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METHOD DEVELOPMENT AND VALIDATION OF LEVODOPA AND CARBIDOPA IN A COMBINED DOSAGE FORM BY RP-HPLC METHOD

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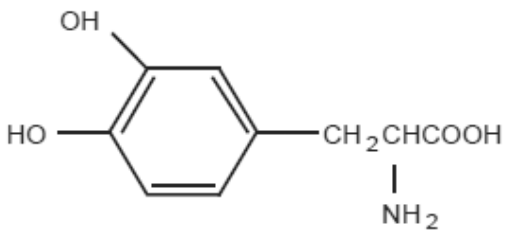
Abstract:

A simple, precise, accurate, and rapid HPLC method has been developed, and validated for the determination of levodopa and Carbidopa simultaneously, in combined tablet dosage form. The mobile phase used was a mixture of phosphate buffer pH 3 and Acetonitrile (90:10% v/v). The detection of levodopa and Carbidopa was carried out by UV detector at 280 nm. The retention time of levodopa and carbidopa were found to be 3.2 and 4.6 respectively. Results of the analysis were validated statistically, and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.

Key words: Levodopa, Carbidopa, Reverse Phase High Performance Liquid Chromatography and validation.

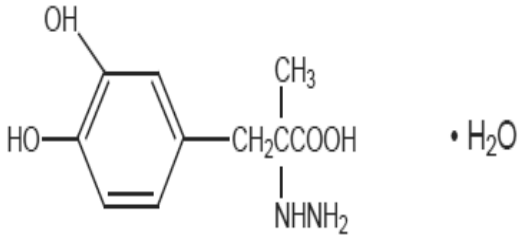
Introduction:

Levodopa, an aromatic amino acid, is a white, crystalline compound, slightly soluble in water, with a molecular weight of 197.2. It is designated chemically as (—)-L-α-amino-β-(3,4-dihydroxybenzene) propanoic acid [1]. Its empirical formula is $C_9H_{11}NO_4$, and its structural formula is:



Carbidopa, an inhibitor of aromatic amino acid decarboxylation, is a white, crystalline compound, slightly soluble in water, with a molecular weight of 244.3. It is designated chemically as (—)-L-α-hydrazino-α-methyl-

β-(3,4-dihydroxybenzene) propanoic acid monohydrate [1]. Its empirical formula is $C_{10}H_{14}N_2O_4 \bullet H_2O$, and its structural formula is:



Syndopa is a combination of carbidopa and levodopa for the treatment of Parkinson's disease and syndrome.

Parkinson's disease is a neurological degenerative disorder of extra pyramidal nervous system. The symptoms of the disease include tremor, rigidity, bradykinesia and loss of control of skeletal muscular system[2]. The disease is caused when blood fails to produce enough dopamine. Dopamine cannot be administered directly because it cannot cross blood-brain barrier, so levodopa is used as a source of dopamine because it is decarboxylated to dopamine by aromatic L-amino acid decarboxylase on entering the brain[3] .Levodopa is generally co-administered with an extra cerebral decarboxylase inhibitor like carbidopa. Carbidopa inhibits decarboxylation of levodopa in peripheral tissues resulting in greater amount of levodopa dose to be transported to brain. Combination therapy of carbidopa and levodopa leads to a marked reduction in both the required levodopa dose and occurrence of side effects[4] eg. Syndopa

Syndopa (carbidopa-levodopa) is supplied as tablets in four strengths[5]:

SYNDOPA 110 tab Levodopa 100mg, Carbidopa 10mg

SYNDOPA 275 tab Levodopa 250mg, Carbidopa 25mg

SYNDOPA PLUS tab Levodopa 100mg, Carbidopa 25mg

SYNDOPA CR tab Levodopa 200mg, Carbidopa 50mg

Various methods such as, spectrofluorimetry[6,7] gas chromatography[8,9] , high performance liquid chromatography(HPLC)[9,10] , radio immunoassay,10 chemiluminescence[11-12] and voltammetric determination[13,14] have been reported in the literature for the determination of these compounds in various biological samples and pharmaceutical preparations.

REAGENTS, STANDARDS AND SAMPLES:

Water HPLC Grade, LevodopaWorking Standard, CarbidopaWorking Standard, Acetonitrile, Potassium dihydrogen phosphate and Syndopa plus tablets.

