



## Compatibility and stability studies of levodopa, carbidopa, entacapone and natural bioenhancer mixture

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### ABSTRACT

Levodopa, Carbidopa and Entacapone are used for the treatment of Parkinsonism disease. Piperine is an alkaloid from Piper species is reported to enhance the oral bioavailability of co-administered drugs. Piperine can improve the bioavailability of Levodopa in solid oral dosage forms. The present study was aimed to added Piperine natural bio-enhancer as a formulation additive in oral formulations of Levodopa, Carbidopa and Entacapone. The physical mixture of Levodopa, Carbidopa, Entacapone and Piperine in the ratio of 1:1 and the mixture were stored up to four weeks at 40°C/75%RH. In that samples are analyzed by validated Liquid chromatography techniques. The stability of the mixture was assessed by the use of ICH guidelines. Statistical experimental designs enable the user to obtain the maximum amount of information, i.e., the degradation effects of additives (Piperine) and their interactions on the stability of the drug substance. The percentage impurities of Levodopa, Carbidopa and Entacapone in Piperine after four weeks at 40°C/75%RH and are same like Initial results. The above studies have proved that Piperine can be used as a formulation additive in oral formulations of Levodopa, Carbidopa and Entacapone.

**Keywords:** Compatibility studies, Stability, Piperine, Related substances.

### INTRODUCTION

The development process of a drug product can be divided into two different steps<sup>1</sup>. The first step involves stability studies that enable the product's designer, usually a pharmaceutical technologist, to gain information about the stability of the drug substance and its compatibility with potential additives, called also excipients. The first type of study is called the stress study of the drug substance and the second is the compatibility study<sup>2-6</sup>. Stress studies of the drug substance are usually carried out

at stress storage conditions that involve higher temperature and/or higher relative humidity, the influence of sun or artificial sun light, (Photo stability chamber) presence of an oxidation agent such as peroxide, solutions having different pH values acidic and basic<sup>3-6</sup>. The main goal of these stability studies is to obtain information about different factors such as temperature, pH, humidity, light that affect the stability of the drug substance, potential degradation pathways of the substance, main degradation products that appear under degradation of the substance at different stress conditions and evaluation of

stability-indicating nature of the analytical method that is employed to detect different degradation products<sup>3-6</sup>.

Compatibility studies are usually the last activity done during pre-formulation profiling. All pre-formulation studies, except compatibility studies, are carried out on pure drug substance. Compatibility studies are aimed at studying the interactions of drug substance with other excipients. Selection of excipients is vital for development of a quality of drug product. Choice of excipients is guided by the type of proposed dosage form. For example for a tablet dosage form, excipients belonging to categories of diluent, binder, disintegrant, lubricant, glidant, sometime bio-enhancers are usually included in the compatibility studies. Based on the need, optionally solubilizer, stabilizing agent, buffer and rate controlling polymer can also be included<sup>7-8</sup>. The pure form of drug substances and additives the corresponding 1:1 (w/w) physical mixtures and the samples are analyzed quantitatively using UV spectroscopy or HPLC. Additional data on solid form changes can also be generated using conventional tools like powder X ray diffraction, FTIR and solid state NMR<sup>6-8</sup>.

Levodopa, Carbidopa and Entacapone has been the most widely used for the treatment of Parkinsonism for over 30 years. L-DOPA is converted into dopamine in the dopaminergic neurons by dopa decarboxylase. Since motor symptoms are produced by a lack of dopamine in the substantia nigra, the administration of L-DOPA temporarily diminishes the motor symptoms. Only 5–10% of L-DOPA crosses the blood–brain barrier. The remainder is often metabolized to dopamine elsewhere, causing a variety of side effects including nausea, dyskinesias and joint stiffness. Carbidopa and Benserazide are peripheral dopa decarboxylase inhibitors, which help to prevent the metabolism of L-DOPA before it reaches the dopaminergic neurons, therefore reducing some side effects and increasing bioavailability. Entacapone is a selective, reversible catechol-O-methyl transferase (COMT) inhibitor for the treatment of Parkinson's disease. When administered concomitantly with levodopa and a decarboxylase inhibitor (e.g., carbidopa), increased and more sustained plasma levodopa concentrations are reached as compared to the administration of levodopa and a decarboxylase inhibitor<sup>9-13</sup>.

