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Analytical method for development and validation of Dess-nitro and EDA in Entacapone tablet by using RP-HPLC method

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ABSTRACT

A simple, rapid, accurate and reproducible reverse phase high performance liquid chromatography method for the quantitative determination of Dess-nitro and EDA in Entacapone tablets is developed and validated.Entacapone (INN) is a medication commonly used in combination with other medications for the treatment of Parkinson's disease. Entacapone together with levodopa and carbidopa allows levodopa to have a longer effect in the brain and reduces Parkinson's disease signs and symptoms for a greater length of time than levodopa and carbidopa therapy alone.

Chromatography was carried out on a COSMOSIL πNAP column (250×4.6mm, 5µ) in gradient mode. The mobile phase consisted of buffer and methanol, pumped at a flow-rate of 0.8 ml/min. The UV detection was employed at 300nm. The retention time of dess-nitro and EDA was found to be 36.14 and 58.07 minutes respectively. The calibration curves were linear in the range. The method is accurate and precise with recovery of dess-nitro and EDA in the range of 95-105%. The proposed method which is rapid, simple and does not require any separation process has been successfully applied to the assay of commercial fixed dose formulations.

1. INTRODUCTION

Entacapone (INN) is a medication commonly used in combination with other medications for the treatment of Parkinson's disease. Entacapone together with levodopa and carbidopa allows levodopa to have a longer effect in the brain and reduces Parkinson's disease signs and symptoms for a greater length of time than levodopa and carbidopa therapy alone.

Entacapone is known as a selective and reversible inhibitor of the enzyme catechol-O-methyltransferase (COMT). When taken together with levodopa (L-DOPA) and carbidopa, entacapone stops catechol-O-methyltransferase from breaking down and metabolizing levodopa, resulting in an overall increase of levodopa remaining in the brain and body.

Entacapone is developed by Orion Pharma and marketed by Novartis under the trade name Comtan. Stalevo, another medication developed by Orion Pharma and marketed by Novartis, is a single tablet formulation that contains levodopa, carbidopa, and entacapone.

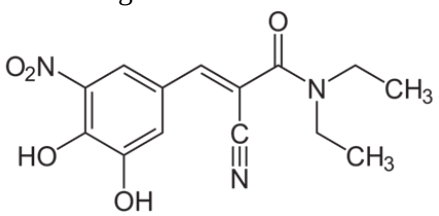

Figure.1.Structure of Entacapone

2. MATERIALS AND METHODS

Standard drugs are obtained from the vendors and test sample from the pharmaceutical organisation, Hetero labs ltd, Hyderabad. Standard drug: Dess-nitro and EDA AN-436 by AN Pharmatech, Sample drug: Entacapone sample by HETERO organization.

HPLC with gradient technology, Pump: Analytical HPLC pump, Detector: UV detector with data handling system.Software: Empower3 software, Column:COSMOSIL π NAP (250 X 4.6mm)5µm.

Method development: All HPLC experiments were carried out on a Waters E 2695 separation module, with waters 2489UV/VISIBLE detector in gradient mode using Auto sampler. Data collections and processing was done using EMPOWER 3 software. The analytical column used for the separation was COSMOSIL πNAP,

250× 4.6 mm I.D., 5µm particle size, Analytical balance (Mettler toledo), pH meter (pH METER PICO$^+$,Labindia), sonicator (EN-200 U S).

Preparation of buffer: dissolve about 2.34g of Sodium dihydrogen phosphate in 1000ml of water and mix. Adjust pH of the solution to 2.1 ± 0.05 with dilute Orthophosphoric acid and mix.

Solvent A: prepare a mixture of buffer and methanol in the ratio of 90:10(v/v). Filter and degas through the 0.45µm membrane filter paper.

Solvent B: prepare a mixture of buffer and methanol in the ratio of 10:90(v/v). Filter and degas through the 0.45 µm membrane filter paper.

Diluent: prepare a mixture of Tetrahydrofuran and methanol in the ratio of 30:70(v/v).

Preparation of Standard solution: Weigh accuratley about each 5.0mg of Dess-nitro and EDA standard into a 50ml volumetric flask, dissolve and dilute the volume with diluent and mix. Dilute 1.0ml of this solution to 20ml with diluent and mix.

Preparation of sample solution: 10.35mg of test sample was taken and dissolved with 20ml diluent. rom the literature survey it is clear that there is no specific and simple method for analysing the dess-nitro and EDA in Entacapone by RP-HPLC. So that the trial and error method were conducted for optimising the simple method of Entacapone by RP-HPLC method.

