the 1st order derivative spectrums, ROPI showed reasonable absorbance at 237nm whereas acid, base and peroxide degraded samples showed zero absorbance at this wavelength. So, 237 nm was found to be zero crossing point for acid, base and peroxide degraded sample of ROPI, it was finally selected for the estimation of ROPI in presence of its degradation products.

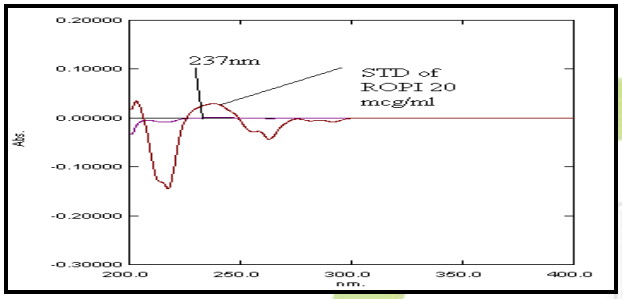

Figure: 1a Overlain First order derivative spectra of standard solution of ROPI & its 100% acid degraded solution

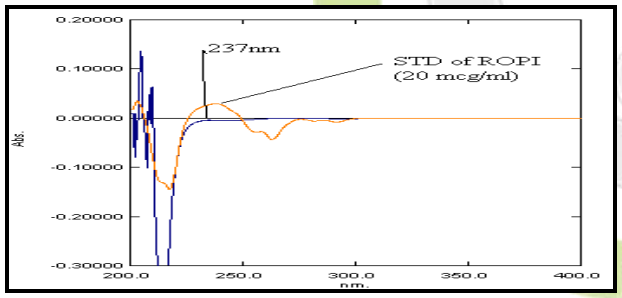

Figure: 1b Overlain First order derivative spectra of standard solution of ROPI & its 100% NaOH degraded solution

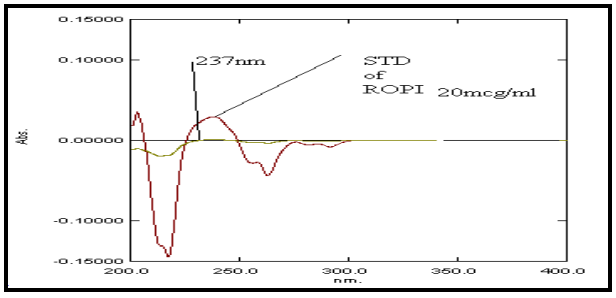

Figure: 1c Overlain First order derivative spectra of standard solution of ROPI & its 100% Oxidative degraded solution

VALIDATION OF THE METHOD

Validation of the method was done by studying various parameters as per ICH guideline[19]. Linearity was studied by analyzing six concentrations of the drug diluted in water in the range of 5-50 µg mL^{-1} using six replicates and fitting the data in the best fitted curve. Precision was verified by repeatability and intermediate precision studies. Repeatability was established by analyzing three different concentrations in six replicates on the same day whereas intermediate precision was checked by repeating the studies on different days.

Accuracy of the method was tested by adding three concentrations of standard drug solution sequentially to a mixture of degraded solution and determining the recovery of the added drug. Specificity of the method towards the drug was studied by analyzing a mixture containing standard drug and the stressed samples.

RESULTS AND DISCUSSION

ROPI has an amino group in its molecular structure making it amenable to acidic and basic hydrolysis. As well as presence of indol moiety in its molecular structure making more vulnerable to ROPI for oxidative degradation (Figure: 2).