Piperine (1-piperoyl piperdine) is a major component of Piper species. This species has been used widely as spices and various systems of Indian medicine. Piperine has been shown to enhance the bioavailability of drugs like

Vascicine, Curcumin, Barbiturate, Oxyphenyl-butazone, Propranolol, Theophylline and Ampicillin<sup>14</sup>. Piperine is having enhancement of Bioavailability activity of Levodopa and used to improved memory impairment and neurodegeneration<sup>15-16</sup>. Piperine (bioactive compound of Piper nigrum) inhibits neuronal cell apoptosis and the anti-inflammatory effect of Piperine on 6-OHDA induced Parkinson's disease<sup>16</sup>. Piperine has been shown to have a wide range of activity, including MAO inhibitory activity<sup>17</sup>. Piperine was examined for inhibitory activity of monoamine oxidase (MAO) A and B from rat brain mitochondrial. Piperine was shown to inhibit MAO B in a dose-dependent manner with the IC<sub>50</sub> data of 91.3<sup>18</sup>. So the present study was aimed to develop a new concept of inclusion of bioenhancer as a formulation additive by conducting compatibility and stability studies of Levodopa, Carbidopa, Entacapone and Piperine (1:1) mixture.

## MATERIALS AND METHODS

### Chemicals, Materials and Equipments

The liquid chromatographic system were of Waters Alliance 2695 separation module with 2996 PDA Detector, which consisted of following components: a gradient pump, variable wavelength programmable UV/Vis detector, auto injector and column oven. The chromatographic analysis was performed using Empower-2 software (Waters, Milford, USA) on Zorbax SB-C18 (250x4.6) mm, 5µm, Zorbax SB C8 (250x4.6) mm, 5µm & Purosphere RP18c, (250x4.6) mm, 5µm. Newtronics Stability chambers and complies with US FDA 21 CFR Part 11. Piperine sample was procured from Sigma-Aldrich Chemicals Private Limited, Bangalore, India. Analytically pure Levodopa, Carbidopa and Entacapone were procured as gift samples from Hetero Labs, Hyderabad, Andhra Pradesh, India. All other chemicals and reagents used were analytical grade and HPLC grade and purchased from Merck Chemicals, India.

### Compatibility studies

Compatibility studies are an important step in pre formulation studies for developing a dosage form. The physical admixtures of Carbidopa, Levodopa and Entacapone with Piperine were made in various ratios and sifted through # 30 mesh and mixed. The samples were packed in clear glass vials and sealed with unpunctured Low density poly ethylene (LDPE) closures. The samples were subjected to 40°C/75 % RH and 25°C/60 % RH and

observed periodically up to 4 weeks. Along with any physical change like color and appearance the chemical compatibility of drug substances with the Piperine was also evaluated by determining the related substances in the admixtures by HPLC analysis.

### Stability studies

Stability studies as an important part of preformulation studies. The stability of the drug substances depends upon the container and conditions in which it is stored and other excipients present with the drug. The storage temperature for Levodopa, Carbidopa and Entacapone at room temperature (15°C to 30°C)<sup>13</sup>. The stability studies of Levodopa, Carbidopa, Entacapone and Piperine physical mixture (0.3:0.3:0.3:1) was carried out as per ICH guidelines<sup>4</sup>. The mixture was encapsulated in hard gelatin capsule shells, the capsules were then transferred to high density poly ethylene (HDPE) bottles. The studies was carried out as accelerated condition for six months by storing at 40°C/75% RH and Long term condition for one year at 25°C/60% RH the sampling time points 0, 1, 2, 3, 6 and 0, 3, 6, 9, 12 months respectively.

### Related substances Determination

The determination of related substances of Levodopa, Carbidopa and Entacapone by RP-HPLC method in two different chromatographic methods. In first method for Levodopa and Carbidopa Related substances and Second for Entacapone Related substances determination.

### Gradient Program

Time in minutes	%Mobile phase A (v/v)	%Mobile phase B (v/v)
0	100	0
25	95	5
45	40	60
50	40	60
52	10	90
60	10	90
62	100	0
75	100	0

### Preparation of Standard solution

Standard solution of Levodopa and Carbidopa were prepared by dissolving 200mg of Levodopa and 50mg of Carbidopa in to a 50ml volumetric flask and dissolved with diluent. This solution was further diluted to obtain

### Chromatographic conditions for Levodopa and Carbidopa Related Substances Determinations (I)

#### Preparation of Buffer pH 4.0 (Mobile phase-A)

Add 1ml of Trifluoroacetic acid in 1000ml of water. Adjust the pH to 4.0± 0.05 with dilute potassium hydroxide solution. Filter through 0.45µm nylon membrane filter.

