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HPLC METHOD FOR ESTIMATION OF DRUG RELEASE OF ENTACAPONE IN ENTACAPONE, LEVODOPA AND CARBIDOPA TABLETS

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Keywords:

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ABSTRACT: Today's generic drugs manufacturing industry need robust analytical methods for analysis of generic drug products to provide best quality pharmaceutical formulation. Entacapone, Levodopa and Carbidopa tablets is such a product which is used for the treatment of Parkinson's disease. This paper presents a robust HPLC method that has been developed for estimation of % drug release of Entacapone in this multi component drug formulation. HPLC column used for separation was Cosmosil 5PE-MS 150mm x 4.6 mm with 5 micron particle size. Combination of phosphate buffer pH 2.5, acetonitrile and methanol was used for mobile phase in isocratic mode with UV detection at 280 nm. The method was validated to ensure suitability of the method for quantitative determination of % drug release of Entacapone in presence of Levodopa and Carbidopa in this triple drug combination pharmaceutical formulation. Proposed method was found to be specific, precise, robust, accurate and linear in range 0.066 to 0.33 mg/mL of Entacapone.

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. But administration of dopamine is ineffective in the treatment of Parkinson's disease. This is because it does not cross the blood-brain barrier. However, levodopa **(Fig. 1A)**, the metabolic precursor of dopamine, does cross the blood-brain barrier, and presumably is converted to dopamine in the brain.

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Carbidopa **(Fig. 1B)** inhibits the decarboxylation of peripheral levodopa, making more levodopa available for transport to the brain. There is no pharmacopoeial or literature reference of a suitable HPLC method to estimate % drug release of Entacapone **(Fig. 1C)** in presence of other two drugs in the proposed triple combination formulation. Proposed method was developed to cater to this need of pharmaceutical industry Entacapone is a selective and reversible inhibitor of catechol-O-methyltransferase (COMT). When entacapone is given in conjunction with levodopa and carbidopa, plasma levels of levodopa are greater and more sustained than after administration of levodopa and carbidopa alone[1]. There is no pharmacopoeial or literature reference of a suitable HPLC method to estimate % drug release of Entacapone in presence of other two drugs in the proposed triple combination

formulation. Proposed method was developed to cater to this need of pharmaceutical industry.

Literature survey revealed few methods for individual or combination product analysis such as; Method for *in-vitro* release of drugs is also found but with longer run time and for single drug product [2, 3, 4], spectroscopic methods for simultaneous estimation of levodopa and carbidopa [5], Estimation methods by liquid chromatography for levodopa and carbidopa have been reported using electrochemical detector [6] and fluorescence detector [7]. Spectrophotometric determination of entacapone was reported in single drug product [8, 9], HPLC method in combination product was also reported [10, 11], USP monographs for determination of Levodopa and carbidopa for single and dual drug combination have also been reported [12-17].

In the present study, we propose a rapid HPLC method for estimation of % drug release of Entacapone [(2E)-2-cyano-3-(3, 4-dihydroxy-5-nitrophenyl) - N, N-diethyl-2-propenamide] in presence of Levodopa and carbidopa in triple drug combination product.

