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Original Research Article

Degradation Study of Entacapone Bulk Drug by HPLC with PDA Detector

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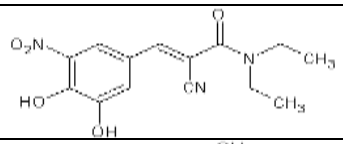
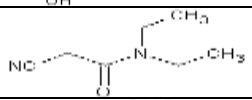
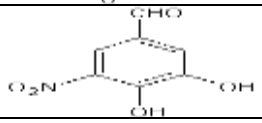
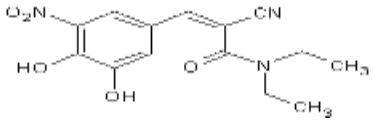
Abstract: A simple and inexpensive method was developed with high performance liquid chromatography with PDA detection for determination of Entacapone. The chromatographic separations were achieved on (250×4.6 mm), 5.0 µm make: X-terra C18 column employing Acetonitrile: 0.1% v/v phosphoric acid in Water (35:65, v/v) as mobile phase at flow rate 1.0 mL/min was chosen. The detector wavelength of 210 nm was employed.
Keywords: Forced degradation, HPLC, Method validation, Entacapone.

INTRODUCTION

Entacapone API is nitrocatechol derivative and it is used for treatment of Parkinson's disease [1, 2]. Maximum recommended daily dose for adults is 2000 mg per day if necessary. Parkinson's disease is a neurodegenerative, slow progressive disorder, resting tremor, rigidity and postural reflex impairment with associated characteristic eosinophilic cytoplasmatic inclusions. Entacapone should be used in combination with levodopa [3]. It is film coated tablet containing 200 mg Entacapone API in one tablet [4, 5]. It is orally taken medicine. Entacapone is rapidly absorbed in the gastro-intestinal tract and undergoes extensive first pass metabolism. entacapone is converted to its (cis)-isomer i.e. Z-entacapone, the main metabolite in plasma followed by direct glucuronidation to inactive glucuronide conjugates [6, 7]. High performance liquid chromatography (HPLC) is a technique used for analysis of drug substance, drug product and determination and quantification of known as well as unknown impurities at lower level, food and drug administration (FDA) also trust on the purity method of analysis by using HPLC, because of high accuracy and reproducibility of results [8]. By using this technique we can separate drug related process impurities, degradation impurities as well as reactants [9, 10]. In the present study, a novel HPLC method was developed and successfully validated for entacapone and its degradation compounds. The details are given below.

Table 1: Entacapone and its degradation compounds

Name	IUPAC Name	Structure	Origin
Entacapone	**(2E)-2-cyano-3-(3, 4-dihydroxy-5-nitrophenyl)-N, N- diethyl-2-propenamide**		-
Impurity -I	**N, N-Diethyl cyano acetamide**		Key raw material
Impurity - II	**3,4-Dihydroxy-5-nitrobenzaldehyde**		Key raw material
Z-isomer	**(2Z)-2-cyano-3-(3,4-dihydroxy-5-nitro phenyl)N,N-diethyl-2-propenamide**		Process impurity

MATERIALS AND METHODS

Materials

Standard gift samples of Entacapone were provided by Dr Benarji Patrudu, Associate Professor, Gitam University, and Hyderabad. All the chemicals and reagents used were of analytical grade.

HPLC Chromatographic Parameters

Chromatographic separation was performed on The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (X-terra C18). Column oven temperature was maintained at 30°C and flow rate 1.0 mL/min An HPLC method was developed for entacapone and related impurities by using photo diode array detector. Entacapone and all related impurities were injected into HPLC system by changing the different composition of Acetonitrile: 0.1% v/v phosphoric acid in Water (35:65, v/v). The absorption maxima for entacapone, Impurity –I, Impurity-II, Z-isomer was found to be at 210 nm. The compound was scanned form 200 – 400 nm. Column temperature was set up at 30°C and injection volume as set to 10µL. By follow this analytical method conditions, entacapone and degradation impurities were separated. The representative blank and standard linearity chromatograms were showed in Figure 1 and Figure 2.