CHROMATOGRAPHIC PARAMETERS:

Equipment	: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column	: Symmetry C18 (4.6 x 150 mm, 3.5 □m, Make: XBridge) or Equivalent.
Flow rate	: 0.5 ml per min
Mobile phase	: pH-3 Phosphate buffer: acetonitrile [90:10]
Wavelength	: 280 nm
Injection volume	: 20**µ**l
Column oven	: Ambient
Run time	: 8 min

Preparation of Phosphate buffer:

Weigh 7.0 grams of Potassium dihydrogen Phosphate into a 1000 ml beaker, dissolve and diluted to 1000 ml with HPLC water. Adjusted the pH to 3.0 with Orthophosphoric acid.

Preparation of mobile phase:

Mix a mixture of above buffer 900 ml (90%) and 100 ml of Acetonitrile HPLC (10%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Mobile Phase as diluent.

Assay:

Preparation of the Levodopa and Carbidopa Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 25 mg of Levodopa and 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of Levodopa & Carbidopa the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer powdered tablet Equivalent to 25 mg of Levodopa and 6.25 mg of Carbidopa [48.3 mg] into a 10 ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 **µ**L of the standard, sample into the chromatographic system and measure the areas for the Levodopa and Carbidopa peaks and calculate the %Assay by using the formulae.

System Suitability:

Tailing factor for the peaks due to Levodopa & Carbidopa in Standard solution should not be more than 2.0

Theoretical plates for the Levodopa & Carbidopa peaks in Standard solution should not be less than 2000

PRECISION:

Accurately weigh and transfer 25 mg of Levodopa and 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent Mix well and filter through 0.45 µm filter.

INTERMEDIATE PRECISION/RUGGEDNESS:

Accurately weigh and transfer 25 mg of Levodopa and 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

ACCURACY:

Preparation of Standard stock solution:

Accurately weigh and transfer 25 mg of Levodopa and 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.4 ml of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 12.5 mg of Levodopa and 3.125 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution).

Further pipette 0.4 ml of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 25.0 mg of Levodopa and 6.25 mg of Carbidopa working standards into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4 ml of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 37.5 mg of Levodopa and 9.375 mg of Carbidopa working standards into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4 ml of Levodopa & Carbidopa of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 25 mg of Levodopa and 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (50 ppm of Levodopa & 12.5 ppm of Carbidopa):

0.2 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (75 ppm of Levodopa & 18.75 ppm of Carbidopa):

0.3 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100 ppm of Levodopa &25 ppm of Carbidopa):

0.4 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (125 ppm of Levodopa & 31.25 ppm of Carbidopa):

0.5 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (150ppm of Levodopa & 37.5ppm of Carbidopa):

0.6 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

LIMIT OF DETECTION:

LEVODOPA

Preparation of 100µg/ml solution:

Accurately weigh and transfer 25 mg of Levodopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.17% solution At Specification level (0.17µg/ml solution):

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Pipette 0.17 ml of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

CARBIDOPA

Preparation of 25µg/ml solution:

Accurately weigh and transfer 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.4% solution At Specification level (0.1µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

Pipette 0.4 ml of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

LIMIT OF QUANTIFICATION:

LEVODOPA

Preparation of 100 µg/ml solution:

Accurately weigh and transfer 25 mg of Levodopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.17% solution At Specification level (0.17µg/ml solution):

Further pipette 1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Pipette 0.17 ml of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

CARBIDOPA

Preparation of 25 µg/ml solution:

Accurately weigh and transfer 6.25 mg of Carbidopa working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute upto the mark with diluent.

Preparation of 1.38 % solution At Specification level (0.34 µg/ml solution):

Further pipette 1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Pipette 1.38 ml of 1µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A) The flow rate was varied at 0.4 ml/min to 0.6 ml/min.

Standard solution 100 ppm of Levodopa & 25 ppm of Carbidopa was prepared and analysed using the varied flow rates along with method flow rate.

B) The Organic composition in the Mobile phase was varied from 15% to 5%.