#### Preparation of Mobile phase (B)

Prepare a mixture of Mobile phase-A and Acetonitrile in the ratio of 50:50 v/v and degas.

#### Preparation of Diluent (0.1N Hydrochloric acid)

Diluted 8.5ml of concentrated Hydrochloric acid (35%) In 1000ml of water and mix well.

Column*	: Zorbax SB C8 (250x4.6) mm, 5µm & Purosphere RP18c, (250x4.6) mm, 5µm
Flow rate	: 1.0ml/minute
Wavelength	: 205nm
Injection volume	: 15µL
Column oven temperature	: 30°C
Sample temperature	: 10°C
Run time	: 75 minutes

\*Note: Connect both the columns in series Zorbax SB C8 (250x4.6) mm, 5µm followed by Purosphere RP18c, (250x4.6) mm, 5µm

final concentrations of Levodopa 80 µg/ml and Carbidopa 20 µg/ml.

### Preparation of Sample solution

The Sample equivalent to 200mg of Levodopa was transferred to 50ml volumetric flask and treated with 30ml of diluent using ultra sonicator for 15minutes. Dilute to volume with diluent and mix well. Centrifuge the solution to get a clear supernatant solution.

### Chromatographic conditions for Entacapone Related Substances Determinations (II)

#### Mobile Phase A

Dissolved 5.76gm of ammonium dihydrogen Orthophosphate acid in 1000ml of water, adjusted pH to

2.2 with 10% Orthophosphoric acid (OPA) and filtered through 0.45µm membrane filter.

#### Mobile Phase B

Prepared a mixture of methanol and acetonitrile in the ratio of 75:25% v/v and degassed.

#### Diluent

Prepared a mixture of mobile phase A and mobile phase B in the ratio of 60:40% v/v and degassed.

### Chromatographic conditions

Column	: Zorbax SB C18 (250 x 4.6)mm, 5µm
Flow rate	: 1.2ml/minute
Wavelength	: 210nm
Injection volume	: 20µL
Column oven temperature	: 50°C
Sample temperature	: 10°C
Run time	: 70minutes

### Gradient Program

Time (Minutes)	% of mobile phase A	% of mobile phase B
0	90	10
10	90	10
35	65	35
60	65	35
62	90	10
70	90	10

### Preparation of Standard solution

Standard solution of Entacapone was prepared by dissolving 40 mg of Entacapone into a 100 ml volumetric flask and dissolve with 40ml mobile phase B, the volume was made up with mobile phase A. This solution was further diluted to obtain final concentrations of Entacapone 4 µg/ml with diluent.

### Preparation of Sample solution

The Sample equivalent to 80mg of Entacapone was transferred to 200ml volumetric flask and treated with 80ml of mobile phase B using ultra sonicator. This solution was filtered through 0.45µm membrane filter and used for analysis.

### RESULT AND DISCUSSION

The results of subjected samples are no any physical changes like color of the mixture, odor and taste of the mixture. The chemical compatibility of drug substances with the Piperine was determined the related substances of Levodopa, Carbidopa and Entacapone by RP-HPLC method. Based on the physical and chemical properties of drug substances and impurities two different Liquid chromatographic method developed. In first method Related substances of Levodopa and Carbidopa and the second method Related substances of Entacapone was determined. Those methods are developed with proper system suitability parameters like resolution, tailing factor and theoretical plates and the developed methods are validated as per ICH guideline<sup>19</sup>. In that validated methods Initial and 4 week samples are prepared as per

above mentioned method and injected. The results of Levodopa, Carbidopa and Entacapone related substances maximum total impurities 0.044%, 0.447% and 0.740% respectively. The results are tabulated in Table No.1-4. The known and unknown impurity results are same like initial results and no degradation peaks in both the method. Figure No.1- 4.

The physical mixtures of drug substances and Piperine are stable in both accelerated and long term stability conditions. The %difference of assay found less than 5.0 compare to initial results and for total impurities less than 15.0. Those results within the specifications indicate Levodopa, Carbidopa and Entacapone in Piperine are stable in stability studies. The results are tabulated in Table No.5-6.

**Table-1: Compatibility study data of (Levodopa, Carbidopa and Entacapone) and Piperine**

Physical admixture	Drug: Additive	Initial Description	40°C/75%RH			25°C/60%
			1 week	2 week	4 week	RH
Levodopa + Piperine	1:1	Off white powder	√	√	√	√
Carbidopa + Piperine	1:1	Off white powder	√	√	√	√
Entacapone + Piperine	1:1	Yellow powder	√	√	√	√
Levodopa + Carbidopa + Entacapone + Piperine	0.3:3:3:1	Light yellowish powder	√	√	√	√

√ No changes in physical appearance.