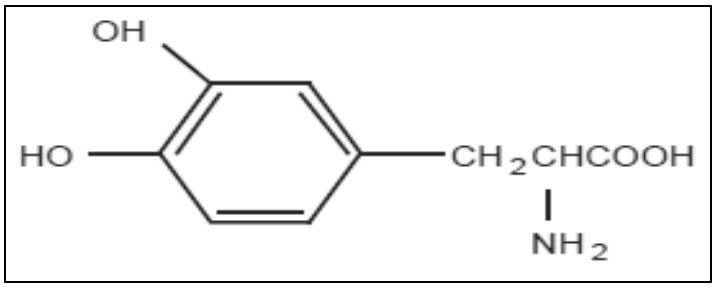


FIG. 1A: CHEMICAL STRUCTURES OF LEVODOPA

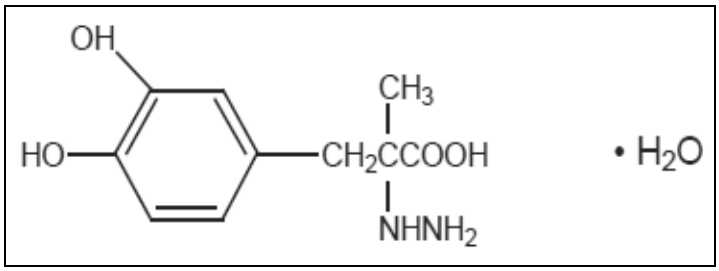


FIG. 1B: CHEMICAL STRUCTURES OF CARBIDOPA

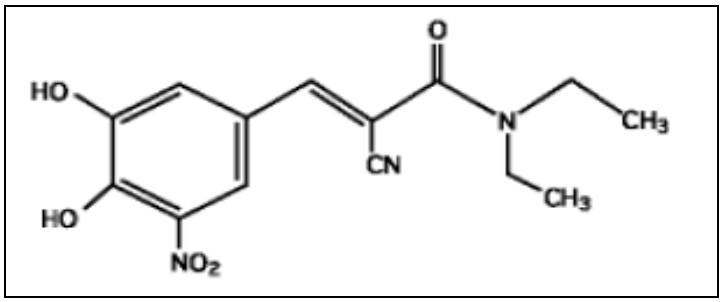


FIG. 1C: CHEMICAL STRUCTURES OF ENTACAPONE

MATERIAL AND METHODS:

Reagents and Materials: All analytical reagent grade (AR Grade) reagents were used for method development purpose. Acetonitrile (Merck) was used for standard solution preparation. Orthophosphoric acid (Rankem) and Potassium dihydrogen orthophosphate (Merck) were used for mobile preparation. Milli-Q water (HPLC grade) was used for all solution preparations. Working standards of Entacapone, Levodopa and Carbidopa were obtained from Macleods Pharmaceuticals Limited, Mumbai, India.

Chromatographic System and Conditions: Development study was performed on Shimadzu HPLC, consisting of UV-Visible, photodiode array detector and a quaternary gradient pump. Sample loop in the system was of 100µl capacity. Cosmosil 5PE-MS 150 x 4.6 mm, 5 µ (Nacalai Tesque, USA) HPLC column was used for chromatographic

separation. Mobile phase consisted of mixture of phosphate buffer, acetonitrile and methanol in isocratic mode. Buffer consisted of 10 mM potassium dihydrogen orthophosphate solution with pH adjusted to 2.5 using orthophosphoric acid. Flow rate was 1.0 mL/min and detection was carried out at 280 nm based on their wavelength maxima as per UV spectrum. Lab solutions software was used for data collection. For intermediate precision study, Shimadzu HPLC system with gradient pump, UV-visible detector and Chemstation software was used.

Solution Preparation:

Standard Preparation: About 45 mg of Entacapone was accurately weighed and transferred to a 200 mL volumetric flask. 10 mL of acetonitrile was added and sonicated to dissolve Entacapone completely. Further diluted to volume with dissolution medium and mixed.

Sample Preparation: 900 mL of dissolution medium was poured in each vessel. Sufficient time was allowed for the dissolution medium to equilibrate at 37 °C ±0.5 °C. Stirring element speed was adjusted to 125 rpm. One tablet was placed in each basket. The apparatus was lowered in the dissolution medium and started.

At the end of specified time, 10 mL aliquot was withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating basket using a sampling cannula with pre - filter attached to the end of it and filtered immediately through Whatman GF/C (25 mm) filter, discarding first 5 ml of the filtrate.

Method Validation: Once optimum separation conditions are achieved, method was validated to ensure its suitability and reliability for routine use in estimation of % drug release of active ingredient in a pharmaceutical formulation. Validation parameters adopted are as follows:

Specificity: Specificity for blank, placebo, Levodopa and Carbidopa was established by injecting blank solution, placebo solution, Levodopa standard solution, Carbidopa standard solution and Entacapone standard solution.

