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A Stability indicating of Rotigotine in Bulk Drugs by HPLC Assay method

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ABSTRACT

A new HPLC method was developed for selective and simultaneous determination of of Rotigotine. The developed method is also applicable for the related substances determination in bulk drugs. The chromatographic separation was achieved on a Zorbax SB C-18, 4.6 x 250mm, and 5µ column. The mobile phase consisted of buffer and methanol (30:70, *v/v*) delivered at a flow rate of 2.0 mL min^{-1}. Buffers consisted of dissolve 5.22 g of dipotassium hydrogen orthophosphate in 1000 mL of water and add 2 mL of triethylamine, adjust pH to 5.5 with ortho phosphoric acid. The mobile phase was pumped at a flow rate 1.0 mL per minute and detector of UV at 225 nm. In the developed HPLC method, the resolution between Rotigotine and its potential impurities, namely Dethienyl ethyl rotigotine, Depropyl Rotigotine, Acetyl rotigotine, Methyl rotigotine, Thiophene tosyl, Dithienyl Ethyl Rotigotine, Rotigotine toluene sulphonic acid ester, Rotigotine thienyl ethyl ether and 3- Thiophene Rotigotine was found. Accuracy found by % recovery from 100.4 – 100.6 at 80.0% to 120.0% level and the linearity results for Rotigotine and its related compounds in the specified concentration calibration curves linear with coefficient of variation (r) not less than 0.99 The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Result seems to be rapid degradation observed when Rotigotine sample solution exposed to heat at 105^0C the sample was decomposed and slow degradation was observed when Rotigotine sample solution exposed to heat at 60^0C. Considerable rapid degradation was found to occur at Oxidation conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 95% - 105%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness. The validation was performed according to the current requirements as laid down in the ICH guidelines.

Key words: Rotigotine; forced degradation; RP-HPLC;

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INTRODUCTION

Rotigotine (Neupro) is a non-ergoline dopamine agonist indicated for the treatment of Parkinson's disease (PD) and restless legs syndrome (RLS) in Europe and the United States. It is formulated as a once-daily transdermal patch which provides a slow and constant supply of the drug over the course of 24 hours[1-2]Like other dopamine agonists, rotigotine has been shown to possess antidepressant effects and may be useful in the treatment of depression as well [3]. Rotigotine was developed by Aderis Pharmaceuticals. In 1998, Aderis licensed worldwide development and commercialization rights for rotigotine to the German pharmaceutical company Schwarz Pharma (today a subsidiary of the Belgian company UCB S.A.)[4]. Neupro, with the active ingredient rotigotine, is a non-ergolinic dopamine receptor-agonist formulated as a transdermal delivery system, a patch, designed for once-a-day application. Rotigotine is designed to mimic the action of dopamine, a naturally-produced neurotransmitter crucial for proper motor functioning. The system is applied to the skin once a day and provides rotigotine continuously to the body for 24 hours. Multinational clinical studies in patients with early stages of Parkinson's disease were completed at the end of 2003. In 15 clinical trials, more than 1,500 patients with Parkinson's disease have been treated with rotigotine transdermal system. The clinical trials have shown efficacy and safety in early Parkinson's disease. Rotigotine exhibits a low potential of pharmacokinetic drug-drug interactions. The administration of rotigotine transdermal system offers the convenience of once daily dosing and easy usage. Rotigotine transdermal system is approved in Europe for the treatment of patients with early and advanced Parkinson's disease in combination with levodopa. Since March 2006, the drug has been available on the European market and has been launched by SCHWARZ PHARMA in 14 countries within Europe, e.g. Germany, the UK, Austria, Denmark, Ireland, Norway, Switzerland, Sweden, Greece, Spain, Finland and Poland. Parkinson's disease is a progressive disorder of the central nervous system. The patients - roughly four million worldwide, including approximately one million people in the U.S. - suffer primarily from a lack of dopamine, a messenger substance in the central nervous system, which is responsible for the coordination of movement. As a result of this shortage, patients are no longer able to control their movements reliably. Dopamine agonists are drugs that attempt to compensate for this lack of dopamine.

The drug has been approved by the EMEA for use in Europe in 2006 and is today being sold in several European countries. In 2007, the Neupro patch was approved by the Food and Drug Administration (FDA) as the first transdermal treatment of Parkinson's disease in the United States. However, as of 2008, Schwarz Pharma has recalled all Neupro patches in the United States and some in Europe because of problems with the delivery mechanism. General side effects for rotigotine may include constipation, dyskinesia, nausea, vomiting, dizziness, fatigue, insomnia, somnolence, confusion, and hallucinations.[5-6]. More serious complications can include psychosis and impulse control disorders like hypersexuality, punding, and pathological gambling[7]. Mild adverse skin reactions at the patch application site may also occur.

