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RESEARCH ARTICLE

DETERMINATION OF ENTACAPONE IN PHARMACEUTICAL FORMULATIONS BY RP-HPLC METHOD

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Abstract: Entacapone is a selective, reversible catechol-O-methyl transferase (COMT) inhibitor for the treatment of Parkinson's disease. A simple, precise, specific, and accurate reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for the estimation of entacapone in bulk and its pharmaceutical dosage forms. The method was carried out on a X timate™ HPLC C18 (250×4.6 ,5µ) column using a mixture of acetonitrile and 0.02M potassium dihydrogen orthophosphate , pH 6.0 adjusted with dilute tri ethylamine in the ratio of 55:45 at flow rate of 1ml/min with UV detection at 310 nm. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 20 to120 µg/ml , R^2 value was found to be as 0.999. The percentage of RSD was found to be lower than 2% proves that the method is precise. The excipients present in the formulations do not interfere with the assay procedure. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental analysis were indicative of satisfactory precision and reproducibility. Hence the developed method was successfully applied to determine entacapone in pharmaceutical formulations.

Key words: Entacapone, RP- HPLC, Tablets, Validation.

INTRODUCTION:

Parkinson's disease is a progressive, neurodegenerative disorder of the extrapyramidal nervous system affecting the mobility and control of the skeletal muscular system. Symptoms of parkinson's disease are related to depletion of dopamine[1-2]. Parkinson's disease (PD) is a neurodegenerative, slowly progressive disorder characterized by bradykinesia, resting tremor, rigidity and postural reflex impairment with associated characteristic eosinophilic cytoplasmatic inclusions. Entacapone is a nitrocatechol derivative and it is chemically known as 2-cyano-3(5-dihydroxyamino-3,4-dioxo-1-cyclohexa-1,5-di enyl)-*N*,*N*-diethyl-prop-2-enamide and belongs to the class of antiparkinson agents(ENT, Fig.1). Entacapone is a selective and reversible inhibitor of catechol ortho-methyltransferase (COMT), with mainly peripheral actions[3-4]. It is used in the treatment of Parkinson's disease as an adjunct to Levodopa/Carbidopa therapy[5].

Literature review revealed that only pharmacological and clinical studies [6-8] have been reported for the determination of entacapone and a pharmacokinetic study which used LC-MS method for the determination of entacapone[9]. HPLC has become a widely used tool for the routine determination and separation of drugs either alone in pure form or in admixture with other drugs or degradation products and in pharmaceutical formulations.[10-13]Existing literature reveals that there are only few methods for the assay of entacapone in bulk and dosage forms [14].

There is no official method for the estimation of entacapone in the pharmacopoeia`s. Hence an attempt has been made to develop a new simple, reliable, and reproducible, isocratic RP-HPLC methods to estimate the entacapone in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility respectively. The proposed method was validated as per ICH guidelines [15].

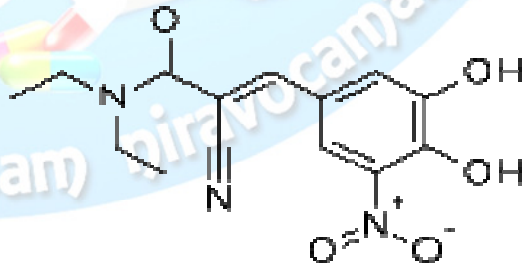

Fig.1 : Structure of entacapone

EXPERIMENTATION:

Equipment

Chromatographic separation was performed on ELICO-LI 120 HPLC system consist of UVdetector and Rheodyne injector with 20µl loop volume. Elichrome software was applied for data collecting and processing.

Reagents and chemicals

Acetonitrile, Methanol and water of HPLC grade were procured from Rankem lab ltd. Entacapone was received as gift samples from Hetero Labs Ltd., Hyderabad, India, respectively. Entacapone® tablets were purchased from local market.

