Chemical Degradation and Mass Balance Study of Entacapone API by HPLC

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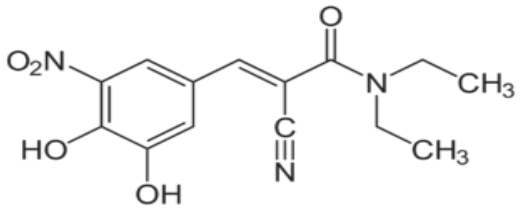
Abstracts:

By looking the importance of HPLC technique, a simple, linear and accurate related substance method was developed for the quantification of Z-isomer impurity and 3, 4-dihydroxy-5-nitrobenzaldehyde intermediate in entacapone active pharmaceutical ingredient by using high performance liquid chromatography technique. Separation between impurities and Entacapone was achieved on Phenomenox Kinetex C18, (100 x 4.6) mm, 2.6 micron column. Mobile phase consisted of 0.1% Orthrophosphoric acid in water: Methanol in ratio 58:42:0.1 in isocratic mode for RSD of standard. Gradient method used for sample preparation and blank with flow rate of 0.8 ml/min at 300 nm and column oven temperature at 50°C. RSD for standard preparation under system precision and percentage recovery was observed with in acceptance range. RSD for retention time was observed 0.05% which shows reproducibility during replicate injections. Limit of detection (LOD) and limit of quantification (LOQ) was achieved at 0.1 ppm and 0.2 ppm level respectively which indicates the lowest level of detection and quantification. The linearity range was achieved from 0.2 ppm to 0.75 ppm level for entacapone, Z-isomer and intermediate. After successful development of this method chemical forced degradation performed by external acid, alkali and peroxide treatment. No any degradation peak was interfering with any impurity. This newly developed innovative method found suitable for analysis of Entacapone API.

Key words: HPLC, Mass balance, Entacapone API, Mass balance.

Introduction:

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]. Food and drug administration (FDA) highly appreciate the purity method of analysis by using HPLC, because this technique is well known for highly accurate, reproducible results. By using this technique we can separate drug related process impurities, degradation impurities as well as reactants. Entacapone API is nitrocatechol derivative and it is used for treatment of Parkinson's disease. Parkinson's disease is a neurodegenerative, slow progressive disorder, resting tremor, rigidity and postural reflex impairment with associated characteristic eosinophilic cytoplasmatic inclusions. It is orally taken medicine which contains effect of a selective and reversible inhibitory effect on catechol-O-methyl transferase (COMT) enzyme. Entacapone consists of two isomeric forms E = trans-isomer and the Z = cis-isomer. The E-isomer was selected because it is easy for synthesis. 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. 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[5-6], European patent[7] for this drug. These reference methods discuss about analytical purity method, but were found not suitable in terms of gradient equilibration and run time.



(2E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N,N-diethylprop-2-enamide [Entacapone][8]

This work presents related substance quantification method by HPLC to quantify entacapone Z isomer, 3, 4-dihydroxy-5-nitrobenzaldehyde as intermediate, degradation and unknown impurities present in drug.

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 LC-solution software.

Development of method was initiated by keeping aim to develop simple gradient, easy setup of experimentation and cost effective short run time related substance HPLC method for entacapone API, in such a way that we will get accurate and reproducible results in short period of time. Also chemical forced degradation was performed to determine formation of any degradation impurities. The referred USP method-29 method was not found suitable in terms of composition of mobile phase[9]. Tekale et al[10] also extend the research for quantification from tablet. Shashikant Aswale et al[11] continued our research from assay method of entacapone by using HPLC technique. Dhawan et.al[12] also works on degradation in tablets.

During selection of HPLC column, Short length, lower micron size C18 column was selected to reduce run time and to achieve excellent separation with sharp peak shapes. Also C18 carbon load in column helps for separation and lower particle size column gives sharp peaks as well as more separation. Higher column oven temperature increase the kinetic energy of components by which we can achieve good and sharp peak. Also it reduces viscosity of mobile phase which helps to reduce the back pressure of column. In this proposed method mobile phase is also easy to setup, which is degassed solution of 0.1% orthtophosphoric acid in HPLC grade water as mobile phase-A and pure HPLC grade Methanol used as mobile phase-B. Gradient method was used for elution of all impurities.