**Table-2: Data of Related substances for Levodopa with Piperine (40°C/75%RH):**

Physical admixture	Duration	Related substances (% Area)						
		1	2	3	4	5	6	7
Levodopa + Piperine	0 days	0.000	0.007	0.000	0.000	0.007	0.007	0.043
	4 weeks	0.000	0.009	0.000	0.000	0.008	0.007	0.031
Levodopa + Carbidopa + Entacapone + Piperine	0 days	0.000	0.010	0.000	0.000	0.006	0.008	0.044
	4 weeks	0.000	0.007	0.000	0.000	0.007	0.007	0.025

1.Related compound-A, 2.L-Tyrosine, 3.Related compound B, 4.1-veratrylglycine, 5.3-Aceyl-L-Tyrosine, 6.Highest unknown, 7.Total impurities.

**Table-3: Data of Related substances for Carbidopa with Piperine (40°C/75%RH)**

Physical admixture	Duration	Related substances (% Area)						
		1	2	3	4	5	6	7
Carbidopa + Piperine	0 days	0.282	0.000	0.000	0.009	0.093	0.050	0.473
	4 weeks	0.264	0.000	0.000	0.024	0.060	0.037	0.447
Levodopa + Carbidopa + Entacapone + Piperine	0 days	0.260	0.000	0.000	0.007	0.071	0.036	0.411
	4 weeks	0.255	0.000	0.000	0.017	0.084	0.033	0.440

1. Methyl dopa, 2.Related compound-A, 3.Methyl dopa methyl ester, 4.Cyclohexylidene carbidopa methyl ester, 5.3,4-Dihydroxy phenyl acetone, 6.Highest unknown, 7.Total impurities.

**Table-4: Data of Related substances for Entacapone with Piperine (40°C/75%RH):**

Physical admixture	Duration	Related substances (% Area)				
		1	2	3	4	5
Entacapone + Piperine	0 days	0.000	0.047	0.148	0.236	0.714
	4 weeks	0.000	0.043	0.185	0.238	0.680
Levodopa + Carbidopa + Entacapone + Piperine	0 days	0.000	0.041	0.151	0.236	0.698
	4 weeks	0.000	0.046	0.190	0.239	0.740

1.2-Cyano-N, N-diethyl acetamide, 2.3,4-Dihydroxy 5-nitrobenzaldehyde, 3.Z-Isomer, 4.Highest unknown, 5.Total related substance.

**Table-5: Results of Accelerated Stability studies (40°C/75%RH)**

Months	% Assay			Total impurity (% Area)		
	Levodopa	Carbidopa	Entacapone	Levodopa	Carbidopa	Entacapone
Initial	99.9	99.7	99.6	0.047	0.474	0.695
1	99.4	99.4	99.3	0.049	0.481	0.708
2	99.2	99.2	99.0	0.050	0.486	0.717
3	98.8	98.8	98.7	0.050	0.492	0.729
6	98.5	98.4	98.4	0.051	0.494	0.736

**Table-6: Results of Long term studies 25°C/60%RH**

Months	% Assay			Total impurity (% Area)		
	Levodopa	Carbidopa	Entacapone	Levodopa	Carbidopa	Entacapone
Initial	99.9	99.7	99.6	0.047	0.474	0.695
3	99.7	99.7	99.5	0.047	0.480	0.707
6	99.6	99.5	99.4	0.048	0.484	0.708
9	99.5	99.4	99.2	0.048	0.489	0.716
12	99.3	99.3	99.2	0.051	0.491	0.719

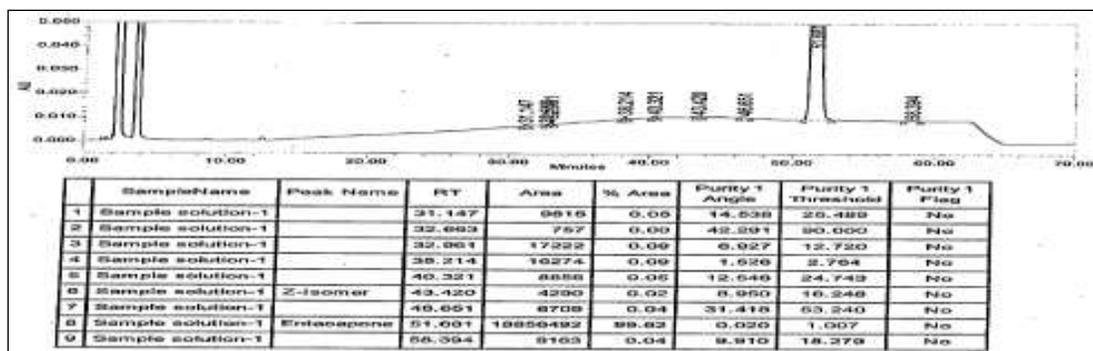
**Figure-1: Chromatogram of Sample solution for Entacapone (Initial)**