Table 1: Table for Assay

Tablet formulation	Drug	Amount present (mg/tab)	Amount found* (mg/tab)	% label claim*
T1	ENT	100	99.861	99.30%
T2	ENT	200	200.032	100.66%

*T1 and T2 are two different brands of tablet formulations. ENT denotes Entacapone respectively.*Each value is average of six determinations.*

Fig .2 Assay Chromatogram of entacapone

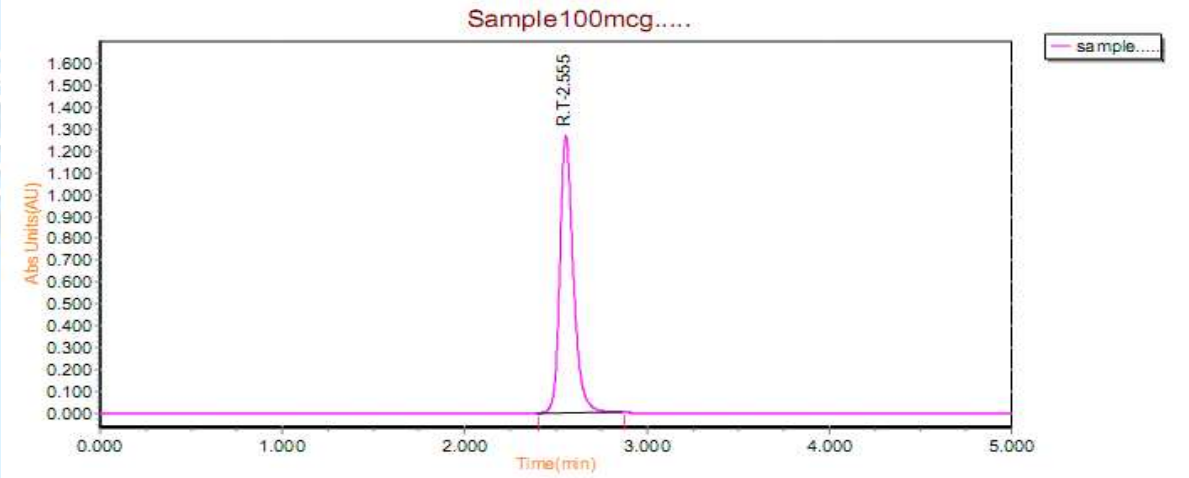

Table 2. System suitability results of entacapone

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor (TF)
1	2.527	6517	6689	1.174
2	2.533	6547	6748	1.123
3	2.533	6587	6859	1.147
4	2.527	6520	6547	1.156
5	2.527	6591	6697	1.146
6	2.540	6534	6795	1.185
Mean	2.512	6549	-	-
SD	0.0052	32.561	-	-
%RSD	0.22	0.497	-	-