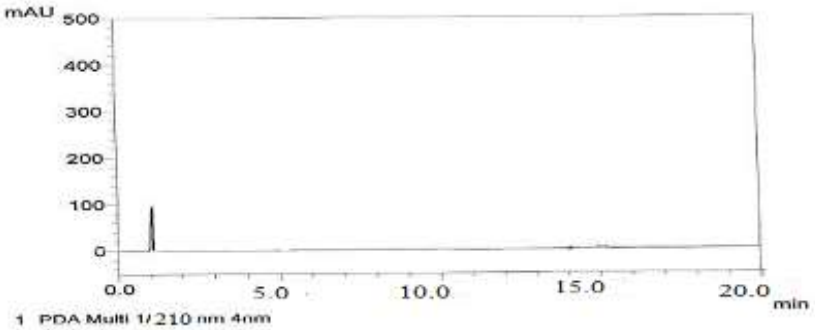


Fig-1: Representative chromatogram of diluent

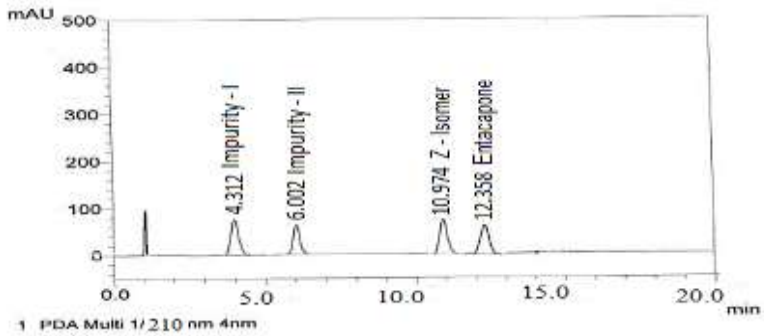


Fig-2: Representative chromatogram of standard

Method of analysis

Standard solution preparations

Weigh and transfer about 100 mg of Entacapone reference standard into a 100mL volumetric flask dissolve and make up to with diluent. Transfer 5mL of this solution into a 100 mL volumetric flask and make up to the volume with diluent.

Evaluation of system suitability

Inject blank, followed by standard solution for five times into HPLC and evaluate the chromatogram. The system is suitable for analysis, in standard chromatogram the USP tailing factor determined for Entacapone peak should not be more than 2.0 and the percentage relative standard deviation of peak area of Entacapone in the five replicate injections should be not more than 2.0.

Sample solution preparations

Weigh and transfer about 100 mg of sample into a 100mL volumetric flask dissolve and make up to with diluent. Transfer 5mL of this solution into a 100mL volumetric flask and make up to the volume with diluent.

Procedure

If the system suitability passes, Inject standard preparation and record the chromatograms. Determine the area responses of Entacapone peak in standard and sample preparations. The expected retention time of Entacapone peak is about 12.0 minutes.

Degradation Study

Analyze the impurities and Entacapone individually as per the method to verify the retention times. In order to assess the stability indicating nature

of the HPLC method, Entacapone samples will be stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples will be analyzed using a PDA detector for determining the peak purity.
Diluent : Acetonitrile: Water (50:50, v/v). The solution was injected (blank).

Test solution

Weigh and transfer about 100 mg of sample into a 100mL volumetric flask dissolve and make up to the volume with diluent. Transfer 5mL of solution in to a 100mL volumetric flask dissolve and make up to the volume with diluent.

Table 2: Acid Hydrolysis

Exposure conditions	At room temperature	At 60˚C temperature
Acid Hydrolysis	Weigh and transfer about 100 mg of sample into a Quality Control 100mL volumetric flask, add 20 mL of diluent and add 2.0 mL of 1.0 N hydrochloric acid solution. Keep the solution at room temperature for three hours and then neutralize with 2.0 mL of 1.0 N sodium hydroxide solution and dilute to 100mL with the diluent. Further dilute to 5mL of solution into100mL volumetric flask and dilute to the volume with the diluent.	Weigh and transfer about 100mg of sample into a 100mL volumetric flask, add 20 mL of diluent and add 2.0 mL of 1.0 N hydrochloric acid solution. Keep the solution at 60°C for three hours and then neutralize with 2.0 mL of 1.0 N sodium hydroxide solution and dilute to 100mL with the diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent.

Table 3: Base Hydrolysis

Exposure conditions	At room temperature	At 60˚C temperature
Base Hydrolysis	Weigh and transfer about 100 mg of sample into a 100mL volumetric flask, add 20 mL of diluent and add 2.0 mL of 1.0 N Sodium hydroxide solution. Keep the solution at room temperature for three hours and then neutralize with 2.0 mL of 1.0 N hydrochloric acid solution and dilute to 100mL with the diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent	Weigh and transfer about 100mg of sample into a 100mL volumetric flask, add 20 mL of diluent and add 2.0 mL of 1.0 N Sodium hydroxide solution. Keep the solution at 60°C for three hours and then neutralize with 2.0 mL of 1.0 N hydrochloric acid solution and dilute to 100mL with the diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent.

Table 4:Oxidation

Exposure conditions	At room temperature	At 60˚C temperature
Oxidation	Weigh and transfer about 100 mg of sample into a 100mL volumetric flask, add 20 mL of diluent and add 2.0 mL of 5% hydrogen peroxide solution. Keep the solution at room temperature for three hours and dilute to 100mL with the diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent.	Weigh and transfer about 100mg of sample into a 100mL volumetric flask, add 20 mL of diluent and add 2.0 mL of 5% hydrogen peroxide solution. Keep the solution at 60°C for three hours and dilute to 100mL with the diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent.

UV light degradation

Weigh and transfer about 100mg of substance into a 100mL volumetric flask and dissolve with 20 mL of diluent and expose the solution to UV light at 365 nm for three hours and dilute to 100mL with diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent.

Heat degradation

Weigh and transfer about 100mg of sample into a 100mL volumetric flask and dissolve with 20 mL of diluent and keep the solution at 60°C temperatures for three hours and dilute to 100mL with the diluent. Further dilute to 5mL of solution into 100mL volumetric flask and dilute to the volume with the diluent.