Chromatographic conditions:

Column name : Phenomenox Kinetex C18, (100 x 4.6) mm, 2.6 micron
Flow rate : 0.8 ml/min
Wavelength : 300 nm
Column Oven temperature : 50°C
Run time : 9 minutes (For standard in isocratic mode, MP-A: MP-B in ratio 58:42)
Injection volume : 5 µl
Diluent : Water: Methanol (50:50)

Gradient:

Time (mins)	Mobile phase-A	Mobile Phase-B
0.0	58	42
8	58	42
15	40	60
18	40	60
20	58	42
26	58	42

Degradation:

Acid degradation:

Enatacpone test sample treated chemically by adding 5 ml 1N and 2N hydrochloric acid and kept at room temperature for about 23 hrs separately. By performing slow degradation, degradation impurities were not observed. Then by increasing concentration of acid i.e. 5 ml, 5 N hydrochloride acid added in solution of entacapone and heat at 80°C for 3 hrs in water bath. By giving extreme condition sample was not degraded.

Alkali degradation:

Enatacpone test sample treated chemically by adding 1 ml 0.01 N sodium hydroxide and injected immediately but degradation was not observed. Hence volume increases to 3 ml and test sample treated with 3 ml 0.01 M sodium hydroxide by keeping solution at room temperature for 1 hr. By doing this also degradation was not achieved. Hence compound treated by extreme condition but nature of compound changed.

Oxidative degradation:

Entacpone test sample treated chemically by adding 5 ml 3% peroxide solution at keep at room temperature for about 23 hrs. By doing this degradation was not achieved with in acceptance criteria. Hence gives extreme treatment to compound by adding 5 ml 30 % peroxide at heat the solution for 3 hrs at 80°C. By doing this also degradation was not achieved.

Results and discussion:

Chromatography:

0.5 ppm standard solution of entacapone was prepared by accurate weighing of entacapone standard and performing exact dilution in volumetric flask.

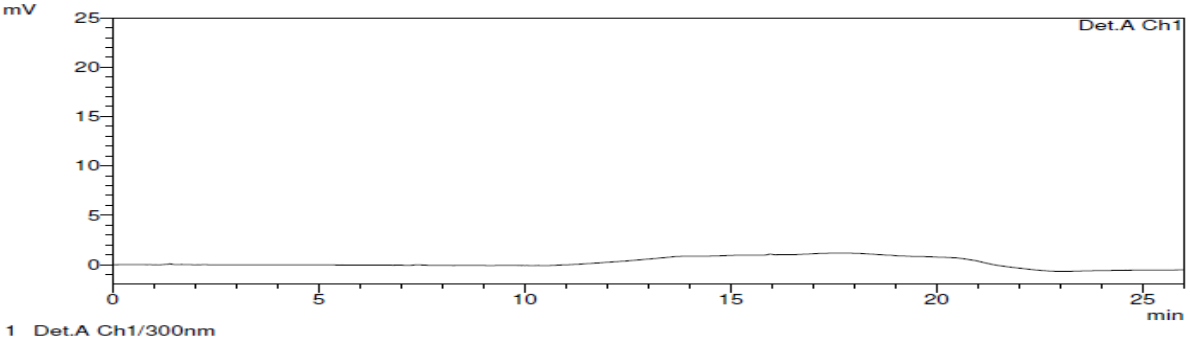


Figure: - 1 Chromatogram of blank preparation.

PeakTable

Detector A Ch1 300nm

Peak#	Ret. Time	Area	Tailing Factor	Name
1	6.666	14040	1.149	Entacapone
Total		14040		

Figure: - 2 Chromatogram of Standard preparation.

Figure 1 is blank preparation chromatogram, from the standard chromatogram (Fig. 2) it was concluded that the Retention time for Entacapone was 6.6 ± 0.05 minutes and tailing factor was 1.14 which as per USP should not be more than 1.5.