Fig3. Linearity curve of entacapone

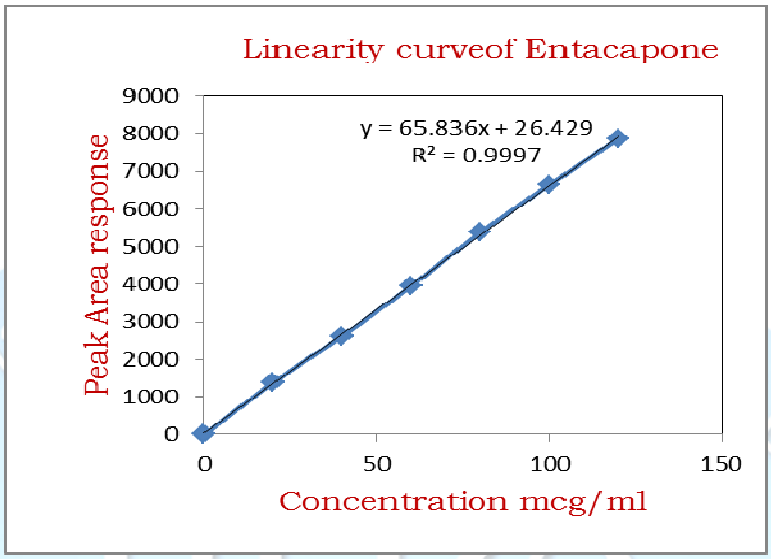

Fig. 4: Linearity over lain chromatogram of entacapone

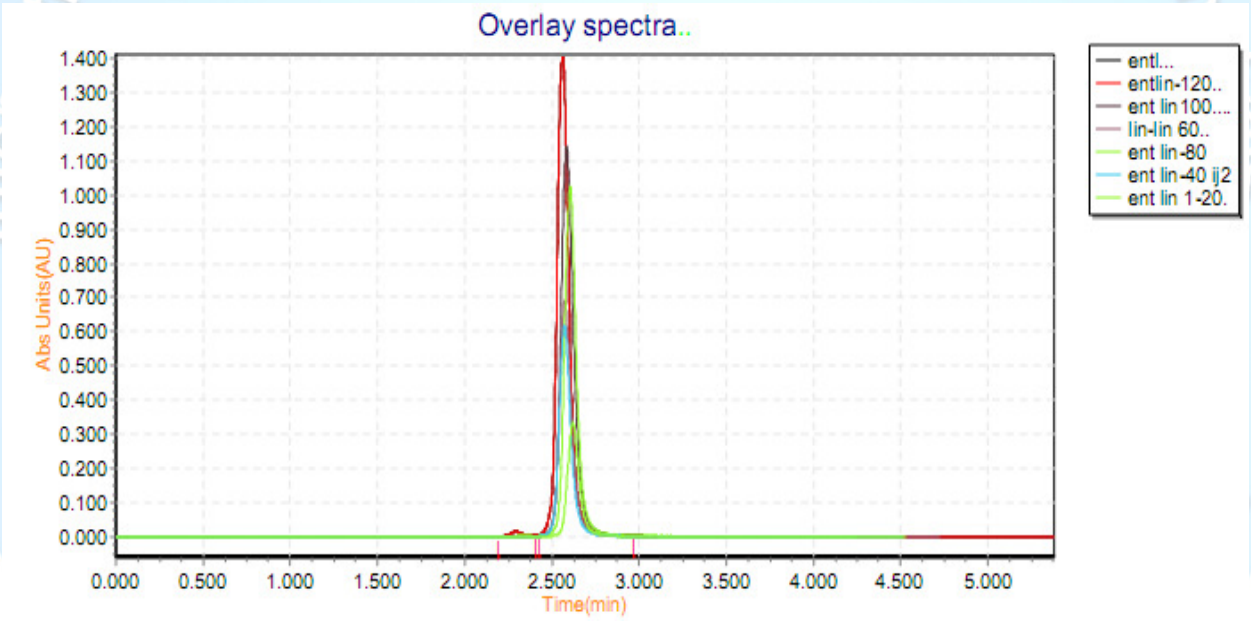

Table 3: Analytical performance parameters of linearity curve

S.No	parameters	Entacapone
1	Linear dynamic range(µg/ml)	20-120
2	Correlation coefficient ©	0.9997
3	Slope (m)	65.836
4	Intercept ©	26.429
5	Curve fitting	99.97
6	LOD(µg/ml)	0.5
7	LOQ(µg/ml)	1.5

Table 4.Recovery studies of entacapone

Labeled	Amount mg/tab Found*	%Label Claim (n=6)	Recovery Studies (n=3)			
			Amount added (mg)	Amount recovered (mg)	%Recovery	% RSD
Entacapone 100mg	100.923	100.25±0.2971	80	80.11±0.125	80.921	0.274
			100	100.64±0.23	99.24	0.525
			120	120.07±0.02	118.14	0.541

**Average of six or three determinations, Mean ± Standard Deviation*

Table 5: Precision studies of Entacapone in dosage forms

	System Precision		Method Precision	
	Entacapone		Entacapone	
S.No.	Retention time	Peak Area	Retention time	Peak Area
1	2.528	6541	2.548	6517
2	2.505	6587	2.258	6547
3	2.571	6599	2.571	6598
4	2.592	6534	2.480	6611
5	2.533	6591	2.572	6597
6	2.581	6548	2.512	6602
Avg	2.511	6566	2.490	6578
Stdev	0.0344	28.72	0.1192	37.69
%RSD	**0.136**	**0.043**	**0.471**	**0.057**

