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Research Article

RP-HPLC Method Development and Validation for the Analysis of Levodopa and Carbidopa in Bulk and Tablet Dosage Forms.

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ABSTRACT:
the simultaneous evaluation of levodopa and carbidoxopa. The chromatogram was performed using Sunfire (250mm 4.6mm, 5µ). A mobile phase consisting of KH2PO4, acetonitrile in a 60:40 v/v ratio was injected across the column at a flow rate of 1.0 ml/min. The temperature was kept steady at 30°C. The optimal wavelength for levodopa and carbidogopa was 280 nm. Levodopa and carbidogpa's percentage RSDs were 1.5 and 0.6, respectively. The retention durations for levodopa and carbidogopa were found to be 2.183 and 2.755 minutes, respectively. The LOD and LOQ values obtained from the Levodopa and Carbidopa regression equations were 0.003, 0.009, and 0.06, 0.18, respectively. The regression equation for levodopa is y = 85498x + 14640, whereas that for carbidogopa is y = 80352x + 11509.

Key Words: Levodopa, Carbidopa, Rp Hplc, Validation.

INTRODUCTION

Parkinson's disease is a progressive neurological movement illness. It results in the weakening, destruction, and death of nerve cells (neurones) in some areas of the brain, which produces symptoms like stiffness, tremor, difficulty moving, and poor balance.[1] Although there is no cure for Parkinson's disease, medications can help manage its symptoms. Often, medications are highly effective. Surgery may be necessary for certain people when medication is no longer effective.[2] Carbidopa and levodopa are the cornerstone of pharmaceutical treatment for Parkinson's disease (PD), a progressive neurodegenerative condition characterised by dopamine insufficiency in the basal ganglia.[3] Carbidopa is chemically written as (2S)-3-(3,4-dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid. It effectively inhibits aromatic amino acid decarboxylase (DDC), and its chemical characteristics prevent it from crossing the blood-brain barrier. Because of its action, carbidopa is always provided in conjunction with levodopa.[4] Levodopa, chemically written as (2S)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid, is a prodrug of dopamine, is supplied to individuals with Parkinson's because of its ability to pass the blood-brain barrier.[5] When used with levodopa, carbidopa prevents aromatic L-amino acid decarboxylase from decarboxylating oxitriptan to serotonin and from peripherally converting levodopa to dopamine. As a result, there is more oxitriptan and levodopa accessible for delivery to the central nervous system. Additionally, carbidopa increases levodopa's bioavailability by blocking its GI tract metabolism.[6]

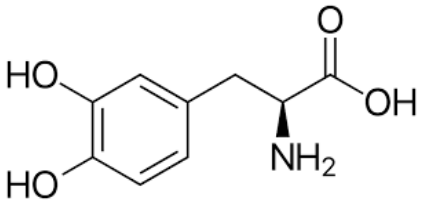

Figure 1: structure of Levodopa

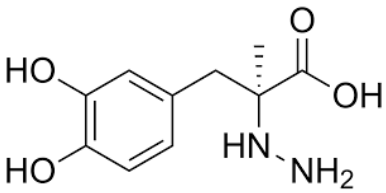

Figure 2: Structure of Carbidopa

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Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Levodopa, Carbidopa, and their medicinal dose form using RP-HPLC [7- 13]must be validated and developed as per ICH guidelines

Materials and Methods: Spectrum pharma Research Solution with Levodopa and Carbidopa pure drugs (API) gift samples and Combination Levodopa and Carbidopa tablets (**Syndopa CR 125**). The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to fulfill ICH standards, we need to design and test an HPLC technique that can detect Carbidopa and Levodopa in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and KH2PO4 (60:40 v/v)
Flow rate	1 ml/min
Column	Sunfire C18 (4.6 x 150mm, 5µm)
Detector wave length	280 nm
Column temperature	30°C
Injection volume	10mL
Run time	5.0 min
Buffer	KH2PO4

Buffer Preparation: 0.01N KH2PO4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.0 with dil. Orthophosphoric acid solution.