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MATERIALS AND METHODS

Experimental

The instrument used was a Waters Model Alliance 2695 separation module equipped with auto sampler, Waters 2998 PDA Detector and the data recorded using empower software. The mobile Phase consisted of buffer and methanol by using the column Zorbax SB C-18, 4.6 x 250mm, and 5µ column and detector of UV at 225 nm, 1.0 mL/min as a Flow rate.

Reagents and chemicals

HPLC grade Methanol was obtained from Ranbaxy Fine Chemical Limited, New Delhi, India. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard volumetric glassware.

Reference solution

Accurately weight and transfer about 5 mg each of Dethienyl ethyl rotigotine impurity, Depropyl Rotigotine impurity and Rotigotine standards and 20 mg of Acetyl rotigotine impurity into a 100 mL volumetric flask, dissolve in and dilute to volume with diluent. Dilute 1.0 mL of this solution to 50 mL with diluent.

Sample solution

Accurately weigh and transfer about 100 mg sample into a 50 mL volumetric flask, dissolve in and dilute to volume with diluent. Prepare in duplicate.

System suitability

Theoretical plates for Rotigotine peak from first chromatogram of standard should be not less than 3000, Tailing factor for Rotigotine peak from first chromatogram of standard not more than 2.0 and % RSD for replicate standard injections not more than 5.0.

RESULTS AND DISCUSSION

Method development

Precision

The system precision was performed by analysing system suitability standard solution six times. Results of Peak area of the API and the impurities. The peak area variation observed for Rotigotine and impurities was less than 5.0%. The results comply with the acceptance criteria and indicating acceptable precision of the system. The percentage relative standard deviation of Peak area of six replicate injections for each impurity ≤ 5.0.

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The Precision of the method was determined by analyzing a sample of Rotigotine solution spiked with impurities at 100% of the specification limit of six replicate sample preparations. The percentage relative standard deviation of recovery obtained for each impurity less than or equal to 5.0. Prior to this, system suitability parameters were calculated by injecting the system suitability solution. The %RSD was found to be 3.01.

Limit of detection and Limit of quantification

The limit of detection (LOD) is determined by calculating the signal to noise ratio and by comparing test results from samples with known concentrations of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. The result obtained for each impurity is listed in Table 1. The limit of detection values obtained for each impurity was within the acceptance criteria. Signal to noise ratio should be about 3: 1 and the detection less than 0.15%.

Limit of Quantification (LOQ) values were determined from the same experiment as mentioned in the limit of detection section. The LOQ values obtained are in Table 2. Signal to noise ratio should be about 10:1 and the quantification limit to be less than level of specification preferably much less.

Table 1: Limit of detection (LOD) for Rotigotine and impurities.

Component	%Impurity w.r.to working strength	Concentration(mg/ml)	Signal to noise	LOD (%)
Rotigotine	0.0011	0.00002219	3.5:1	0.001
Dethienyl ethyl rotigotine	0.0014	0.00002809	2.7:1	0.001
Depropyl Rotigotine	0.0014	0.00002759	3.1:1	0.001
Acetyl rotigotine	0.0008	0.00001511	3.5:1	0.001

Table2: Limit of Quantitation for Rotigotine and impurities.

Component	%Impurity w.r.to working strength	Signal to noise
Rotigotine	0.004	9.7:1
Dethienyl ethyl rotigotine	0.005	9.5:1
Depropyl Rotigotine	0.005	10.0:1
Acetyl rotigotine	0.003	9.9:1

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Linearity and Range

The linearity of the HPLC method was demonstrated for Rotigotine related substances solutions ranging from LOQ 20%, 40%, 80%, 100%, 120% and 150% .Results obtained are shown in Table3. The linearity results for Rotigotine e and impurities in the specified concentration range were found satisfactory, with a correlation coefficient greater than 0.99.

Table 3: linearity method.

Component	Slope	Intercept	Correlation coefficient (R)	R^2	Intercept value w.r.to 100% conc.std response
Rotigotine	35373637.75	697.14781	0.9990	0.9979	1.79
Dethienyl ethyl	29608419.44	-586.1013	0.9999	0.9999	-0.50
Depropyl	30311345.27	375.5343	1.0000	0.9999	1.23
Acetyl rotigotine	66859263.97	-884.5145	0.9996	0.9992	-1.34

Specificity

Each known impurity and Rotigotine solutions were prepared individually at a concentration of 0.10 mg/ml and a solution of all known impurities spiked with Rotigotine was also prepared. All these solutions were analyzed using the PDA detector as per the HPLC method. Rotigotine and its known impurities (Dethienyl ethyl, Depropyl and Acetyl rotigotine e) elute at different retention times and the impurities should be adequately resolved as shown in the fig 2.

