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Assay Method of Active Pharmaceutical Ingredient Entacapone by High Performance Liquid Chromatographic Technique

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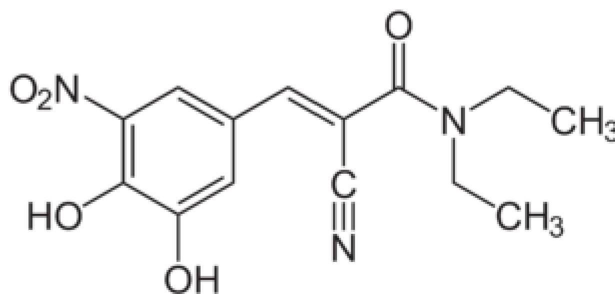
Abstracts:

A simple, linear and reproducible assay method was developed for the quantification of entacapone API by using high performance liquid chromatography. The method was developed by using Phenomenox Kinetex C18, (100 x 4.6) mm, 2.6 micron column. Mobile phase consisted of Water: Methanol: Orthrophosphoric acid in ratio 50:50:0.1 with flow rate of 1.0 ml/min at 300 nm and column oven temperature at 50°C. RSD for standard preparation under system precision was observed 0.10%. RSD for retention time was observed 0.15% which shows reproducibility during replicate injections. The linearity range was achieved from 200 to 600 ppm level for entacapone. Comparative data of method and intermediate precision shows average assay value 99.81% on as is basis. The method was applied for quantification of assay of entacapone API.

Key words: HPLC Assay, Entacapone API.

Introduction:

High performance liquid chromatography (HPLC) is a worldwide accepted technique used for analysis of drug substance, drug product and quantification of known as well as unknown impurities at lower level[1]. Entacapone API is nitrocatechol derivative and it is used for treatment of Parkinson's disease. Entacapone should be used in combination with levodopa. It is film coated tablet containing 200 mg Entacapone API in one tablet. 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 is orally taken medicine which contains effect of a selective and reversible inhibitory effect on catechol-O-methyl transferase (COMT) enzyme. It contains two stereoisomeric forms E = trans-isomer and the Z = cis-isomer. The E-isomer was selected because of early synthetic route. Entacapone is rapidly absorbed from 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. Elimination of this conjugates is mainly via urine as glucuronide conjugates and Z- isomer[2]. A HPLC method for Entacapone was published by T. Wikberg[3] and Ramakrishna[4] from rats and humans. The quantification method was reported by Siddiqui[5] and Sivasubramanian[6] for this drug.



(2E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylprop-2-enamide [Entacapone][7]

Current work presents assay quantification method by HPLC to quantify entacapone API against standard.

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Experimental:

HPLC grade methanol as a solvent (Spectrochem), orthrophosphoric acid AR grade (Rankem) and HPLC grade water (Merck) were used. Entacapone test sample was received from Ramdev Chemical as a free sample. Z-Isomer of Entacapone is procured from LGC Promochem India limited. HPLC instrument of make Shimadzu LC 2010C HT with a quaternary gradient pump system and a fixed dual wavelength UV detector having LC-solution software with auto sampler tray with cooling facility and column oven temperature compartment is available was used for measurements.

Development of method was initiated by keeping aim to develop simple isocratic, easy setup of experimentation and cost effective short run time assay quantification HPLC method for entacapone API, in such a way that we will get accurate and reproducible results in short period of time. The referred USP method-2[8] method was not found suitable in terms of composition of mobile phase[8]. Tekale et al[9] also extend the research for quantification from tablet.

Short length, lower micron size C18 column was selected to reduce run time and to achieve excellent separation with sharp peak shapes. Because of short length compound eluted faster and C18 carbon load in column helps for separation and lower particle size column gives sharp peaks as well as more separation. Column oven temperature study was performed in order to reduce the backpressure of column ensuring that it does not impact on separation. Some basic compounds have property to get retained in column and because of such compounds get stuck inside the column and when they eluted it gives more tailing to peak. In this case higher column oven temperature increases kinetic energy of compound for early elution with good separation and symmetric peak shape will be obtained. Also it reduces viscosity of mobile phase which helps to reduce the back pressure of column. In our method mobile phase is also easy to setup, which was premixed and degassed solution of HPLC grade water: Methanol: Orthrophosphoric acid in ratio 50:50:0.1 was used. Run time was kept 7 minutes and 5 micro liter injection volume was injected to avoid excess of sample loading on column. Since excess sample loading on column baseline may get disturbed, hump and broad peak shape may not observed.

Mobile phase and other working solutions were filtered through 0.45 micron membrane filter and degassed by sonication. Slowly the flow rate was increased to achieve the column oven temperature and equilibrate column in mobile phase. Direct application of flow rate creates high pressure on column and may damage it. Diluent (water: methanol 50:50) was injected as blank preparation. No peak was observed in blank preparation at retention time of entacapone means there is no any blank interference. Five replicate injections of standard preparation were injected for calculation of % RSD.