API Preparation:

Preparation of Standard stock solutions: Accurately weighed 50mg of Levodopa, 12.5mg of Carbidopa and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of Levodopa and 250µg/ml of Carbidopa)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml Levodopa of and 25µg/ml of Carbidopa)

Formulation Preparation:

Preparation of Sample stock solutions: Average of one tablet weight was transferred into a 100 ml volumetric flask, 20ml of diluents was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of Levodopa and 250µg/ml of Carbidopa)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Levodopa and 25µg/ml of Carbidopa)

System suitability parameters: Levodopa (100 ppm) and Carbidopa (25 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in

order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S.no	Levodopa			Carbidopa			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.124	2902	1.43	2.682	6548	1.27	3.7
2	2.135	2899	1.39	2.690	6883	1.31	3.7
3	2.136	2864	1.38	2.696	6742	1.26	3.7
4	2.148	2989	1.38	2.703	6934	1.30	3.8
5	2.173	2915	1.42	2.743	6969	1.27	3.8
6	2.176	2930	1.38	2.746	6737	1.30	3.8

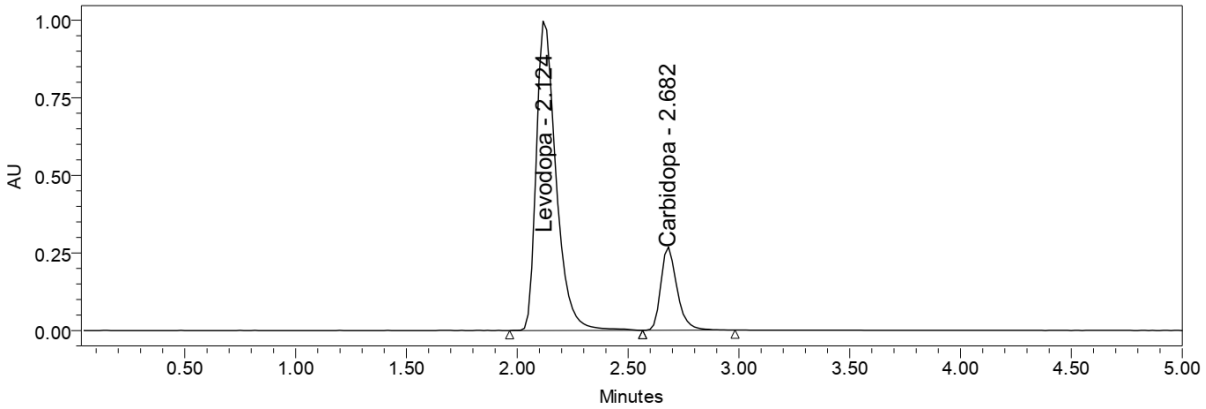

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time	Area	Plate count	Tailing	Resolution
Levodopa	2.183	8697542	3120.0	1.3	
Carbidopa	2.755	2027355	4263.9	1.3	3.2

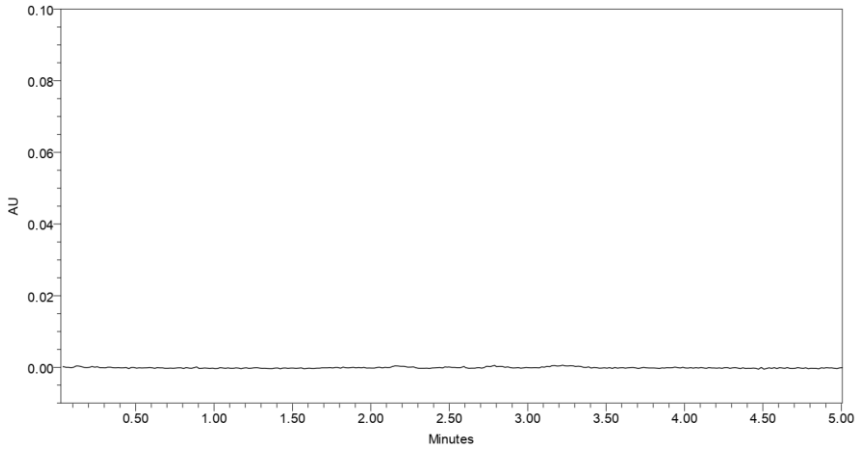

Figure.4 Specificity of Levodopa and Carbidopa

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 6, 7

Table 4: Calibration data of Levodopa and Carbidopa

Levodopa		Carbidopa	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
25	2051074	6.25	501753
50	4283239	12.5	1011000
75	6529234	18.75	1539295
100	8696265	25	2045375
125	10749939	31.25	2557330
150	12679363	37.5	2972032