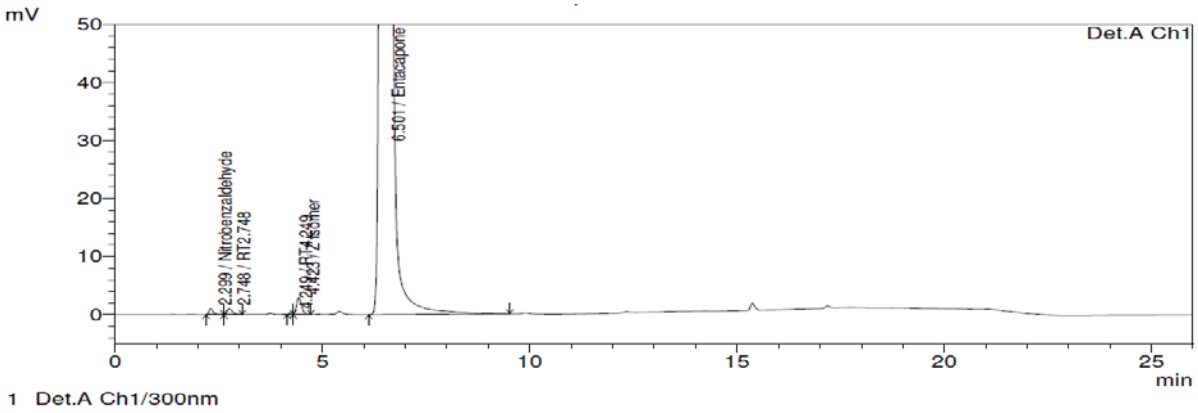


Figure: - 3 Chromatogram of Spike preparation (in Which Z Isomer an Intermediate spike with Test sample)

Fig (3) shows that impurity, intermediate as well as unknown impurities are also separated from entacapone peak. Where 3, 4-dihydroxy-5-nitrobenzaldehyde is an intermediate and Z isomer is impurity in entacapone.

Linearity[13] was performed from limit of quantification (LOQ) to 150% level with respect to test concentration level in which linearity range was covered from 0.20 ppm to 0.75 ppm level. It was concluded from linearity graph that test concentration is linear. Observed value of correlation coefficient is 0.99 for intermediate, Z isomer and Entacapone API.

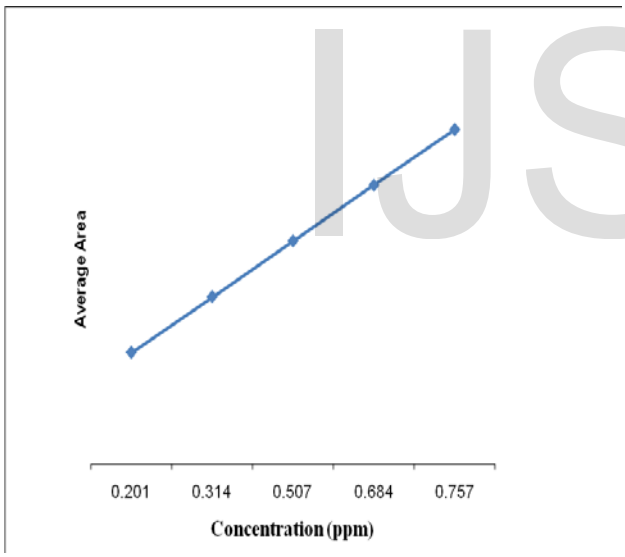


Figure: 4 Linearity graph for 3,4-dihydroxy-5-nitrobenzaldehyde (Intermediate)

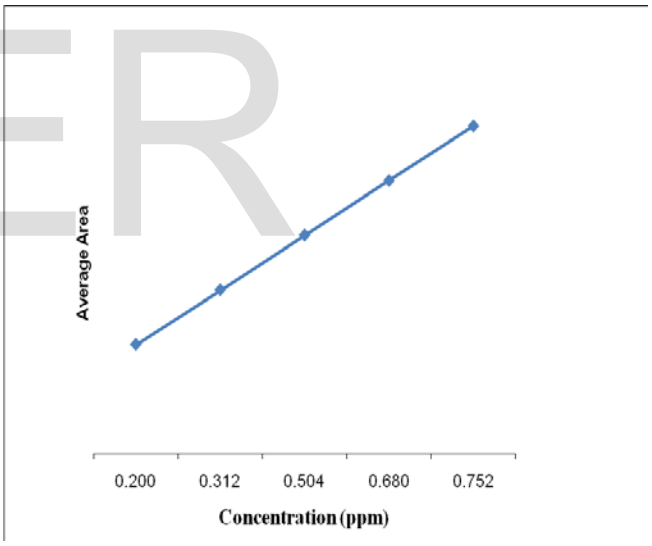


Figure: 5 Linearity graph for Z Isomer (Impurity)