Solid state stability

Perform the solid-state stability study by exposing the Entacapone to heat, white fluorescent light and UV light. Analyze the heat, white fluorescent light and UV light exposed samples along with control

sample using a photodiode-array detector for determining the peak purity.

Exposure and sample preparations

Preparation of Control sample

Weigh and transfer about 100mg of sample into a 100mL of volumetric flask dissolve and make up to volume with diluent. Transfer 5mL of this solution into a 100mL volumetric flask and make up to the volume with diluent.

Exposure to white fluorescent light

Transfer about 3000.0 mg of the Entacapone sample into a petri dish, spread uniformly and expose to the white fluorescent light for 1.2 million LUX hours for a period of 23 hours.

After Exposure to white fluorescent light

Weigh and transfer about 100mg of sample into a 100mL volumetric flask dissolve and make up to with diluent. Transfer 5mL of this solution into a 100mL volumetric flask and make up to the volume with diluent.

Exposure to UV light at 365 nm

Transfer about 3000.0 mg of the Entacapone sample into a petri dish, spread uniformly and expose to the UV light at 365 nm for 200 W/m2 for a period of 48 hours.

After Exposure to UV light at 365 nm

Weigh and transfer about 100mg of sample into a 100mL volumetric flask dissolve and make up to with diluent. Transfer 5mL of this solution into a 100mL volumetric flask and make up to the volume with diluent.

Exposure to Heat at 105°C

Transfer about 3000.00 mg of the sample in a petri dish, spread uniformly and expose to heat at 105°C for ten days.

After 24 hours

After twenty-four hours weigh and transfer about 100mg of sample into a 100mL volumetric flask, dissolve and make up to the volume with diluent. Transfer 5mL of this solution into a 100mL volumetric flask and make up to the volume with diluent.

After 10 days

After ten days weigh and transfer about 100mg of sample into a 100mL volumetric flask dissolve and make up to with diluent. Transfer 5mL of this solution into a 100mL volumetric flask and make up to the volume with diluent.

RESULTS AND DISCUSSION

Table 5: Table Acid Hydrolysis

Exposure conditions	At room temperature	At 60˚C temperature
Acid Hydrolysis	From the 2D plot at 210nm, no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.	The solution was injected after being exposed to temperatures of 60 ˚C for 3, 6, 12, and 24 hours. The heating treatment shows no degradation of the product at 210nm, no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle

Table 6: Base Hydrolysis

Exposure conditions	At room temperature	At 60˚C temperature
Base Hydrolysis	From the 2D plot at 210nm, no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.	The solution was injected after being exposed to temperatures of 60 ˚C for 3, 6, 12, and 24 hours. The heating treatment shows no degradation of the product at 210 nm, no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle

Table 7:Oxidation

Exposure conditions	At room temperature	At 60˚C temperature
Oxidation	From the 2D plot at 210nm, no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.	The solution was injected after being exposed to temperatures of 60 ˚C for 3, 6, 12, and 24 hours. The heating treatment shows no degradation of the product at 210nm, no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.

UV light degradation

The solution was injected after being exposed to UV light at 365nm for 3, 6, 12, and 24 hours. From the 2D plot at 210nm no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Tolterodine tartrate peak, this because the Purity threshold is greater than the purity angle.

Heat degradation

The solution was injected after being exposed to temperatures of 60 ˚C for 3, 6, 12, and 24 hours. From the 2D plot at 210nm no impurities can be noted on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Tolterodine tartrate peak, this because the Purity threshold is greater than the purity angle.

Solid state stability

After Exposure to white fluorescent light

The control sample and the sample prepared after exposure the sample to fluorescent light for a period of 23 hours was injected to the HPLC system contains PDA detector. No degradation of the product at 210nm indicates no additional peak was observed on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.

After Exposure to UV light at 365 nm

The control sample and the sample prepared after exposure the sample to UV light for a period of 48 hours was injected to the HPLC system contains PDA detector. No degradation of the product at 210 nm, shows no additional peak was observed on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.

Exposure to Heat at 105°C

After 24 hours

The control sample and the sample prepared after exposure to 105°C for a period of 24 hours was injected to the HPLC system contains PDA detector. No degradation of the product at 210 nm, shows no additional peak was observed on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.

After 10 days

The control sample and the sample prepared after exposure to 105°C for a period of 10 days was injected to the HPLC system contains PDA detector. No degradation of the product at 210 nm, shows no additional peak was observed on the chromatogram. Reviewing the PDA plots namely the purity plot, it can be said that there are no impurities that fall under the Entacapone peak, this because the Purity threshold is greater than the purity angle.

CONCLUSION

From the results obtained in this study, one can conclude that the Entacapone is stable to all the conditions tested in this study, hence it can be conclude that the product shows no appreciable degradation in the conditions tested in this study.

Results also show that the HPLC method used in this study is able to detect any degradation of the product and separate any degradation impurities formed, thus making it a suitable stability indicating method.

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