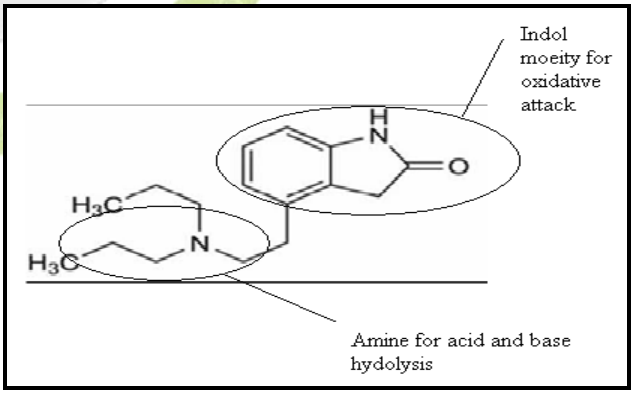

Figure: 2 Structure of ROPI shows presence of functional group responsible for degradation

The TLC studies showed that Rf value for the standard ROPI was found to be 0.71, while for its HCL, NaOH and H_2O_2 treated solutions appeared at 0.88, 0.85 and 0.44, respectively. HPTLC densitogram of ROPI & its 100 % degraded solutions in acid/base hydrolytic and

oxidative conditions is depicted in Figure: 3. It shows that no peak was found for acid, base, and peroxide degraded sample of ROPI after 100 % degradation of drug at the Rf value (0.71) for standard ROPI.

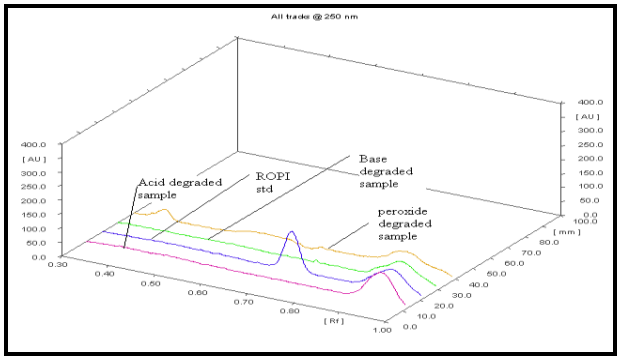

Figure: 3 Densitogram of ROPI standard and its 100% degraded samples by acid, base and oxidative stress condition

The spectroscopic studies of the stressed samples of ROPI suggested following behavior of the drug under various stressed conditions. It was observed that around 50% of ROPI was degraded on heating at 50° for 6 hr in 3N HCL. The drug was totally degraded if heated at 60° for 6 hr in 5N HCL (Figure: 4a). The degradation was very rapid under basic condition. It was observed that around 50% of ROPI was degraded on heating at 50° for 60 min in 3N NaOH. The drug was totally degraded if heated at 80° for 90 min in 5N NaOH (Figure: 4b). The degradation was slow at oxidative condition. It was observed that around 50% of ROPI was degraded on heating at 40° for 22 hr in 10% H_2O_2.

