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APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number:	214869
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Submission Type:	Original NDA (505(b)(2))
Associated IND:	135441
Applicant:	Riverside Pharmaceuticals Corporation.
Submission Date:	October 14, 2020
Brand Name:	DHIVY
Generic Name	Carbidopa / Levodopa
Dosage Form:	IR Tablet
Dosage Strength:	25 mg/100 mg
Proposed Indication:	Parkinson's disease
Proposed Dose:	Each DHIVY IR tablet contains carbidopa 25 mg and levodopa 100 mg. The initial dose of DHIVY is one tablet, three times a day, that provides 75 mg of carbidopa and 300 mg of levodopa. The optimum daily dose of DHIVY must be determined by careful titration for each patient. The tablet is functionally scored to facilitate precise fractionated dosing. DHIVY tablet has a total of four segments, and each segment contains carbidopa/levodopa 6.25/25 mg. The maximum dosage is eight tablets of DHIVY per day, i.e., 200/800 mg.
OCP Division:	DNP
Primary Reviewer:	Srinivas R. Chennamaneni, Ph.D.
Secondary Reviewer:	Bilal S. AbuAsal, Ph.D.
Team Leader:	Bilal S. AbuAsal, Ph.D.

Contents

1. Executive Summary

Riverside Pharmaceuticals Corporation submitted a New Drug Application (NDA214869) under Section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act for a 25 mg/100 mg carbidopa/levodopa immediate release (IR) oral tablet (DHIVY). This submission relies on the findings of safety and efficacy of SINEMET® (NDA 017555, carbidopa/levodopa), IR tablet approved on 5/2/1975, as the listed drug[1]. SINEMET is a combination of carbidopa and levodopa for the treatment of Parkinson's disease and syndrome. SINEMET tablets are available in a 1:4 ratio of carbidopa to levodopa (SINEMET 25-100) as well as 1:10 ratio (SINEMET 25-250 and SINEMET 10-100). SINEMET tablet is approved for an initial dose of SINEMET 25-100, one tablet, three times a day with a maximum daily dose of eight tablets of SINEMET 25-100 in a 24 hour period. The optimum daily dosage of SINEMET must be determined by careful titration for each patient. Patients should be monitored closely during the dose adjustment period.

DHIVY is a redesign tablet of carbidopa and levodopa 25/100 mg. DHIVY IR tablet employed a (b) (4) scored design. (b) (4) (b) (4), and the (b) (4) scoring design allows splitting the tablet into fourths (25/100 mg, 18.75/75 mg, 12.5 mg/50 mg, or 6.25 mg/25 mg) enabling fractionated dosing and gradual upward dose titration.

The application is supported by results from a pivotal bioequivalence study (190051), a three-way crossover study conducted in healthy subjects with DHIVY (carbidopa/levodopa 25/100 mg), and the carbidopa/levodopa 25/100 mg generic IR tablet (ANDA 074260, Actavis Elizabeth LLC) listed in the FDA's Orange Book. The sponsor used generic carbidopa/Levodopa IR tablet, due to the global shortage of SINEMET. Hereafter, carbidopa, and levodopa will be referred to as CD and LD or CD/LD, respectively in this review. The agency accepted the use of generic CD/LD IR oral tablet, approved and listed in FDA's Orange Book as therapeutically equivalent to SINEMET IR tablets. The sponsor used ANDA 074260 as a reference drug[2]. The study 190051 demonstrated bioequivalence between DHIVY (carbidopa/levodopa 25/100 mg) and the generic carbidopa/levodopa 25/100 mg IR tablet (ANDA 074260) based on C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

A consult request for clinical and bioanalytical site inspections for Study 190051 was sent to the Office of Study Integrity and Surveillance (OSIS) on December 4, 2020. The inspection request was declined by OSIS based on the recent inspectional history, and the final classification was No Action Indicated (NAI) for clinical and bioanalytical sites indicating the data can be accepted without an inspection. See OSIS's "Decline to conduct an on-site inspection" memo (12/16/2020) for details[3].

The focus of this review was to confirm the bioequivalence of DHIVY to SINEMET and evaluate the effect of food and dose proportionality of split tablets.

[1] SINEMET® (carbidopa and levodopa) USPI: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/017555s072lbl.pdf

[2] https://darrts.fda.gov/darrts/ViewDocument?documentId=090140af8050bfc5

[3] https://darrts.fda.gov/darrts/ViewDocument?documentId=090140af805bb4c1

1.1 Recommendations

The Office of Clinical Pharmacology (OCP) has reviewed the information submitted in the NDA 2 1 4 8 6 9 and recommends approval of DHIVY IR tablet for the treatment of Parkinson's disease. This recommendation is based on the bioequivalence demonstrated for DHIVY to the generic CD/LD 25/100 mg IR tablet (ANDA 074260), listed in the FDA's Orange Book.

2. Background and Regulatory History

Levodopa is a naturally occurring amino acid and is the immediate precursor of the neurotransmitter dopamine. Carbidopa inhibits peripheral metabolism of levodopa and thereby formation of dopamine in the periphery. The combination of carbidopa/levodopa approved for the treatment of Parkinson's disease in adults in 1975. It is metabolized primarily by aromatic-l-amino-acid decarboxylase and excreted in the urine as its major metabolite, indole acetic acid (IAA). The elimination half-life of LD in the presence of CD is approximately 1.5 hours and it is excreted primarily in the urine as dopamine and smaller percentage as catecholamines.