Optimized Chromatographic conditions:

Column: COSMOSIL πNAP, 250x4.6mm, 5µm
Wavelength: 300nm
Flow rate: 0.8 ml/min
Injection volume: 10µl
Run time: 70min

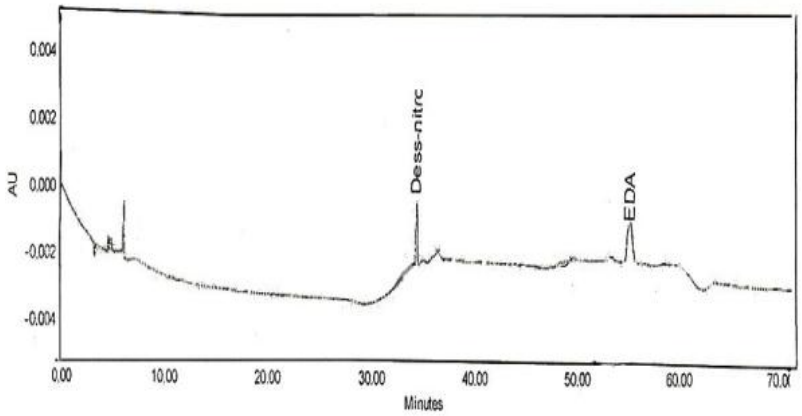

Figure.2.Optimized chromatogram

Observation: In this trail the observed peaks has given good response and good shape so that it is finalized.

Table.1.Results of Optimized method for Standard

Name of Peak	Retention time(min)	Area	USP plate count	USP Tailing	Injection Volume
Dess-nitro	36.29	11527	5264	1.105	10µl
EDA	58.07	20605	5982	1.125	10µl

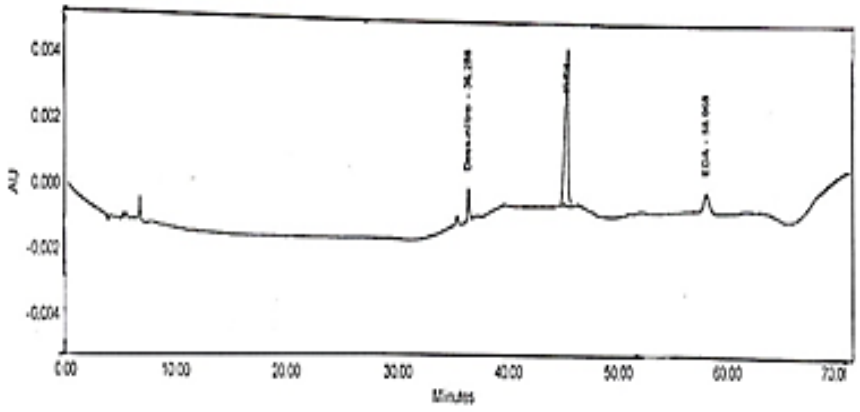

Figure.3.Final chromatogram for sample

Observation: Dess-nitro and EDA peaks are separated well in the injected sample solution and are having good peak shape and resolution. This method is finalized, because dess-nitro and EDA peaks are well separated without any interference and have better resolution

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Table.2.Results of Optimized method for sample

Name of Peak	Retention time(min)	Area	USP plate count	USP Tailing	Injection Volume
Dess-nitro	36.15	11512	5245	1.019	10µl
EDA	57.45	20589	5964	1.126	10µl

Table.3.Results of %Assay of dess-nitro and EDA

Name	As	At	Wt. equivalent taken (mg)	%Assay
Dess-nitro	11527	11512	5.0	98.45
EDA	20605	20589	5.0	78.402

%Assay of dess-nitro and EDA in tablet is found to be 98.45% and 78.402% which are within acceptance criteria 75-125%.

Table.4.Results of %RSD of dess-nitro and EDA

S.NO	Injection no	Peak area of dess-nitro	Peak area of EDA	Acceptance criteria
1	1	11488	20338	The %RSD of peak area of dess-nitro and EDA should not be more than 2.0
2	2	11539	20185	
3	3	11493	20640	
4	4	11463	20332	
5	5	11369	20769	
6	6	11452	20627	
%RSD		0.64	1.11	
Std.dev		73	228	

Table.5.Results from system suitability studies of dess-nitro and EDA

System suitability parameters	Observed value for dess-nitro	Observed value for EDA	Acceptance criteria
Tailing factor	1.12	1.15	NMT 2
Theoretical plate count	5976	5263	NLT 2000

Observation: The %Relative standard deviation of individual area response of six replicate injections of dess-nitro and EDA was found to be 0.64 and 1.11 respectively. The %Relative standard deviation of areas of six replicate injections of dess-nitro and EDA standard were found to be within limit. The tailing factor for dess-nitro and EDA peaks was found to be 1.12 and 1.15 respectively. The tailing factor for dess-nitro and EDA peak was found to be within limit. The number of theoretical plates for dess-nitro and EDA were found to be 5976 and 5263 respectively, it is within the limit.

Table.6.Precision for dess-nitro and EDA

S.NO	Injection no	Peak area of dess-nitro	Peak area of EDA
1	01	11341	19927
2	02	11227	20169
3	03	11260	20025
4	04	11501	20228
5	05	11557	20664
6	06	11374	20066
Mean		11372	20180
%RSD		1.15	1.29

Acceptance Criteria: The Relative standard deviation of area of dess-nitro and EDA from six standard preparations should be not more than 2.0%.