Standard solution 100 µg/ml of Levodopa & 25 µg/ml of Carbidopa was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

CALCULATION:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Average Peak Area obtained with test preparation

AS = Average Peak Area obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Results and Discussions:

System suitability:

Standard solution is injected five times and Flow rate was maintained at 0.5 ml/min. temperature of column kept ambient and the column effluents were monitored at 280 nm, chromatograms were taken and System suitability parameters were computed. The system suitability was calculated as per ICH guidelines (figure no.1,2).

Figure No-1: Levodopa and Carbidopa sample.

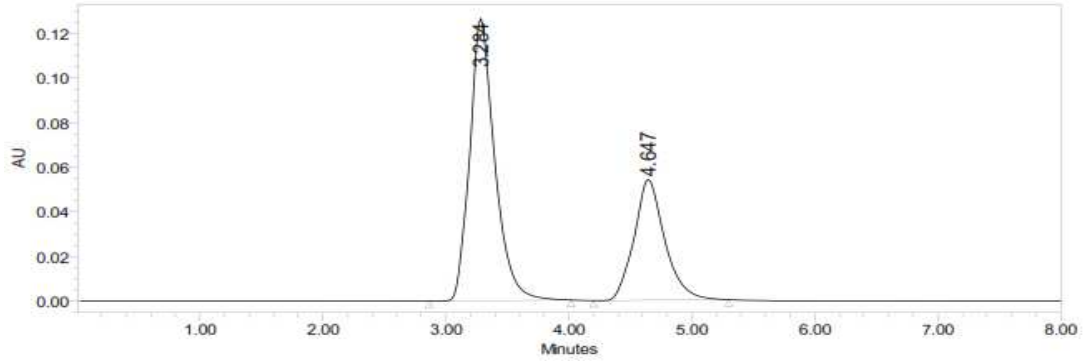
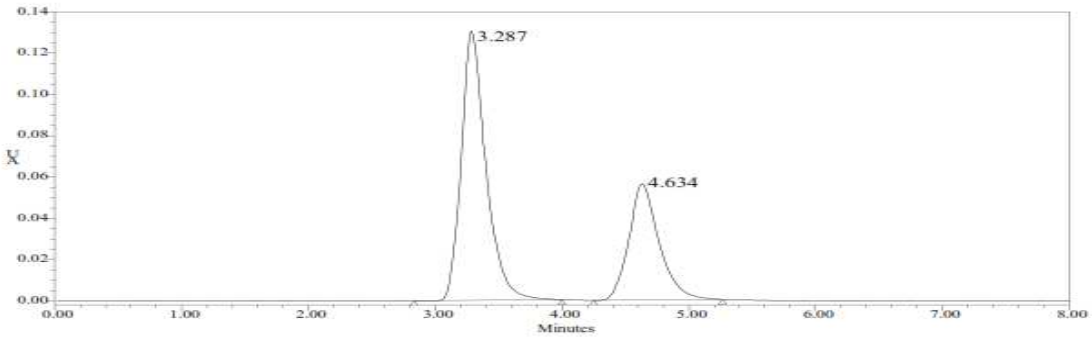


Figure No-2: Levodopa and Carbidopa standard.



Precision:

The sample solution prepared as mentioned in sample preparation was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. (table no.1 & 2).

Table No-1: Summary of levodopa precision:

Injection	**Area**
Injection-1	1800075
Injection-2	1794674
Injection-3	1798698
Injection-4	1799569
Injection-5	1794931
Average	1797590
Standard Deviation	2592.7
%RSD	0.14

Table No-2: Summary of carbidopa precision:

Injection	**Area**
Injection-1	947667
Injection-2	948433
Injection-3	948214
Injection-4	948776
Injection-5	944983
Average	947615
Standard Deviation	1525.3
%RSD	0.16

INTERMEDIATE PRECISION:

Table No-3: Summary of levodopa intermediate precision:

Injection	**Area**
Injection-1	1831344
Injection-2	1835541
Injection-3	1838564
Injection-4	1845898
Injection-5	1865219
Average	1843313
Standard Deviation	13348.8
%RSD	0.72

Table No-4: Summary of Carbidopa intermediate precision:

Injection	**Area**
Injection-1	966843
Injection-2	967136
Injection-3	967611
Injection-4	968601
Injection-5	977143
Average	969467
Standard Deviation	4342.5
%RSD	0.45

ACCURACY (RECOVERY STUDIES):

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 50%, 100% and 150% concentration levels.