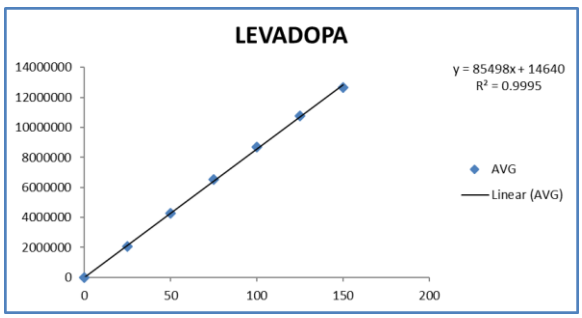

Figure 5 Calibration curve of Levodopa

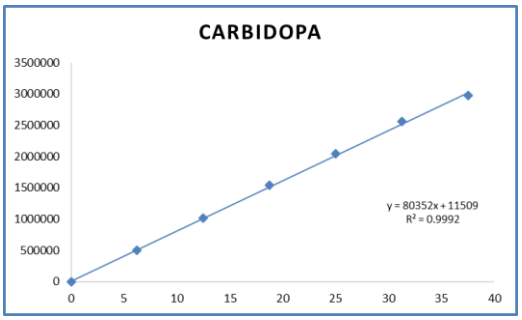

Figure 6Calibration curve of Carbidopa

Table 5: regression data

Parameter	Levodopa	Carbidopa
Conc range (µg/mL)	25 – 150 µg/ml	6.25 – 37.5 µg/ml
Regression Equation	y = 85498x + 14640	y = 80352x + 11509
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Levodopa and Carbidopa

	Levodopa			Carbidopa		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	50	49.210	98.42	12.5	12.406	99.25
	50	49.715	99.43	12.5	12.417	99.33
	50	49.993	99.99	12.5	12.562	100.50
100%	100	99.013	99.01	25	25.342	101.37
	100	99.833	99.83	25	24.792	99.17
	100	99.292	99.29	25	24.766	99.06
150%	150	148.757	99.17	37.5	37.977	101.27
	150	148.888	99.26	37.5	37.231	99.28
	150	149.810	99.87	37.5	37.451	99.87
% recovery	**99.36**			**99.90**		

System precision was performed and the data was shown in table 8

Table 7: System precision of Levodopa and Carbidopa

S. No	Area of Levodopa	Area of Carbidopa
1.	8650464	2045427
2.	8564718	2034804
3.	8576217	2029961
4.	8656080	2000279
5.	8662542	2049523
6.	8788056	2050064
Mean	8649680	2035010
S.D	79962.8	18854.9
%RSD	0.9	0.9

The % RSD for the peak areas of Levodopa and Carbidopa obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Levodopa and Carbidopa and shown in table 8.

Table 8: method Precision

S. No	Area of Levodopa	Area of Carbidopa
1.	8633539	2028749
2.	8628794	2029299
3.	8629945	2059269
4.	8633095	2028099
5.	8621200	2017943
6.	8632115	2053419
Mean	8629781	2036130
S.D	4586.7	16314.0
%RSD	0.1	0.8

From the above results, the % RSD of method precision study was within the limit for Levodopa and Carbidopa.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.0ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Levodopa and Carbidopa.

Condition	%RSD of Levodopa	%RSD of Carbidopa
Flow rate (-) 0.9ml/min	0.9	0.5
Flow rate (+) 1.1ml/min	0.4	0.2
Mobile phase (-) 55B:45A	0.5	0.5
Mobile phase (+) 65B:35A	1.0	0.6
Temperature (-) 27°C	0.3	0.2
Temperature (+) 33°C	0.7	0.3

Sensitivity:

Table 10: sensitivity of Levodopa and Carbidopa

Molecule	LOD	LOQ
Levodopa	0.003 µg/ml	0.009 µg/ml
Carbidopa	0.06 µg/ml	0.18 µg/ml

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	60 mins
Base	2N NAOH	60^0c	60 mins
Oxdation	20% H_2O_2	60^0c	60 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	60 mins