Solution Stability: Solution stability was evaluated by storing sample solution at 25 °C till 48 hrs.

Filter Compatibility: Sample solution was prepared by spiking Entacapone into placebo powder containing Levodopa and Carbidopa equivalent to one dosage unit. At filtration stage, solution was filtered through Whatman GF/C filter (25 mm). First 5.0 mL of filtrate was discarded. The filtrate was collected for further analysis. The unfiltered sample solution was centrifuged. Each of the above solutions thus obtained (filtered sample solution and centrifuged sample solution) were analysed as described in the methodology. The results were calculated.

Filter Saturation: Sample solution was prepared by spiking Entacapone into placebo powder containing Levodopa and Carbidopa equivalent to one dosage unit. At filtration stage, three filtrates were obtained using three separate Whatman GF/C filters (25 mm) by discarding 1 mL, 3 mL and 5 mL respectively. The filtrates were collected for further analysis.

Accuracy: Accuracy study was performed at 50%, 100% and 120 % of the target concentration of Entacapone. Recovery solutions were prepared by spiking Entacapone API to placebo powder containing levodopa and Carbidopa in dissolution medium.

Linearity: A series of solutions were prepared by quantitative dilutions of the stock solution of standard to obtain solutions as mentioned in the following table at 30% to 150 % of the target concentration of Entacapone. Each solution was injected and the peak area was recorded. Slope, Y-intercept and Correlation coefficient of the regression line were calculated.

Repeatability:

Precision: Precision test was carried out by spiking Entacapone into placebo with levodopa and Carbidopa powder equivalent to one dosage unit to obtain solutions at 100 % level of target concentration. Six sample preparations were prepared and injected. The mean and relative standard deviation of the **% drug release** was calculated.

Intermediate Precision: For intermediate precision, analysis was carried out by different analyst, on a different day, using a different HPLC and different dissolution apparatus. The absolute difference

between the mean % release obtained in precision and intermediate precision was calculated.

Robustness: The Dissolution method was carried out as described in the methodology and by making the following alterations in the dissolution conditions. Changing the volume of dissolution medium (890 mL, 910 mL), Changing the pH of dissolution medium (pH = 5.3, pH = 5.7)

RESULTS AND DISCUSSION:

Preliminary Studies: Selection of Dissolution Medium: Dissolution medium was chosen based on USFDA recommendation of Phosphate Buffer pH 5.5 for Entacapone in Carbidopa, Levodopa and Entacapone Tablets.

Selection of Wavelength: Wavelength was selected based on absorbance maxima of Entacapone as per UV spectrum. 280 nm was found to be optimum (**Fig. 2**).