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Chromatographic conditions:

Column name	:	Phenomenox Kinetex C18, (100 x 4.6) mm, 2.6 micron
Flow rate	:	1.0 ml/min
Wavelength	:	300 nm
Column Oven temperature	:	50°C
Run time	:	7 minutes.
Injection volume	:	5 µl
Mobile phase	:	Water: Methanol: Orthrophosphoric acid (50:50:0.1)
Diluent	:	Water: Methanol (50:50)

Results and discussion:

a) Chromatography:

Standard stock solution of entacapone was prepared by weighing accurately 50 mg of entacapone standard in 50 ml standard volumetric flask. 25 ml of methanol was added and sonicated to dissolve and volume was made up to mark with water. Thus 1000 ppm of Entacapone Stock solution was prepared. Further 10 ml of this solution was diluted to 25 ml volumetric flask and made up to mark with diluent. (400 ppm standard solution).

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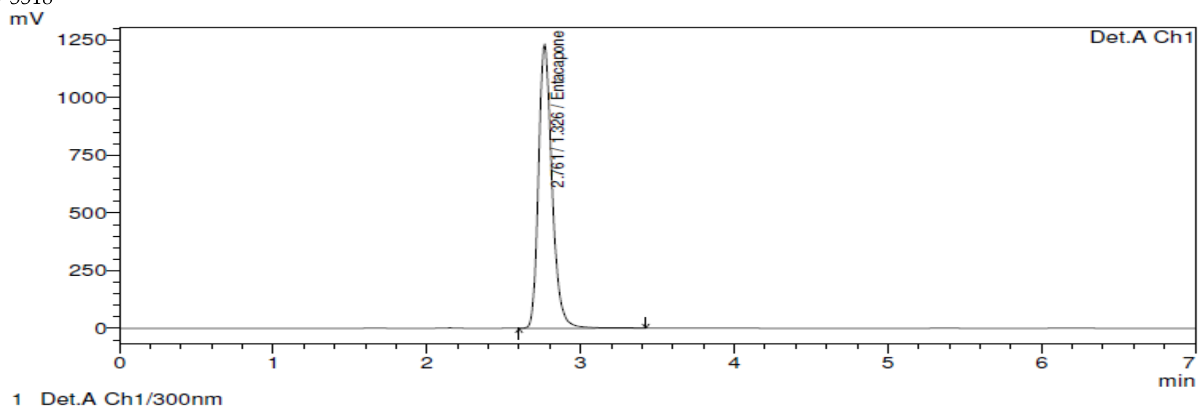

PeakTable

Detector A Ch1 300nm

Peak#	Ret. Time	Area	Tailing Factor	Name
1	2.761	7532099	1.326	Entacapone
Total		7532099		