Results of recovery studies are shown in table no.5.

Table No-5: Summary of Recovery studies of Levodopa.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	873206	12.5	12.2	98.3%	99.8%
100%	1770715	25.0	24.9	99.7%	
150%	2705031	37.5	38.0	101.5%	

Table No-6: Summary of Recovery studies of Carbidopa.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	462040	3.125	3.07	98.2%	99.9%
100%	936766	6.25	6.23	99.6%	
150%	1438287	9.375	9.56	101.9%	

LINEARITY:

Linearity was studied by preparing standard solutions at different concentration levels.

Table No-7: Summary of Linearity for Levodopa.

S.No	Linearity Level	Concentration	Area
1	I	50ppm	613697
2	II	75ppm	1232954
3	III	100ppm	1783436
4	IV	125ppm	2353788
5	V	150ppm	2930827
Correlation Coefficient			1.000

Table No-8: Summary of Linearity for Carbidopa.

S.No	Linearity Level	Concentration	Area
1	I	12.5 ppm	319393
2	II	18.75 ppm	647698
3	III	25 ppm	939367
4	IV	31.25 ppm	1238862
5	V	37.5 ppm	1544535
Correlation Coefficient			0.999

Figure No-3: Linearity Graph of Levodopa.

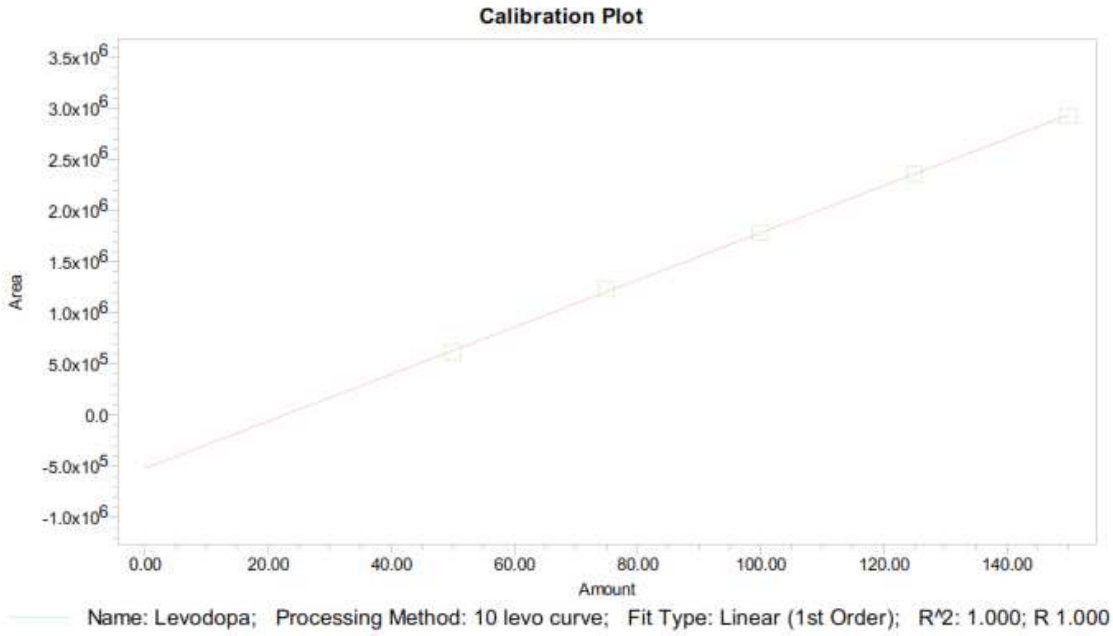
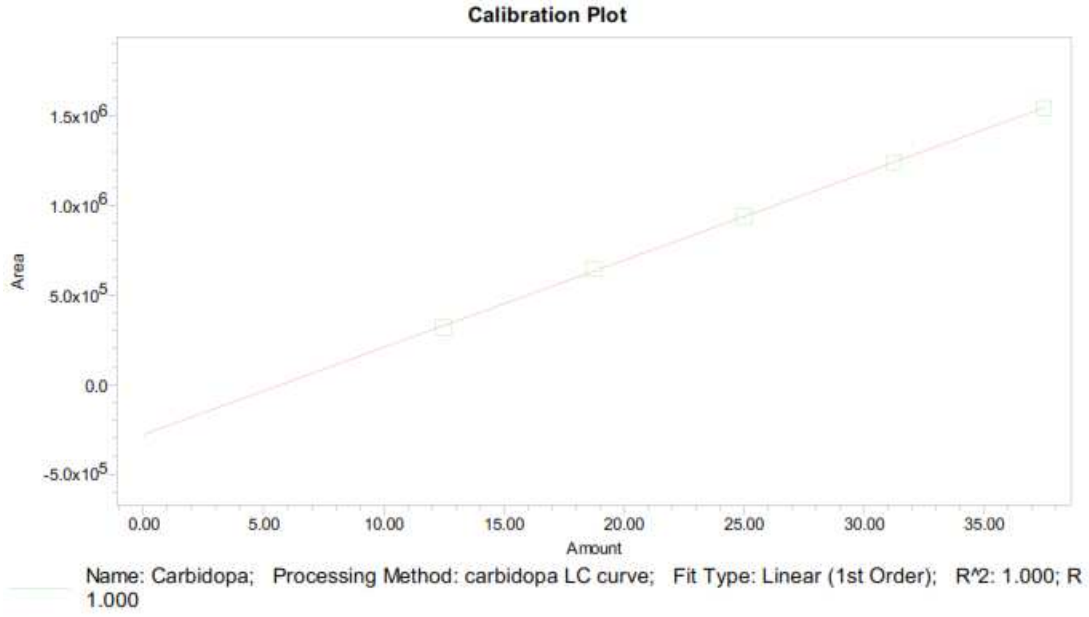