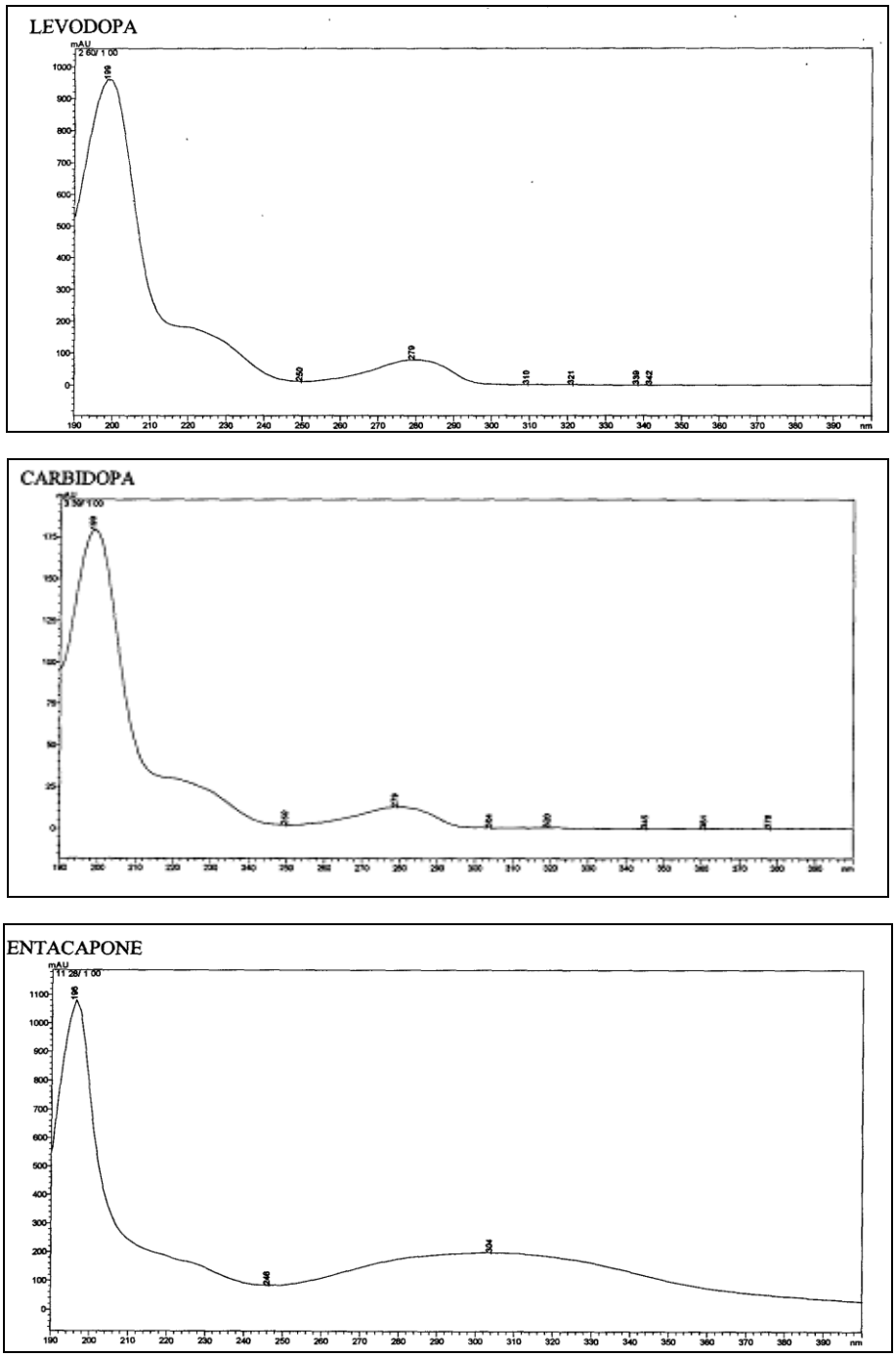


FIG. 2: UV ABSORPTION SPECTRA OF LEVODOPA, CARBIDOPA AND ENTACAPON

Selection of Mobile Phase: Due to non-polar nature of Entacapone, mobile phase was optimized with 50% of methanol and 50% buffer. Looking at the pH range of HPLC column, buffer pH 2.5 was evaluated and found to be optimum.

Selection of HPLC Column: Entacapone is non-polar in nature which makes it elute late on a octadecyl phase. In order to elute it early, a more polar phase was evaluated and selected for method development. Cosmosil 5PE-MS, 150 x 4.6 mm, 5 µ was the column of choice. Conventional Phenyl phase is polar in nature but do not last long at low pH due to its weak bonding. Cosmosil PE column has an ethyl group attached to phenyl group which makes this column a rugged stationary phase with better column life. 150 mm column was chosen to achieve a shorter run time.

Selection of HPLC Pump Mode: Mobile phase with higher ratio of solvent was optimized in isocratic mode for estimation of active ingredients with a flow rate of 1 ml/min and run time of 10 minutes.