Observation: The Relative standard deviation of area of dess-nitro and EDA were found to be 1.15 and 1.29 respectively. Results were found to be within limit.

Specificity: The specificity of the method is performed by separately injecting the blank, standard sample containing dess-nitro and EDA. The interference observed (if any) at the retention times of each analyte in the chromatogram is evaluated.

Table.7.Accuracy for Dess-nitro and EDA

% Spiked concentration	Wt added (mg)dess-nitro	Wt added (mg)EDA	Wt recovered mg(dess-nitro)	Wt recovered mg (EDA)	%Recovery Dess-nitro	%Recovery EDA
50	3.026	8.013	2.986	7.986	98.67	99.66
	3.154	8.045	3.067	7.995	97.24	99.37
	3.089	8.102	2.995	8.016	96.95	98.93
100	5.134	10.023	5.015	9.989	97.68	99.66
	5.056	10.156	4.989	10.069	98.67	99.14
	5.097	10.085	4.995	9.986	97.99	98.97
150	7.054	12.051	6.994	11.998	99.14	99.56
	7.158	12.087	7.026	11.986	98.15	99.16
	7.062	12.023	6.998	11.995	99.09	99.76

Table.8.Results of Robustness for dess-nitro and EDA

S.no	Parameters	Condition		%RSD		USP tailing		USP platecount	
		Dess-nitro	EDA	Dess-nitro	EDA	Dess-nitro	EDA	Dess-nitro	EDA
	Flow rate by ± 5%	0.6	0.6	0.64	0.67	1.10	1.06	4895	4652
		0.8	0.8	0.96	0.98	1.16	1.28	6219	5962
		1.0	1.0	1.15	1.25	1.65	1.62	8542	7521
	Column oven temperature by ± 5°c	30	30	0.84	0.52	1.05	1.07	3256	3561
		35	35	0.95	0.91	1.26	1.26	5248	5942
		40	40	1.05	1.64	1.69	1.85	7562	8125
	Wavelength by ± 5nm	295	295	0.52	0.94	1.09	1.02	3526	3269
		300	300	0.92	1.12	1.12	1.15	5169	5964
		305	305	1.07	1.86	1.84	1.82	8163	7895
	pH of Buffer solution by ± 0.2 units	2.08	2.08	0.75	0.64	1.04	1.02	3169	
		2.10	2.10	0.89	1.09	1.56	1.43	5698	
		2.12	2.12	1.02	1.26	1.75	1.69	7986	

Acceptance criteria: %RSD should not be more than 2%. Thoeritical plates should not less than 2000. Tailing factor should not more than 2.0

Observation: From the obtained values %RSD was found to be within the range of 0.5%-1.86% which states the method is acceptable.

Limit of Detection and Limit of Quantitation:

Table.9.Results of LOD and LOQ

Sample	LOD	LOQ
Dess-nitro	1.176	3.566
EDA	3.358	10.178

Table.10.Summary of validation parameters by RP-HPLC method

Validation	Parameters	Results for	
		Dess-nitro	**EDA**
System suitability	Tailing factor	1.12	1.15
	%RSD	0.64	1.11
	Theoretical plates	5976	5263
Linearity	Correlation coefficient	0.997	0.999
	Slope	204.7	224.5
Precision	%RSD	1.15	1.29
Accuracy	Mean % recovery for 50, 100, 150% respectively	97.12 97.68 98.59	98.92 98.65 99.27
Specificity	Interference	No interference	
Robustness	Flow rate by ± 0.2ml	All the system suitability parameters are within the limit for all the variable parameters, for dess-nitro and EDA	
	Column Oven temperature by ± 5°C		
	Wavelength of analysis ± 2nm		
	pH of Buffer solution by ± 0.2 units		
LOD	Standard deviation method	1.176	3.358
LOQ		3.566	10.178

4. CONCLUSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of dess-nitro and EDA in pharmaceutical preparations.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with 10mm sodium dihydrogen Phosphate buffer (pH 2.1) and methanol in gradient mode with 0.8 ml/min flow rate is quite robust.

COSMOSIL πNAP column was selected with optimum wavelength of 300nm at which better detector response for drug was obtained. The average retention time for dess-nitro and EDA were found to be 36.14mins and 58.07mins respectively. The linearity was observed in the range of 50-150% for the drugs with a correlation coefficient of 0.997 and 0.999 respectively. The low values of % RSD indicate the method is precise and accurate. The mean recoveries were found in the range of 75-125%.

From the above experimental data and results, the developed HPLC method is having the following advantages:

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analysis of formulation.
- Run time required for recording chromatograms were less than 5.0 minutes.
- Suitable for the analysis of raw materials, applicable to dissolution studies and can be used for the content uniformity studies.

Hence, the chromatographic method developed for dess-nitro and EDA is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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