Figure No-4: Linearity Graph of Carbidopa.



LOD and LOQ:

LEVODOPA:

For Levodopa the LOD concentration for obtained is 0.00017 mg/ml (or) 0.17% with respect to working concentration of 0.1 mg/ml and the LOQ concentration obtained is 0.00017 mg/ml (or) 0.17% with respect of working concentration of 0.1mg/ml (table no.9).

Table No-9: Summary of LOD and LOQ.

Component	Working concentration[mg/ml]	LOD concentration[mg/ml]	Signal to Noise Ratio
Levodopa [LOD]	0.1	0.00017	2.96
Levodopa [LOQ]	0.1	0.00017	9.90

CARBIDOPA:

For Carbidopa the LOD concentration obtained is 0.00017 mg/ml (or) 0.17% with respect to working concentration of 0.025 mg/ml and the LOQ concentration obtained is 0.0001 mg/ml (or) 0.4% with respect to working concentration of 0.025 mg/ml (table no.10).

Table No-10: Summary of LOD and LOQ.

Component	Working concentration[mg/ml]	LOD concentration[mg/ml]	Signal to Noise Ratio
Carbidopa [LOD]	0.025	0.00017	3.03
Carbidopa [LOQ]	0.025	0.0001	9.94

ROBUSTNESS:

The flow rate was varied at 0.4 ml/min to 0.6ml/min.

The results are summarized:

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

The method is robust only in less flow condition.

System suitability results for Levodopa:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	**USP Tailing**
1	0.4	2232.3	1.4
2	0.5	2381.7	1.3
3	0.6	2193.0	1.2

System suitability results for Carbidopa:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	**USP Tailing**
1	0.6	2173.3	1.4
2	0.8	2880.9	1.2
3	1.0	2013.7	1.1

The Organic composition in the Mobile phase was varied from 15% to 5%.

The results are summarized

On evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is not robust even by change in the Mobile phase ±1

System suitability results for Levodopa:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	**USP Tailing**
1	10% less	2227.0	1.2
2	*Actual	2381.7	1.3
3	10% more	2168.7	1.3

System suitability results for Carbidopa:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2303.1	1.1
2	*Actual	2880.9	1.3
3	10% more	2288.4	1.2

Conclusion:

The results of the validation study indicate that the analytical method developed for the determination of assay is found to be accurate and precise. The percentage RSD for all parameters was found to be less than two, which indicates the validity of the method and assay results obtained by this method are in fair agreement. The method is both repeatable and rugged.

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