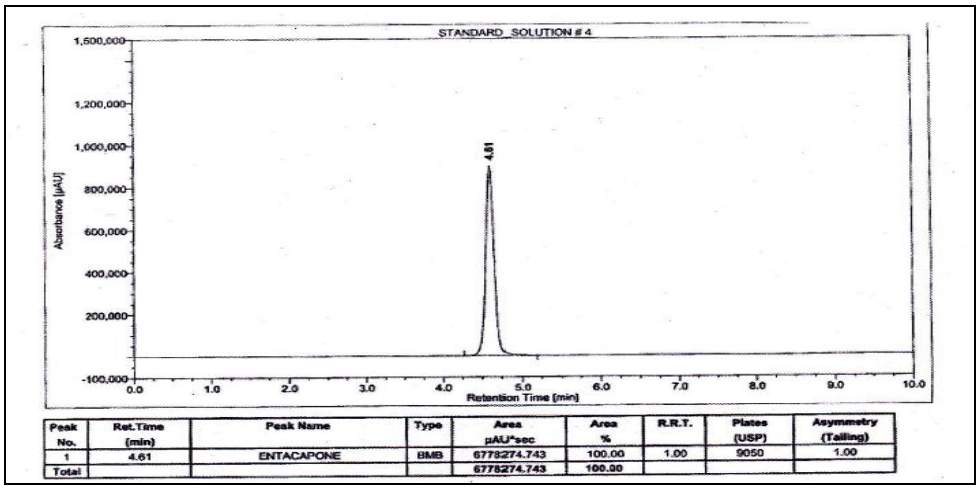
Selection of Diluent: Due to its low solubility in dissolution medium, Entacapone was first dissolved in acetonitrile and then finally diluted with dissolution medium for standard preparation.

Method Validation:

Specificity: As shown in **Table 1** and **Fig. 3**, No interference from blank, placebo, Levodopa and Carbidopa was observed at retention time of Entacapone peak.

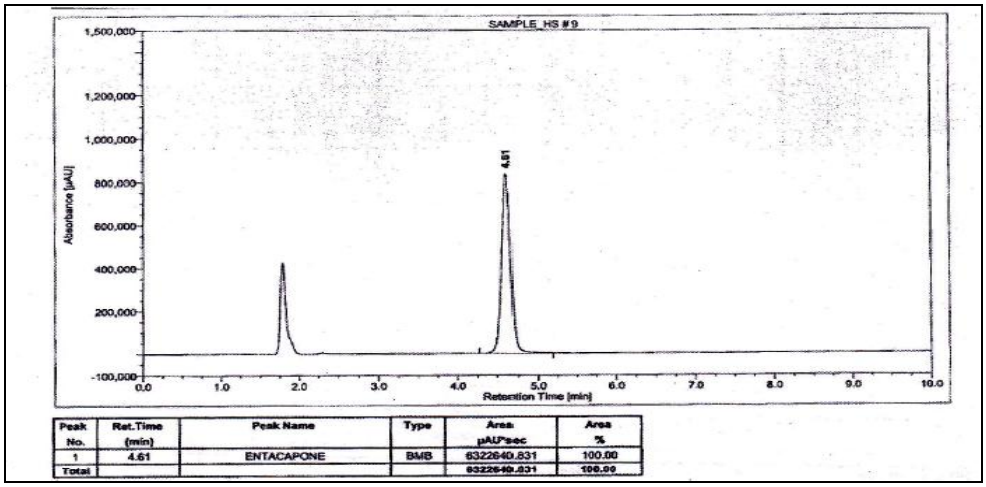
TABLE 1: METHOD VALIDATION

Sr. No	Sample Details	Retention Time (min)
1	Blank	No peak detected
2	Placebo Solution	No peak detected
3	Levodopa	1.79
4	Carbidopa	1.82
5	Entacapone	4.61