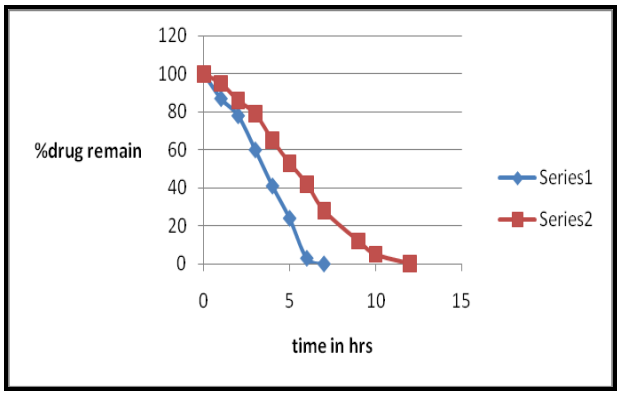

Figure: 4a Degradation of Ropinirole under acidic conditions

(♦—♦—♦) Represents degradation profile of Ropinirole in 3N HCL at 50° C

(■—■—■) Represents degradation profile of Ropinirole in 5N HCL at 80° C

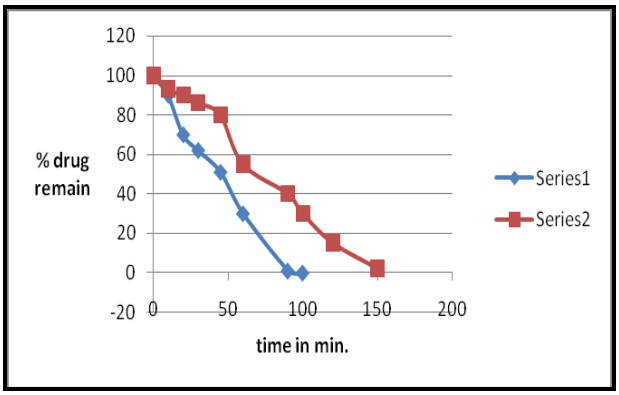

Figure: 4b Degradation of Ropinirole under alkaline conditions

(♦—♦—♦) Represents degradation profile of Ropinirole in 3N NaOH at 50° C

(■—■—■) Represents degradation profile of Ropinirole in 5N NaOH at 60° C

The drug was totally degraded if heated at 55° for 24 hr in 30% H_2O_2 (Figure: 4c). Total degradation under all these conditions was confirmed by TLC. The analysis of ROPI and its degraded products formed under various stressed conditions was possible using spectrophotometric method.

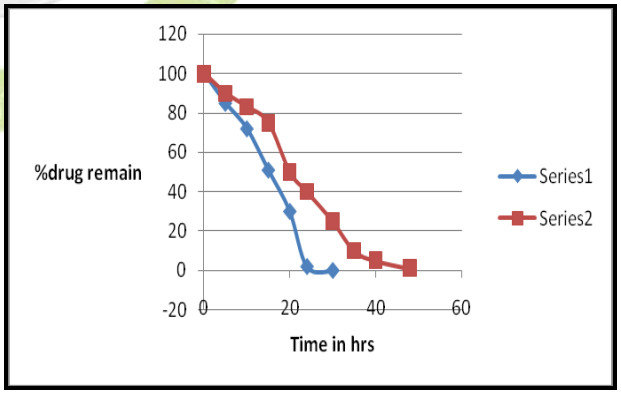

Figure: 4c Degradation of Ropinirole under Oxidative conditions

(♦—♦—♦) Represents degradation profile of Ropinirole in 10% H2O2 at 40° C

(■—■—■) Represents degradation profile of Ropinirole in 30% H2O2 at 55° C

The described method has been validated, apart from specificity, for linearity, accuracy, and intermediate precision. The proposed method was found to be linear over the range of 5-50 µg mL^{-1}. Characteristic parameters for regression equation are given in Table: 1.

Table: 1 Characteristic Regression Parameter for the Proposed Method

Parameters	**First derivative spectroscopy**
λ_{max} (nm)	237
Beer's Range (µg mL^{-1})	5-50
Regression Equation	Y = 0.0012X + 0.0007
Correlation Coefficient (r^2)	0.9966
LOD (µg mL^{-1})	1.6
LOQ (µg mL^{-1})	5.0
Repeatability (CV, %) (n = 6)	1.81

n = Number of determinations

The developed method was found to be precise as indicated by low CV, % values of within a day (1.21, %) and day to day (1.28, %) variations for ROPI.

The accuracy was determined by recovery study of spiked samples. The good recovery (99.03±1.11, %) suggested accuracy of the method. Results of recovery study are shown in Table: 2. The tablet formulations were analyzed by developed method and assay results were found to be 99±0.96 % of the labeled claim.

Conclusions

The spectral characteristics of the drug was totally different from its degradation products, so first order derivative UV spectroscopy method could be used to estimate ROPI in presence of its degradation products. The study showed that ROPI is susceptible to acid/base hydrolytic and oxidative degradations but the developed method can be used as stability indicating method to differentiate the drug from its degradation products. The method has linear response in stated range and is accurate and precise. Though no attempts were made to identify and quantify the degraded products, the method can be used to determine the degradation of drug during storage.

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Table: 2 Recovery Data for the Proposed Method (n=6)

Level	**Amount of drug in degraded sample (µg mL^{-1})**	**Amount of standard drug added (µg/mL)**	**Total amount of drug obtained (µg mL^{-1})**	**Amount of STD drug recovered (µg mL^{-1})**	**Mean % Recovery ± SD**
I	8	4	11.9	3.9	97.50 ± 0.75
II	8	8	16.1	8.1	101.25 ± 1.39
III	8	12	19.8	11.8	98.33 ± 1.2

n = Number of determinations SD = Standard Deviations of six determinations

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