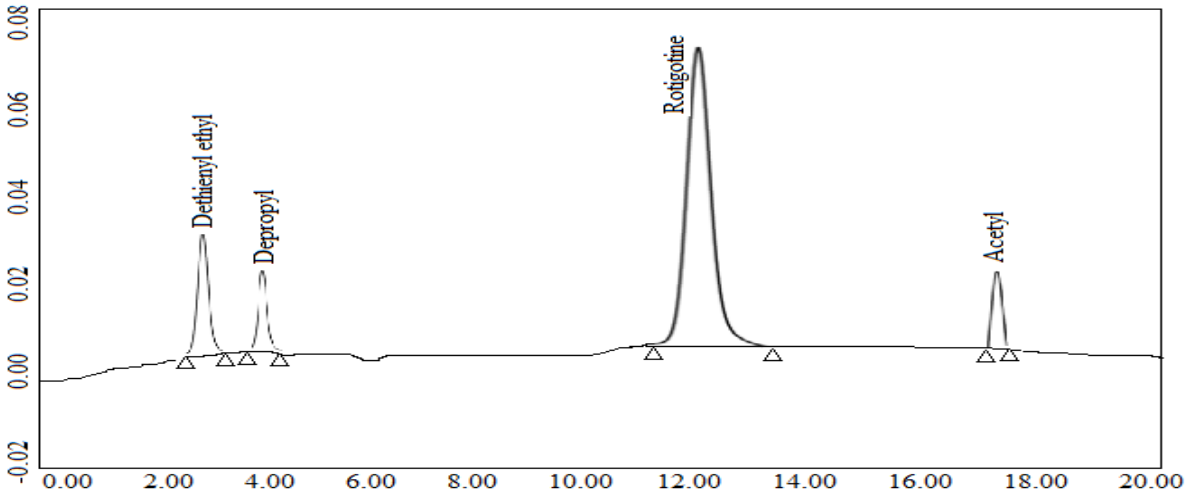

Figure 2) Rotigotine spiked with impurities.

System Suitability

Prepared the reference solution and the solution were analyzed six times as per the HPLC method described.

Accuracy

The accuracy of the method was determined using four solutions containing Rotigotine spiked with the impurities at approximately LOQ, 25%, 50%, 100% and 150% of the working strength of API. % recovery obtained in the range of 100.4 – 100.6 at 80.0% to 120.0%.

Robustness

System suitability followed by a sample analysis was run to assess if these changes had a significant effect on the chromatography. A sample of Rotigotine spiked with known impurities was analyzed for verifying the level of impurities at each variation. The retention time of all the impurities including Rotigotine were effected by slight variation in the flow, pH and column temperature, however the system suitability criteria for the method were fulfilled. The number of theoretical plates for Rotigotine peak not less than 3000. The resolution between the peaks due to intermediate and Rotigotine not less than 2.0. The tailing factor for Rotigotine peak not more than 2.0.

Solution stability

A solution of Rotigotine spiked with the impurities and the standard solution stability were kept at room temperature (24-26°C) as well as in the refrigerator at 2-8° C. The solution stability was monitored at different intervals (Initial, 24 hours and 48 hours). No significant variation in the percentage of impurities was observed upto 48 hours at 2-8° C for reference solution and sample solution. The level of unknown impurity was found to increase in the sample solution stored at room temperature. It is recommended to keep the solutions at 2-8° C for analysis. Record the results and assign the stability of the solution based on the experimental data. For a stable solution, the individual impurity values to be within ±0.03 of the original value and the total impurities to be within ±0.10 of the original value.

Degradation studies

Rotigotine and its impurities were analyzed individually to verify the retention times. In order to assess the stability indicating nature of the HPLC method, Rotigotine samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples were then analyzed a photodiode-array detector.

Diluent: Water and methanol in the ratio of 30:70 v/v.

Standard preparation

Accurately weighed and transferred about 50.0 mg of Rotigotine standard into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

Control sample preparation

Accurately weighed and transferred about 50.0 mg of Rotigotine standard into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

Forced Degradation

Analyze the impurities and Rotigotine individually as per above method to verify the retention time. In order to assess the stability indicating nature of the HPLC method Rotigotine samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples are analyzed using a photodiode-array detector.