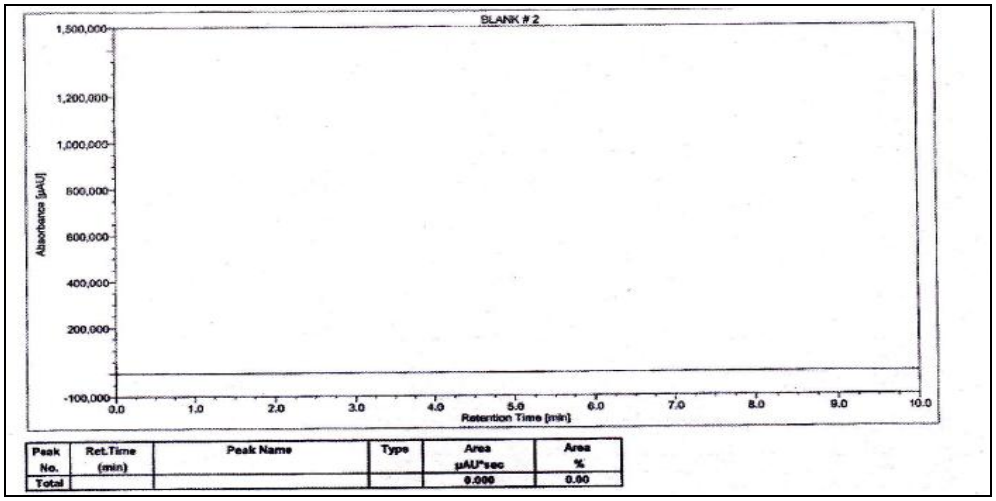
Peak No.	Ret.Time (min)	Peak Name	Type	Area µAU*sec	Area %	R.R.T.	Plates (USP)	Asymmetry (Tailing)
1	4.61	ENTACAPONE	BMB	6778274.743	100.00	1.00	9050	1.00
Total				6778274.743	100.00			

CHROMATOGRAM OF ENTACAPONE STANDARD

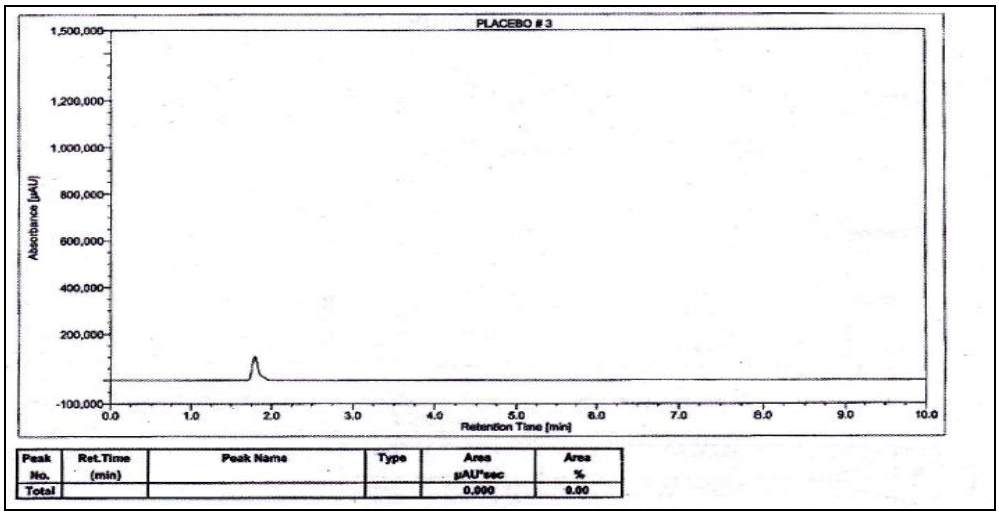


Peak No.	Ret.Time (min)	Peak Name	Type	Area µAU*sec	Area %
1	4.61	ENTACAPONE	BMB	6322640.831	100.00
Total				6322640.831	100.00

CHROMATOGRAM OF SAMPLE PREPARATION



CHROMATOGRAM OF BLANK



CHROMATOGRAM OF PLACEBO WITH LEVODOPA AND CARBIDOPA

FIG. 3: CHROMATOGRAMS OF SPECIFICITY TO CONFIRM NO INTERFERENCE AT RETENTION TIME OF PEAK OF INTEREST

Solution Stability: The absolute difference between the % release of sample solution when stored for 48 hours at 25 °C and % release of initial was within the acceptance criteria of not more than 2 (**Table 2**).