Table 12: degradation data

Type of degradation	Levodopa			Carbidopa		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	8358746	96.44	3.56	1951505	95.70	4.30
Base	8354935	96.40	3.60	1909255	93.63	6.37
Peroxide	8357146	96.42	3.58	1950029	95.63	4.37
Thermal	8387146	96.77	3.23	1914837	93.91	6.09
Uv	8501505	98.09	1.91	1947959	95.53	4.47
Water	8530156	98.42	1.58	1997987	97.98	2.02

Acid degradation chromatogram

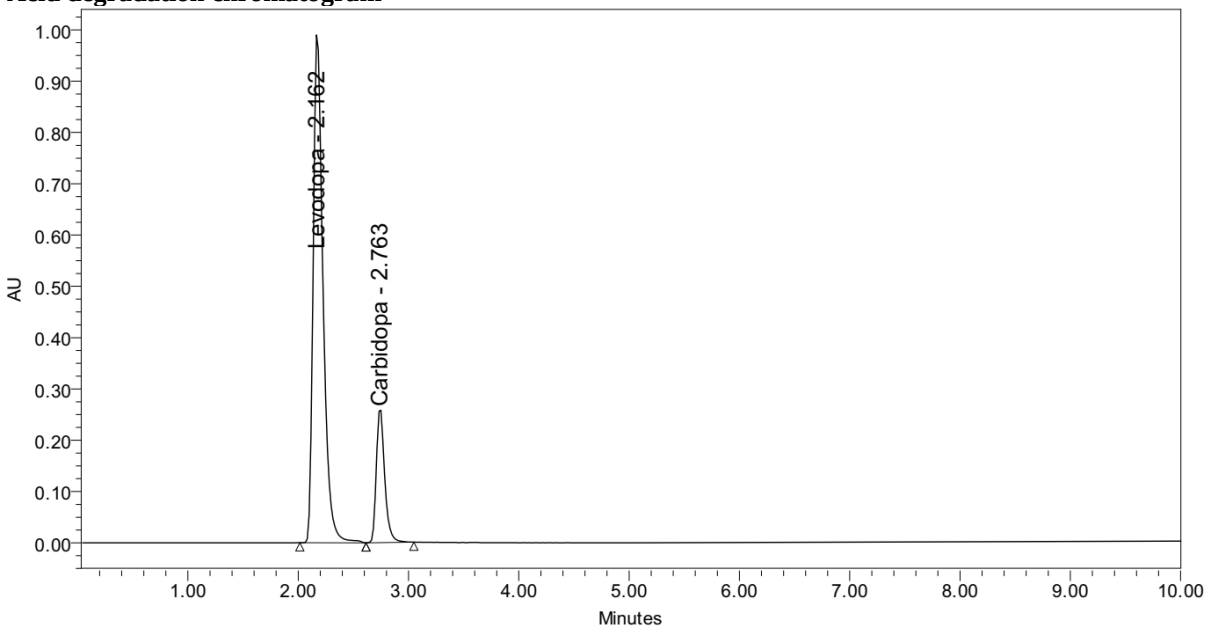

Fig 7 acid

Base degradation chromatogram

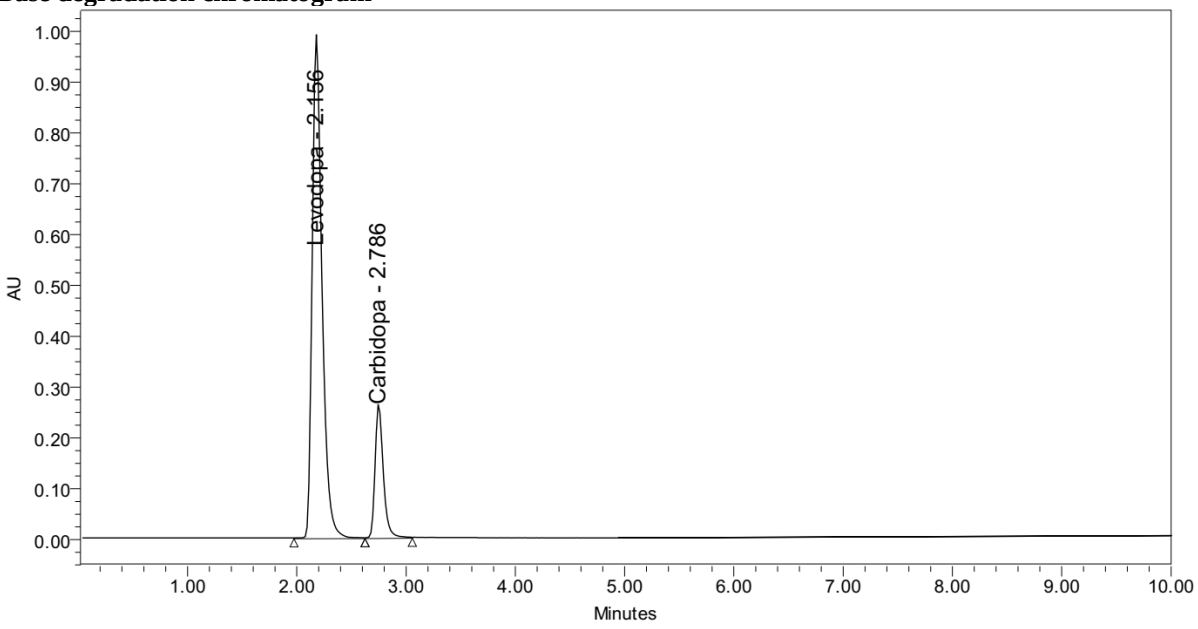

Fig 8 base

Peroxide degradation chromatogram