Figure: - 1 Chromatogram of Standard preparation

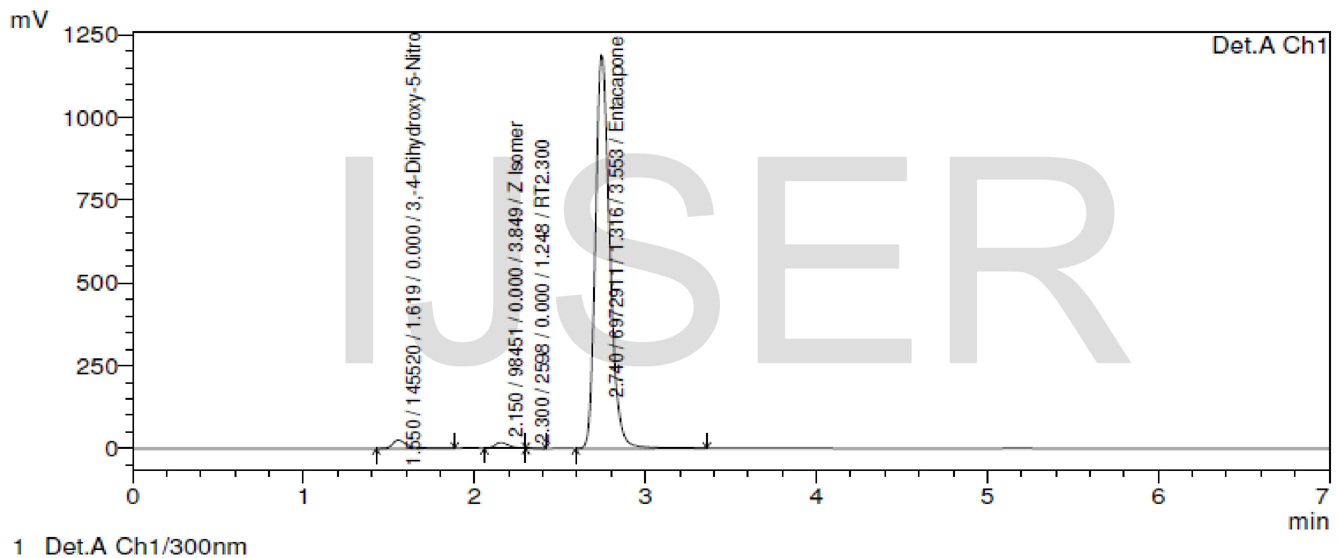

Figure: - 2 Chromatogram of Spike preparation

From the chromatogram (Fig. 1) it was concluded that the Retention time for Entacapone was 2.7 ± 0.05 minutes and tailing factor was 1.32 which as per USP should not be more than 1.5. Refer figure 2 is spike chromatogram of Z isomer, intermediate with entacapone API. It shows that impurity and intermediate is separated from entacapone peak. Where 3, 4-dihydroxy-5-nitrobenzaldehyde is an intermediate and Z isomer is impurity in entacapone. During method development it has to ensure that no any known as well unknown impurity especially any intermediate or raw material from which the product is synthesized should get merged with concern peak. If such peak gets merged then peak area of such peak will also get contributed in concerned peak area and incorrect results will be obtained. In the present work system precision performed by injecting five replicate injections of standard preparation and percent RSD was calculated. Table 1 refers to retention time and % RSD of standard area.

Table- 1: System precision

Sr.No	RT (min)	Area
1	2.761	7547024
2	2.764	7538672
3	2.763	7539123
4	2.768	7527813
5	2.771	7530300
Average	2.76	7536586
% RSD	0.15	0.10

Linearity was performed from 50% to 150% level with respect to test concentration level in which linearity range was covered from 200 ppm to 600 ppm level. It was concluded that linearity graph that test concentration is linear and observed value for correlation coefficient is 0.9997. Linearity10 is an important parameter in validation which needs to be performed to obtain test results which are directly proportional to the concentration of analyte in test sample (Figure 3).

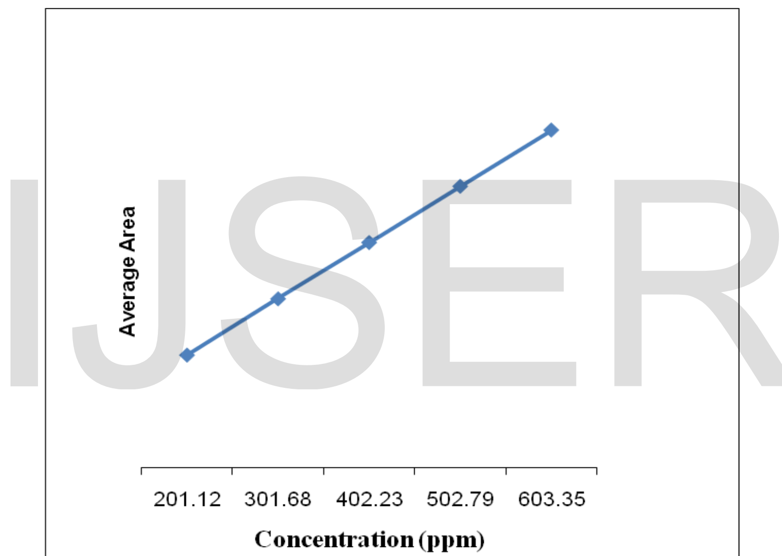

Figure: 3 Linearity graph

Reproducibility of method was checked by performing method precision in which same test preparation was performed six times and each of the preparations was injected in duplicate. Results were calculated against average area of five replicate injections of standard preparation. Reproducibility of method was checked by performing intermediates precision, in which same experiment was performed by different worker, different days etc. From the data obtained it is concluded that our method is reproducible. This is depicted in table 2 for comparative results.

Table: 2 Comparison between method precision and intermediate precision.

Sr.No	Method Precision	Intermediate Precision
1	99.8%	100.6%
2	99.6%	100.0%
3	99.8%	100.1%
4	99.7%	99.9%
5	99.3%	99.2%
6	100.2%	99.5%
Average	99.81%	

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% RSD	0.39%

Robustness[11] of method was checked by changing mobile phase composition, column oven temperature and flow rate and found method is robust in all condition and there is no merging of any peaks and peak shapes is also found good. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. Stability of analytical solution was checked and found that it is stable up to 21 hrs at room temperature. For that freshly prepared solution injected and same solution kept at room temperature and injected after 21 hrs. Area difference found within acceptance criteria.

Conclusion:

The elution of entacapone standard was carried out on Kinetex C18, 4.6 × 100 mm x 2.6 micron column HPLC column, at the flow rate of 1 ml/min in isocratic mode using the mobile phase consisting of Water: Methanol: Orthrophosphoric acid (50:50:0.1) with flow rate of 1 ml/min at 300 nm.

The method precision and intermediate precision of the method was calculated in terms of the relative standard deviation and it was observed 0.39% which indicates accurate reproducibility of method. Linearity graph plotted against average area Vs concentration which is found linear with correlation coefficient 0.9997

The method can be used successfully for identification and quantification of the active pharmaceutical ingredient entacapone from pharmaceutical ingredient. Hence this method can be used for the routine analysis of entacapone.

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