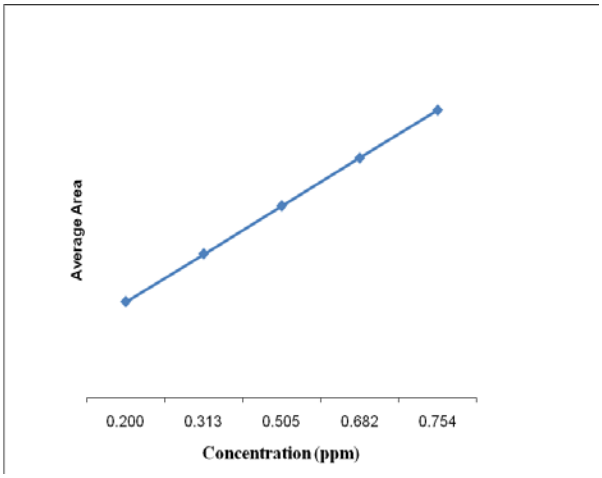


Figure: 6 Linearity graph for Entacapone (API)

Figure 4 shows linearity graph for 3, 4-dihydroxy-5-nitrobenzaldehyde, figure 5 shows linearity graph for Z isomer of entacapone and figure 6 shows linearity graph of entacapone API that line passes through all five concentration points.

Lowest limit of detection (LOD) of intermediate, Z isomer and entacapone i.e. at 0.1 ppm level on visual detection technique. Six replicate injections of 0.1 ppm solution injected and observed consistent and average area observed.

Lowest limit of quantification (LOQ) of intermediate, Z isomer and entacapone i.e. at 0.2 ppm level, six replicate injections of 0.2 ppm solution injected. Consistent, average area was achieved.

The accuracy[14] of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy study performed and successfully achieved.