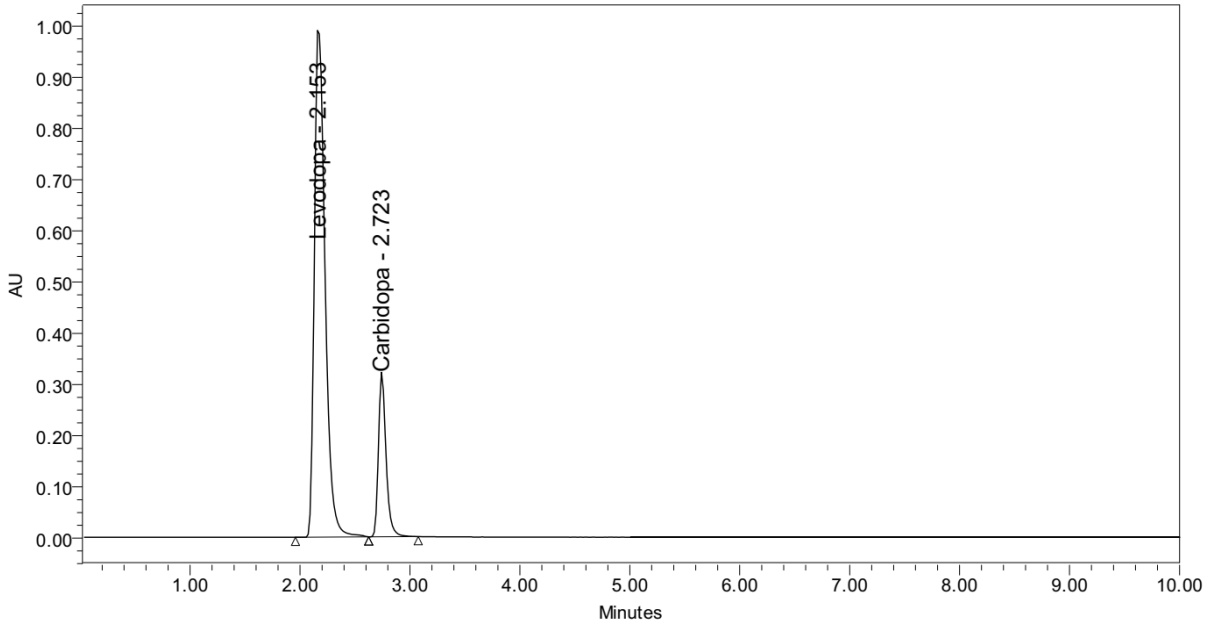

Fig 9 peroxide

Thermal degradation chromatogram

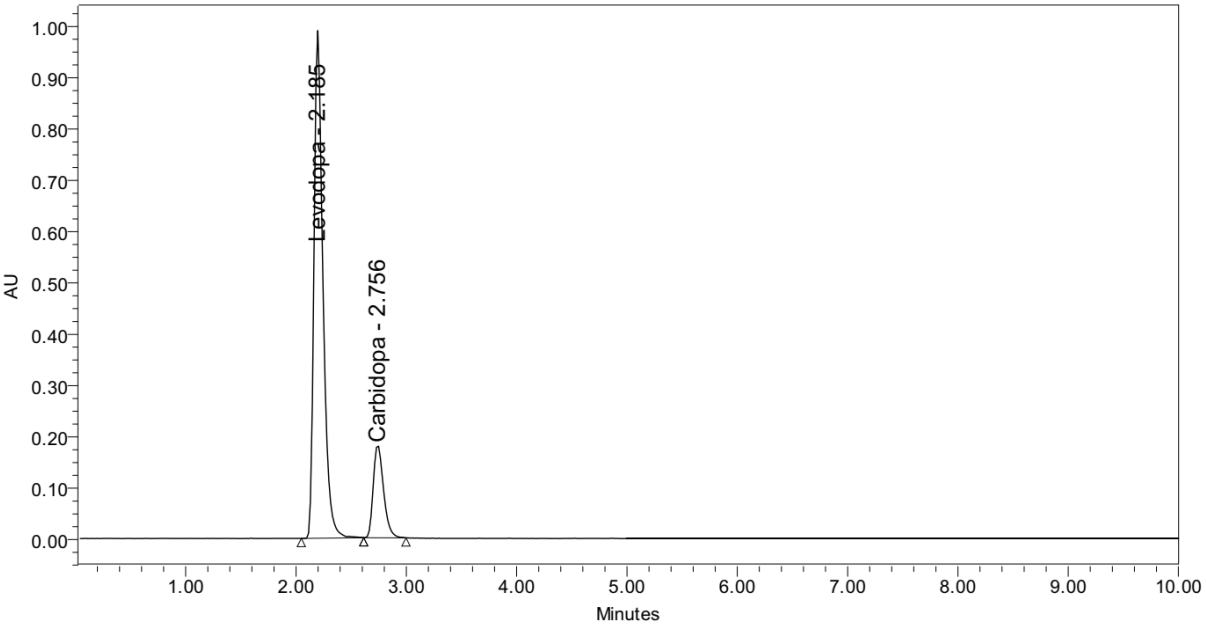

Fig 10 thermal

UV degradation chromatogram

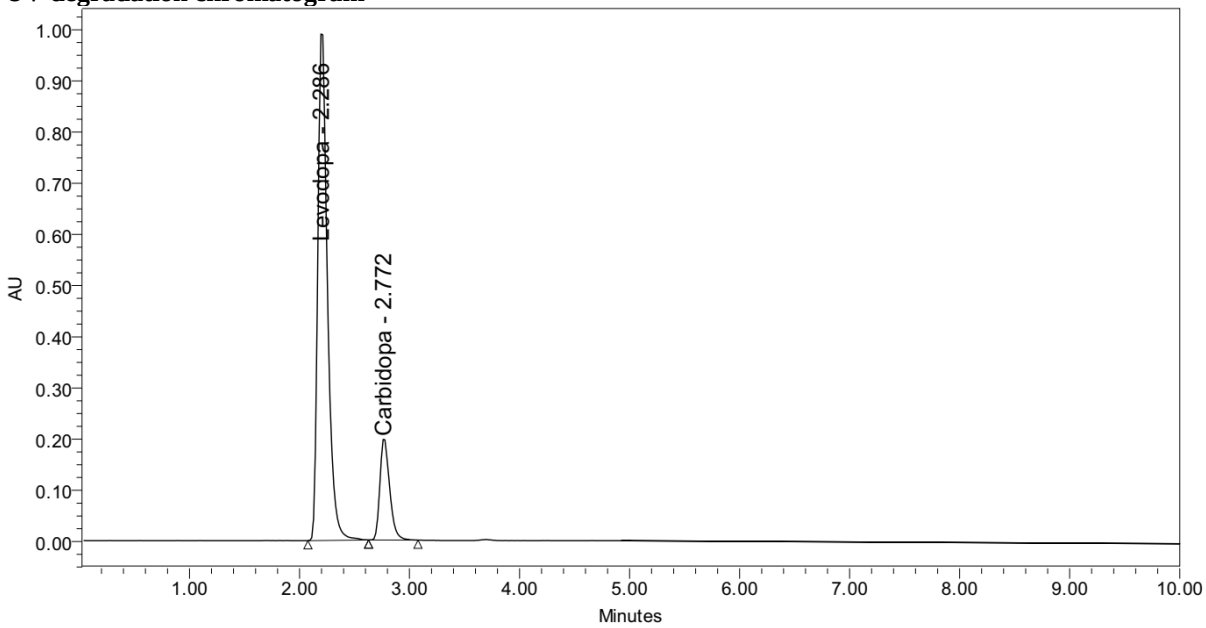

Fig 11 UV

Water degradation chromatogram

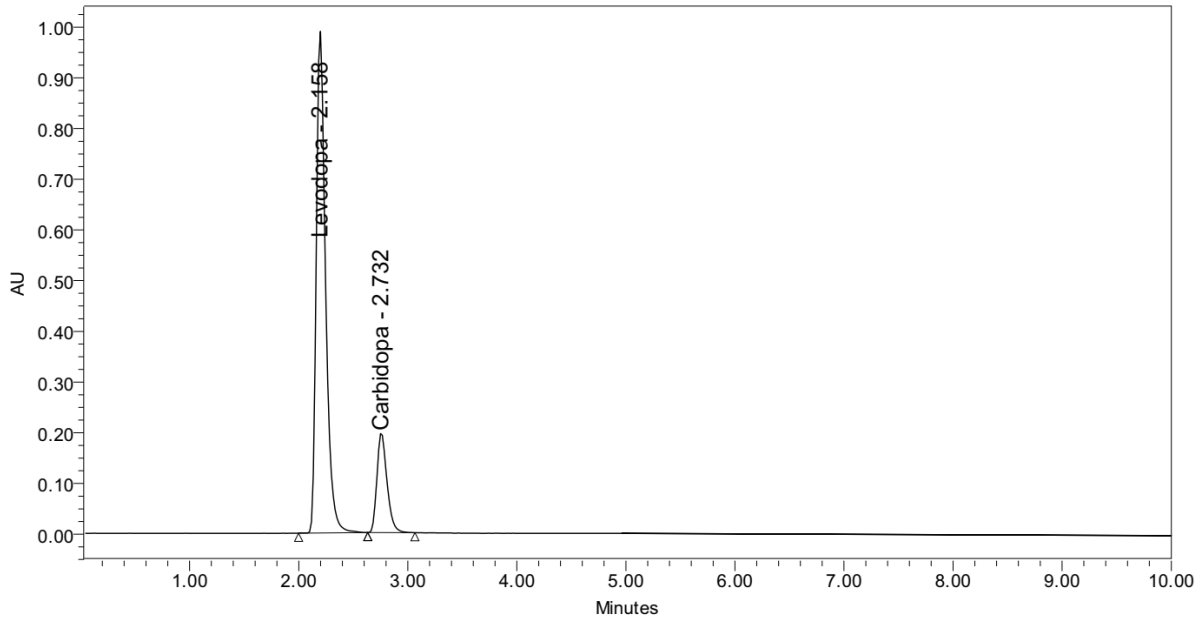

Fig 12 water

Assay: Syndopa CR 125, bearing the label claim Levodopa 100mg, Carbidopa 25mg. Assay was performed with the above formulation. Average % Assay for Levodopa and Carbidopa obtained was 99.43% and 99.46% respectively.

Table 13: assay data

	Levodopa			Carbidopa		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	8650464	8633539	**99.61**	2045427	2028749	**99.49**
2	8564718	8628794	**99.56**	2034804	2029299	**99.52**
3	8576217	8629945	**99.57**	2029961	2059269	**100.99**
4	8656080	8633095	**99.61**	2000279	2028099	**99.46**
5	8662542	8621200	**99.47**	2049523	2017943	**98.96**
6	8788056	8632115	**99.60**	2050064	2053419	**100.70**
Avg	8649680	8629781	**99.57**	2035010	2036130	**99.85**
Stdev	79962.8	4586.7	**0.05**	18854.9	16314.0	**0.80**
%RSD	0.9	0.1	**0.05**	0.9	0.8	**0.80**

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{100} \times P \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 13 formula

CONCLUSION:

The study's findings will be very helpful in evaluating the quality of reasonably priced drugs that contain carbidopa and levodopa. This could be as a result of the study's straightforward sample preparation method, which required little mobile phase and a brief analytical period. The results of evaluating two medications combined in a single dosage demonstrated that the recently created analysis technique was almost entirely successful.

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