Figure-2: Chromatogram of Sample solution for Entacapone (4 weeks)

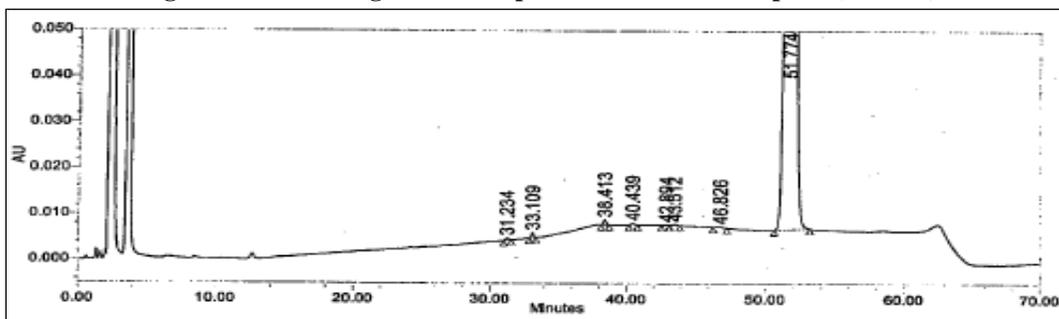


Figure-3: Chromatogram of sample solution for Levodopa and Carbidopa (Initial)

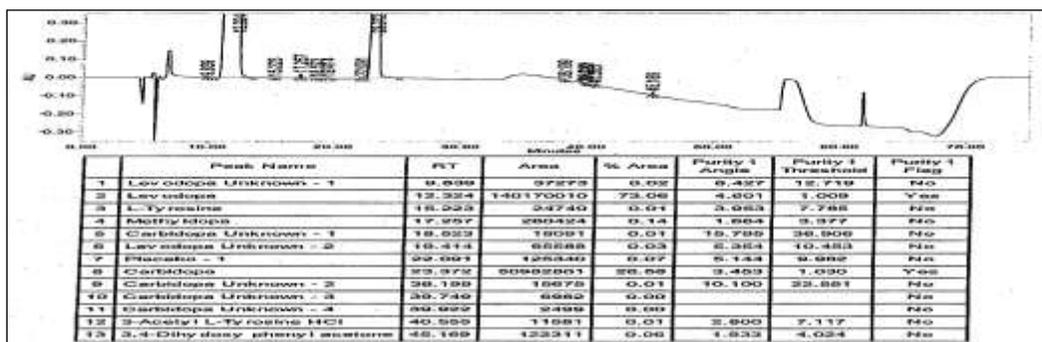
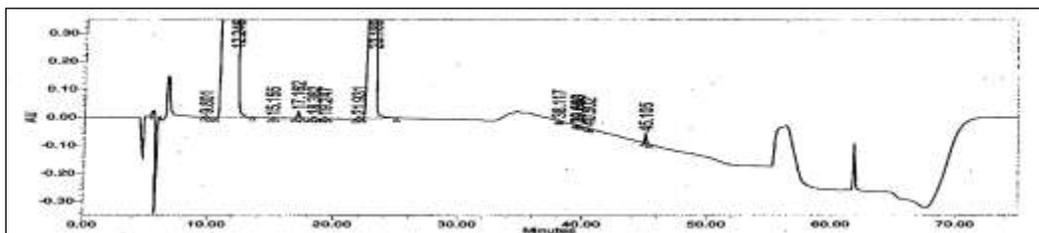


Figure-4: Chromatogram of sample solution for Levodopa and Carbidopa (4 weeks)



**CONCLUSION**

From the above compatibility studies it can be conclude that Piperine can be used as a formulation additive (Bio-enhancer) in oral solid dosage forms of Levodopa, Carbidopa and Entacapone. The addition of Piperine as a

bio-enhancer will result in better oral absorption of Levodopa and reduction its oral dose. The reduction in oral dose will result in minimal adverse effects, reduced cost of the treatment and improved patient compliance.

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