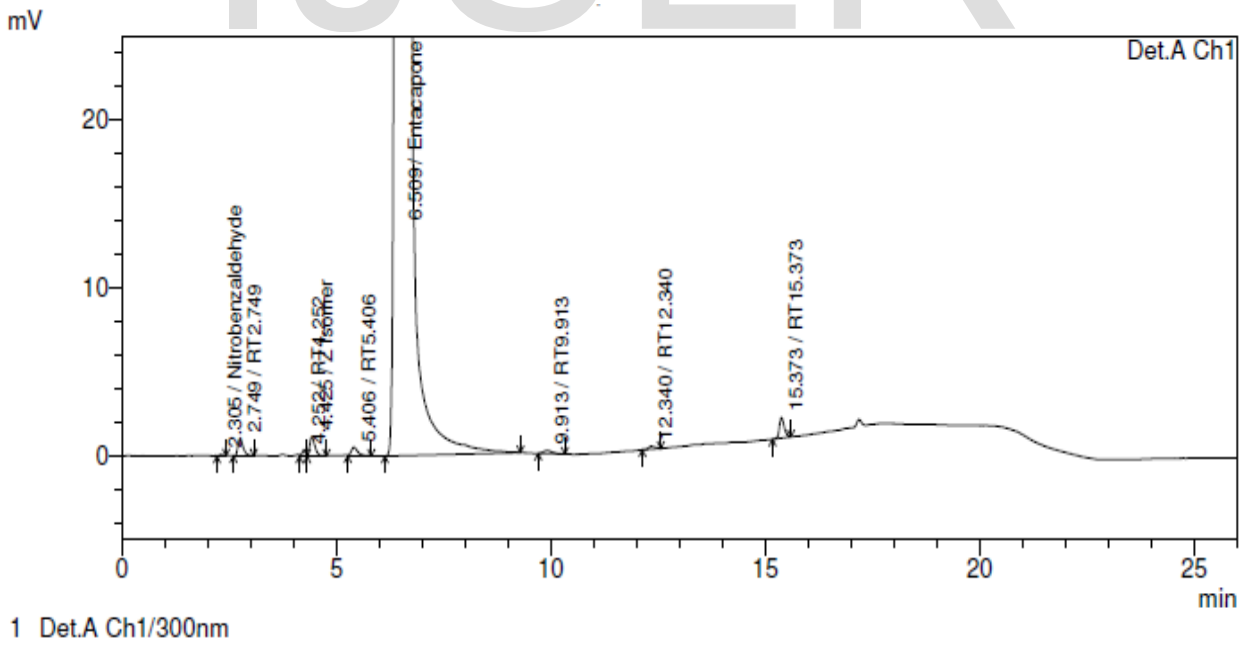


Figure: 7 Test preparation chromatogram of Entacapone API.

Figure 7 shows typical chromatogram of test preparation,

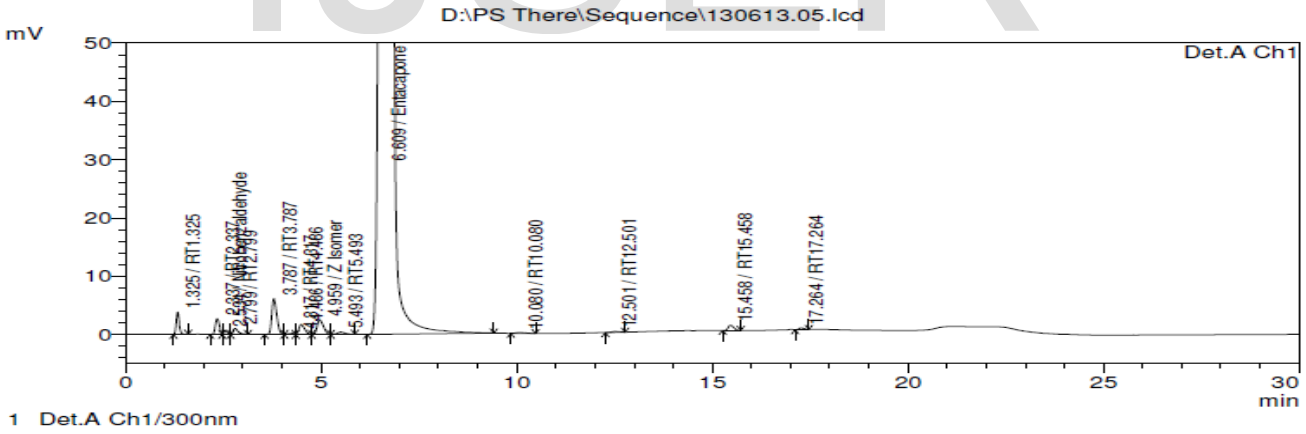
Table-5: Calculation of % w/w of impurities under method precision.

Sr. No	Intermediate (%w/w)	Z Isomer (%w/w)	Highest Unknown impurity (%w/w)	Total Impurities (%w/w)
1	0.003	0.058	0.056	0.24
2	0.003	0.058	0.056	0.24
3	0.003	0.059	0.057	0.25
4	0.003	0.058	0.056	0.24
5	0.003	0.059	0.057	0.24
6	0.003	0.058	0.055	0.24
Average	**0.003**	**0.058**	**0.056**	**0.24**

Reproducibility of method was checked by performing method precision in which same test was prepared six times and each of the preparations was injected. Results were calculated against average area of six replicate injections of entacapone standard preparation. This is depicted in table 5 for comparative results.

Table-6: Degradation data.