Acid hydrolysis

At room temperature Acid hydrolysis of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 1.0N HCl solution to 5.0 mL of 1.0 mg/mL stock solution). The acid treated samples were kept at room temperature for 3 hours, 6 hours and 24 hours, and then the sample solutions were neutralized with 0.2 mL of 1N NaOH solution and diluted to 100 mL with diluent.

At 60^0C Acid hydrolysis of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 1.0N HCl solution to 5.0 mL of 1.0 mg/mL stock solution). The acid treated samples were kept at 60^0C for 3 hours, 6 hours and 24 hours, and then the sample solutions were neutralized with 0.2 mL of 1N NaOH solution and diluted to 100 mL with diluent.

Base hydrolysis

At room temperature base hydrolysis of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 1N NaOH solution to 5.0 mL of 1.0 mg/mL stock solution). The acid treated samples were kept at room temperature for 3 hours, 6 hours and 24 hours, and then the sample solutions were neutralized with 0.2 mL of 1.0N HCl solution and diluted to 100 mL with diluent.

At 60^0C base hydrolysis of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 1N NaOH solution to 5.0 mL of 1.0 mg/mL stock solution). The acid treated samples were kept at 60^0C for 3 hours, 6 hours and 24 hours, and then the sample solutions were neutralized with 0.2 mL of 1.0N HCl solution and diluted to 100 mL with diluent.

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Oxidation

At room temperature Oxidation of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 5% hydrogen peroxide solution to 5.0 mL of 1.0 mg/mL stock solution). The peroxide treated samples were kept at room temperature for 3 hours, 6 hours and 24 hours, and then diluted to 100 mL with diluent.

At 60^0C Oxidation of Rotigotine sample was carried out by using four solutions (adding 0.2 mL of 5% hydrogen peroxide solution to 5.0 mL of 1.0 mg/mL stock solution). The peroxide treated samples were kept at 60^0C for 3 hours, 6 hours and 24 hours, and then diluted to 100 mL with diluent. Observed rapid degradation at 3 hours, hence carried out study for 0 hours, 30 minutes, 1 hour and 2 hour as shown table 4.

Table 4: Assay values for oxidation samples.

Sample	% Assay
Control sample	99.6
At RT for 3 hours	99.0
At RT for 6 hours	98.8
At RT for 12 hours	98.3
At RT for 24 hours	97.3
Control sample	99.1
At 60^0C for 0 hour	100.5
At 60^0C for 1/2 hour	100.0
At 60^0C for 1 hour	98.3
At 60^0C for 2 hour	97.0
Control sample	99.6
At 60^0C for 3 hours	89.6

Heat degradation and Solid state stability

Heat degradation of Rotigotine sample was examined by heating 5.0 mL of 1.0 mg/mL stock solution at 60^0C for 3 hours, 6 hours and 24 hours, and then diluted to 100 mL with diluent. All these degraded samples were analyzed along with control sample using a photodiode array detector for determining the assay.

UV degradation

Transferred 5.0 mL of 1.0 mg/mL stock solution into a 100 mL volumetric flask and diluted to volume with diluent. Exposed the solution to UV light with an integrated near UV energy of not less than 200 watt hours/square meter. All these degraded samples were analyzed along with the control sample using a photodiode array detector for determining the assay.

Solid state stability

Solid state stability was performed by exposing the active pharmaceutical ingredients to white fluorescent light, UV light and heat.

Standard preparation

Accurately weighed and transferred about 50.0 mg of Rotigotine standard into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

Control sample preparation

Accurately weighed and transferred about 50.0 mg of Rotigotine standard into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

Exposure to white fluorescent light

About 200 mg of the sample was taken in a petridish and exposed to white fluorescent light with an overall illumination of not less than 1.2 million lux hours. After exposure, transferring 50 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

Exposure to UV light

About 200 mg of the sample was taken in a petridish and exposed to UV light with an overall illumination of not less than 200 Wh/sq.mt. After exposure, transferring 50 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

Exposure to heat at 105^0C

About 200 mg of the sample was taken in a petridish and exposed to heat at 105^0C for 24 hours: observed sample was decomposed, hence exposed at 60^0C. Accurately weighed and, transferring 50 mg of sample into a 50 mL volumetric flask, dissolved in and diluted to volume with diluent. Further diluted 5.0 mL of this solution to 100 mL with diluent.

CONCLUSION

Hence, it can be concluded that the newly developed RP-HPLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for Rotigotine without any interference from other components or additives.

This method can be employed conveniently, reliably and successfully for the estimation of Rotigotine for routine quality control and stability studies.

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