TABLE 2: OBSERVATION OF SOLUTION STABILITY

Time (hours)	Area	% Release	Absolute difference
Initial	6782464	100.7	-
24	6833824	99.4	1.3
48	6597937	99.1	1.6

The sample solution was found to be stable till 48 hours, when stored at 25°C.

Filter Compatibility: The absolute difference between the results obtained for filtered solution and centrifuged solution was calculated (**Table 3**).

TABLE 3: FILTER COMPATIBILITY RESULTS OF THE PROPOSED METHOD

Filter Type	Area	% Release	Absolute Difference
Centrifuge	6713359	99.6	-
Whatman GF/C filter	6782464	100.7	1.1

Since the absolute difference between the results obtained for filtered sample solution and centrifuged sample solution was within acceptance criteria of NMT 2, Whatman GF/C (25 mm) filter is considered as suitable for sample filtration.

Filter Saturation: Each of the above solutions thus obtained were analysed as described in the methodology. The absolute difference between the results obtained for consecutive filtered solutions was calculated (**Table 4**).

TABLE 4: FILTER SATURATION RESULTS OF THE PROPOSED METHOD

Volume Discarded	Area	% Release	Absolute Difference
1 mL	6727730	99.8	-
3 mL	6777998	100.6	0.8
5 mL	6790666	100.8	0.2

Since the absolute difference between the results obtained for two consecutive filtered solutions was within acceptance criteria of NMT 2, volume of 5 mL was considered as sufficient to saturate the filter.

Accuracy: The % recovery was within 95-105% (**Table 5**). Hence method is considered to be accurate.

TABLE 5: ACCURACY RESULTS OF THE PROPOSED METHOD

Level	API Spiked (mg)	Area	% Recovery
	100.15	3308363	98.9
50 %	100.15	3307003	98.9
	100.15	3306039	98.8
	200.31	6724202	100.5
100%	200.31	6701853	100.2
	200.31	6720652	100.5
	240.37	8020915	99.9
120%	240.37	8060047	100.4
	240.37	8062883	100.4
	Mean % Recovery		99.8

Linearity: Linearity plot (**Fig. 4**) and results (**Table 6**) shows that the correlation coefficient is within acceptance criteria of not less than 0.99. Hence the method is linear.

Repeatability: The absolute difference between the mean % release results obtained in precision (**Table 7**) and intermediate precision (**Table 8**) was within the acceptance criteria of not more than 5.0.

Also difference between precision and intermediate precision was within 5% (**Table 9**).

TABLE 6: LINEARITY RESULTS OF THE PROPOSED METHOD

% Level	Concentration (ppm)	Area
30	67.4	2095012
50	112.3	3499256
80	179.73	5566098
100	224.7	6893248
120	269.6	8283081
150	336.9	10366642
	Slope	30592.4
	Y-Intercept	46167.6
	Correlation coefficient	1.000

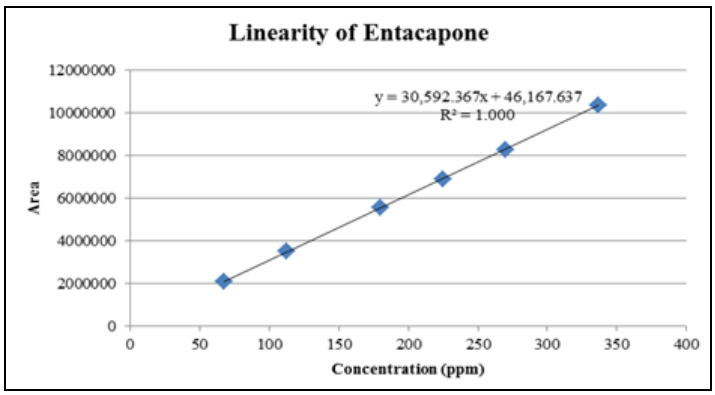


FIG. 4: LINEARITY PLOT

Precision:

TABLE 7: PRECISION RESULTS OF THE PROPOSED METHOD

	Area	% Release
Sample-1	6688850	100.4
Sample-2	6660375	100.0
Sample-3	6707099	100.7
Sample-4	6684719	100.4
Sample-5	6698032	100.6
Sample-6	6666779	100.1
Mean		100.4
% RSD		0.27

Intermediate Precision:

TABLE 8: INTERMEDIATE PRECISION RESULTS OF THE PROPOSED METHOD

	Area	% Release
Sample-1	6892912	98.8
Sample-2	6908731	99.0
Sample-3	7003650	100.4
Sample-4	6998750	100.3
Sample-5	7016739	100.6
Sample-6	7015409	100.6
Mean		100.0
% RSD		0.82

TABLE 9: COMPARISON OF PRECISION AND INTERMEDIATE PRECISION RESULTS OF THE PROPOSED METHOD

Content	Mean % Release in Precision	Mean % Release in Intermediate Precision	Absolute difference
Entacapone	100.4	100.0	0.4

Hence, the method for estimation of % release is precise.

Robustness: The absolute difference in the results obtained under normal condition and robustness study of change in dissolution medium volume was within the acceptance criteria of NMT 10 (**Table 10** and **11**). System suitability parameters were also within acceptance limits (**Table 12**). The absolute difference in the results obtained under normal condition and robustness study of pH 5.7 of dissolution medium was within the acceptance criteria of NMT 10. The absolute difference in the results obtained under normal condition and robustness study of pH 5.3 of dissolution medium was not within the acceptance criteria of NMT 10.

TABLE 10: ROBUSTNESS RESULTS OF THE PROPOSED METHOD

	% Release of Entacapone				
Unit	Unaltered	Volume 890 mL	Volume 910 mL	pH of medium 5.3	pH of medium 5.7
1	95.2	97.3	91.7	82.3	101.7
2	96.7	97.4	92.0	82.9	99.7
3	94.9	95.4	90.5	83.3	99.9
4	97.7	96.2	92.1	83.3	101.8
5	95.9	96.1	91.4	84.6	98.9
6	95.4	96.9	92.4	83.2	99.3
Mean	96.0	96.6	91.7	83.3	100.2
% RSD	1.10	0.81	0.73	0.91	1.23

TABLE 11: COMPARATIVE % RELEASE RESULTS FOR ROBUSTNESS

S. no.	Changed Parameter	Mean % Release	Absolute Difference
1	Unaltered	96.0	-
2	Volume 890 mL	96.6	0.6
3	Volume 910 mL	91.7	4.3
4	pH 5.3	83.3	12.7
5	pH 5.7	100.2	4.2

The system suitability parameters were not significantly changed with altered conditions.

TABLE 12: SYSTEM SUITABILITY RESULTS FOR ROBUSTNESS

S. no.	Changed Parameter	Tailing Factor (NMT2.0)	Theoretical Plates (NLT 2000)	% RSD for Entacapone peak area (NMT 2.0)
1	Unaltered	0.99	8934	0.08
2	Volume 890 mL	1.02	9072	0.03
3	Volume 910 mL	1.00	9050	0.06
4	pH 5.3	1.00	9124	0.08
5	pH 5.7	1.00	9111	0.02

This concludes that pH of dissolution medium is a critical parameter.

CONCLUSION: A simple and efficient HPLC method for estimation of % drug release of Entacapone in triple drug combination product was developed and validated for specificity, accuracy, linearity, precision and robustness ensuring suitability of the method for quantitative analysis. The results indicated that this method is suitable for estimation of % Release of Entacapone in a pharmaceutical formulation.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interests.

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