Impurity Type	RRT	As such test Preparation (% Area)	Acid Degradation Sample (%Area)	Base Degradation Sample (%Area)	Peroxide Degradation Sample (%Area)
Unknown	0.200	Not Detected	0.178	Not Detected	Not Detected
Unknown	0.354	Not Detected	0.169	0.252	0.163
Intermediate	0.383	0.004	0.042	0.191	0.475
Unknown	0.573	Not Detected	0.474	Not Detected	Not Detected
Unknown	0.679	0.019	0.157	Not Detected	0.157
Z Isomer	0.750	0.080	0.230	Not Detected	0.233
Entacapone	1.000	99.673	98.467	99.163	98.735

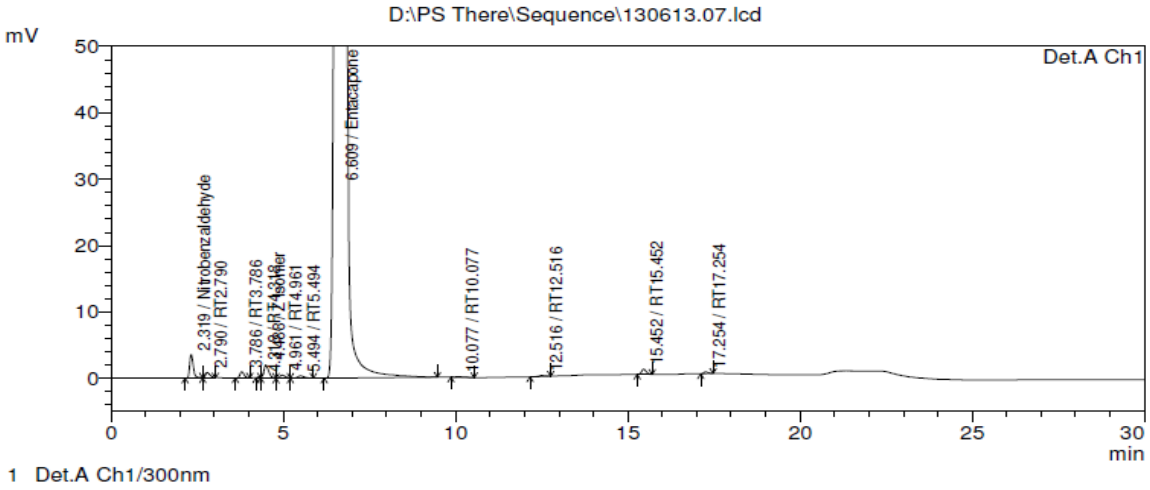


PeakTable

Detector A Ch1 300nm

Peak#	Ret. Time	Area	Area %	ative Retention T	Name
1	1.325	20933	0.178	0.200	RT1.325
2	2.337	19938	0.169	0.354	RT2.337
3	2.534	4929	0.042	0.383	Nitrobenzaldehyde
4	2.799	9397	0.080	0.424	RT2.799
5	3.787	55864	0.474	0.573	RT3.787
6	4.317	2885	0.024	0.653	RT4.317
7	4.486	18469	0.157	0.679	RT4.486
8	4.959	27115	0.230	0.750	Z Isomer
9	5.493	5476	0.046	0.831	RT5.493
10	6.609	11596376	98.467	1.000	Entacapone
11	10.080	2165	0.018	1.525	RT10.080
12	12.501	1895	0.016	1.891	RT12.501
13	15.458	8806	0.075	2.339	RT15.458
14	17.264	2704	0.023	2.612	RT17.264
Total		11776951	100.000		

Figure: 8 Acid degradation test chromatogram of Entacapone API.