Table 6. Method ruggedness of entacapone in dosage forms

%Assay*(n=6)	%RSD ofAssay(n=6)
Day -1 , Analyst-1, Instrument-1&Column-1	
100.71 ± 0.112	0.251
Day -2 , Analyst-2, Instrument-2&Column-2	
100.19 ± 0.241	0.127

**Average of six determinations, mean ± Standard Deviation*

Table 7: Method robustness of entacapone in dosage Forms

S.No	Parameter	Condition	Theoretical plates	Rt	Tailing factor	% RSD
1.	Flow rate (±0.2ml/min)	0.8	6602	2.793	1.250	0.231
		1.2	6541	2.300	1.257	0.164
2.	Wavelength changes (±2nm)	312	6024	2.542	1.317	0.191
		308	6714	2.527	1.325	0.079

HPLC conditions

A XterraC18 (4.6x250 mm,5µ), column was used as the stationary phase. A mixture of acetonitrile and 0.02M potassium dihydrogen orthophosphate , pH 6.0 adjusted with dilute tri ethylamine in the ratio of 55:45 was used as a mobile phase. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 310nm.The injection volumes of samples and standard were 20µl.

Standard solutions

A stock solution containing 1000µg/ml of ENT was prepared by dissolving ENT in mobile phase. A working standard solution containing 20-120µg/ml of ENT was prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

ASSAY OF TABLET FORMULATION:

20 tablets (each tablet contains 200mg of Entacapone (Entacapone®)) were accurately weighed and calculated their average weight. Then it was taken into a mortar and crushed to fine powder and uniformly mixed. A quantity of powder equivalent to100mg of ENT was weighed and transferred to a 100 ml standard flask .The drug was initially dissolved in diluent and sonicated for 10 minutes. The volume was made up to 100 ml with mobile phase. Then the solution was filtered using 0.45-micron syringe filter. After that 1.0 ml of the above filtrate was diluted to 10 ml with the diluent so as to give a concentration of 100µg/ml of entacapone .Then 20µl of this solution was injected in to the column and chromatogram was recorded and shown in Fig.2.Each concentrations of ENT in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in table1.

VALIDATION OF THE METHOD:

System suitability studies

The system suitability test was carried out on freshly prepared stock solution of entacapone to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in table 2. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within ± 3% standard deviation range during routine performance of the method.

Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 20-120µg/ml. 20µl of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in µg/ml(X axis). Peak area of linearity range and the parameters were calculated and presented in table 3 respectively. The linearity curve of entacapone and overlain spectra of linearity chromatograms of entacapone was shown in Fig.3and Fig.4.

Limit of detection and Limit of quantification

The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3X {Residual Standard deviation/Slope}.

The LOD for ENT was confirmed to be 0.5 µg/ml.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ= 10X {Residual Standard deviation/Slope}

The LOQ for ENT was confirmed to be1.5 µg/ml

Accuracy

The accuracy of the method was determined by recovery

experiments. Placebo was spiked with known quantities of standard drugs at levels of 80 to 120% of label claim. The recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in table 4. The mean recovery is well within the acceptance limit, hence the method is accurate.

Precision

a) System precision

The system precision of the method was established by six replicate injections of the standard solution containing entacapone. The percentage RSD were calculated and presented in Table 5. From the data obtained, the developed RP-HPLC method was found to be precise.

b) Method precision

The method precision of the method was established by carrying out the analysis of entacapone (n=6) using the proposed method. The low value of the relative standard deviation showed that the method was precise. The results obtained were presented in table 5.

Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for entacapone in tablet dosage form.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature and refrigerator temperature for 24h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were up to 24h at room temperature and refrigerator temperature.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A4T), Water Alliance HPLC 2695 by different operators using different columns of similar type like Kromosil C_{18} column and Xterra C18 column. Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The results of ruggedness were presented in table 6. The results of robustness were presented in table 7.

CONCLUSION

The proposed RP-HPLC method for the estimation of entacapone in tablet dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw material, formulation and dissolution studies. The method validation shows satisfactory data for all the method validation parameter tested.

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