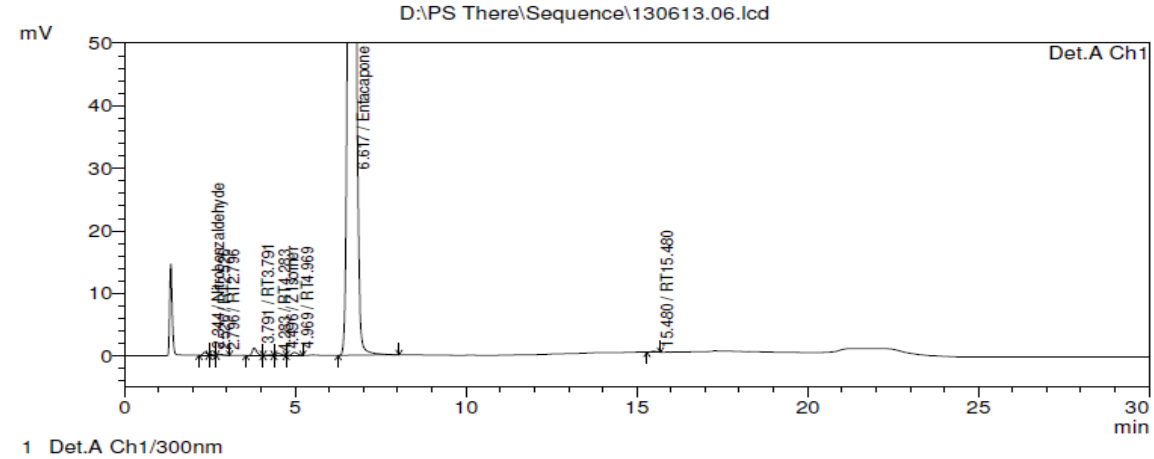


PeakTable

Detector A Ch1 300nm

Peak#	Ret. Time	Area	Area %	ative Retention T	Name
1	2.319	26128	0.252	0.351	Nitrobenzaldehyde
2	2.790	8326	0.080	0.423	RT2.790
3	3.786	8217	0.079	0.574	RT3.786
4	4.318	1324	0.013	0.654	RT4.318
5	4.488	19770	0.191	0.680	Z Isomer
6	4.961	4651	0.045	0.752	RT4.961
7	5.494	4638	0.045	0.832	RT5.494
8	6.609	10277983	99.163	1.001	Entacapone
9	10.077	1974	0.019	1.527	RT10.077
10	12.516	1986	0.019	1.896	RT12.516
11	15.452	7389	0.071	2.341	RT15.452
12	17.254	2366	0.023	2.614	RT17.254
Total		10364751	100.000		

Figure: 9 Alkali degradation test chromatogram of Entacapone API.



PeakTable

Detector A Ch1 300nm

Peak#	Ret. Time	Area	Area %	ative Retention T	Name
1	2.344	3659	0.163	0.355	Nitrobenzaldehyde
2	2.526	1070	0.048	0.382	RT2.526
3	2.796	1565	0.070	0.423	RT2.796
4	3.791	10685	0.475	0.574	RT3.791
5	4.283	1072	0.048	0.648	RT4.283
6	4.496	3523	0.157	0.680	Z Isomer
7	4.969	5251	0.233	0.752	RT4.969
8	6.617	2220575	98.735	1.001	Entacapone
9	15.480	1619	0.072	2.342	RT15.480
Total		2249018	100.000		

Figure: 10 Peroxide degradation test chromatogram of Entacapone API.

Degradation is also important criteria in method development. In this process, compound needs to be degrading by chemical treatment and formation of unknown impurities as well as increasing or decreasing trend of impurities to be monitor. Results obtained from this study can help to decide the storage condition and handling precaution of drug. From table no 6 it was concluded that intermediate and Z isomer shows increasing trend in all three i.e. acidic, basic and oxidative conditions. Acid degradation shows unknown impurity at RRT 0.573 which was not observed in any degradation condition. From this it was concluded that this method is capable to separate out degradation impurities. Figure 8,9 and 10 shows details about chromatogram of acid degradation, alkali degradation and peroxide degradation.

Mass Balance = 100 – total impurities in test sample.

= 100- 0.24

= 99.76 %

Robustness14 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.

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 0.8 ml/min in isocratic mode for standard preparation and in gradient mode for blank and test sample analysis in which degassed solution of 0.1% orthtophosphoric acid in HPLC grade water as mobile phase-A and pure HPLC grade Methanol used as mobile phase-B with flow rate of 0.8 ml/min at 300 nm.

The method precision result shows reproducibility of method. Linearity graph plotted against average area Vs concentration which is found linear with correlation coefficient 0.99 for Z isomer, intermediate and entacapone.

From LOD and LOQ results it was concluded that in this method lowest detection limit is 0.1 ppm and quantification limit is 0.2 ppm with respect to test concentration.

Degradation study clearly indicates that this chromatographic method is capable for separation and quantification of degradation, known as well as unknown impurities in entacapone sample. Also mass balance study shows that theoretically obtained value is very much close with purity obtained under degradation.

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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