Prior to the submission of this NDA on December 11, 2020, the investigational drug was studied under IND 135441. The agency agreed that a food effect study coupled with a bioequivalence study in healthy volunteers would be adequate to bridge with the listed drug to support an NDA (See IND 135441 Meeting Minutes dated 7/17/2017). In the Pre-NDA meeting the applicant was advised to evaluate the impact of food effect on the safety and efficacy of DHIVY in the NDA submission (See IND 135441 Meeting Minutes dated 6/27/2020).

The original plan was to use SINEMET IR tablets as the reference drug. However, the Sponsor was unable to obtain SINEMET IR tablets to conduct the bridging study and since SINEMET was on backorder, the FDA accepted the Sponsor's proposal to use a generic CD/LD IR oral tablet approved and listed in FDA's Orange Book as therapeutically equivalent to SINEMET IR tablets. The sponsor used ANDA 074260 as a reference drug in the pivotal BE study 190051.

3. Summary of Pivotal Bioequivalence Study

Study Title: A Phase 1 Study to Evaluate the Bioequivalence Between DHIVY (Carbidopa/Levodopa 25/100 mg Tablet) and Carbidopa/Levodopa 25/100 mg Tablet (FDA Approved Generic Reference) and to Evaluate the Food-Effect of DHIVY in Normal Healthy Volunteers.

Methodology: Study 190051 was an open label study conducted at a single center (Syneos Health Clinique, Québec, Canada) conducted in 3 sequential parts.

- Part I: BE and food-effect, randomized, open-label, single-dose, 3-period, crossover design.
- Part II: multiple-dose (every 4 hours), open-label, 1-period design.
- Part III: multiple-dose (every 2 hours), open-label, 1-period design.

The primary objectives of this study were to evaluate the bioequivalence (BE) between DHIVY tablet (CD/LD 25/100 mg IR tablet) and the reference tablet and to evaluate the food effect of DHIVY in normal healthy volunteers (Part I). The secondary objective of this study was to compare the PK profile of a fraction of the DHIVY tablet when administered at frequent intervals every 2 hours to the PK profile of a whole tablet administered every 4 hours (Parts II and III).

Blood Sampling for PK: Blood samples were collected for Part I: pre-dose and 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 hours post-dose. For Parts II and III: pre-dose and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, and 12 hours post-first dose. There was a washout period of at least 48 hours between last drug administration of Part I and first dosing of Part II. In Part II, 22 subjects received a whole (b) (4) tablet at 0 hours and at 4 hours post first dose (Treatment D). Thus, subjects received a total dose of CD/LD 50/200 mg over the 8-hour duration of Part II. There was a washout period of at least 40 hours between last drug administration of Part II and first dosing of Part III.

Number of Subjects Enrolled and Randomized:
N=48 healthy adults enrolled and randomized in Part I, and forty-five (45) subjects completed all treatment periods. A total of three (3) subjects (Subject numbers (b) (6), (b) (6), and (b) (6)) vomited after study drug administration during Part I and were discontinued for PK reason. Twenty-two (N=22) subjects who completed Part I were enrolled to complete Part II and Part III. All enrolled subjects completed Part II and Part III.

Main Criteria for Inclusion:
Healthy adults ≥35 and ≤75 years of age, with BMI > 18.5 and < 30.0 kg/m^2, who did not use tobacco or nicotine products within 3 months prior to screening.

Test and Reference Products:
In Part I, subjects were administered each treatment according to the 3-period, 6-sequnce, and block randomization scheme as listed below.

Treatment A (Test- fasting): 1 x DHIVY tablet (immediate release CD/LD 25/100 mg; Riverside Pharmaceuticals Corporation, USA), administered under fasting conditions.

Treatment B (Reference- fasting): 1 x CD/LD 25/100 mg tablet (immediate release CD/LD 25/100 mg; FDA approved generic reference), administered under fasting conditions.

Treatment C (Test- fed): 1 x DHIVY tablet (immediate release CD/LD 25/100 mg; Riverside Pharmaceuticals Corporation, USA), administered under fed conditions.

In Parts II and III, subjects were administered Treatments D and E, respectively as listed below.

Treatment D (Test- every 4 hours): 1 x DHIVY tablet (immediate release CD/LD 25/100 mg; Riverside Pharmaceuticals Corporation, USA) administered at 0 and 4 hours post-first dose, for a total daily dose of CD/LD 50/200 mg

Treatment E (Test- every 2 hours): ½ x DHIVY tablet (immediate release CD/LD 12.5/50 mg; Riverside Pharmaceuticals Corporation, USA) administered at 0, 2, 4, and 6 hours post-first dose, for a total daily dose of CD/LD 50/200 mg.

Criteria for BE Assessment:
Bioequivalence between the test treatment and the reference treatment was concluded if the 90% confidence interval of the ratio (test/reference) of ln-transformed C_{max}, $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ values are within 80.0 to 125%.

Results:

Part I: Bioequivalence/Food Effect:

Results showed that DHIVY tablet is bioequivalent to the reference drug (ANDA 074260) following a single dose under fasting conditions for both LD and CD. The results of the fasting BE study are summarized in the table below (**Table 1**).

Table 1. Levodopa and Carbidopa Geometric mean ratios, and 90% confidence intervals of C_{max} and AUC, fasted bioequivalence study - Study number 190051

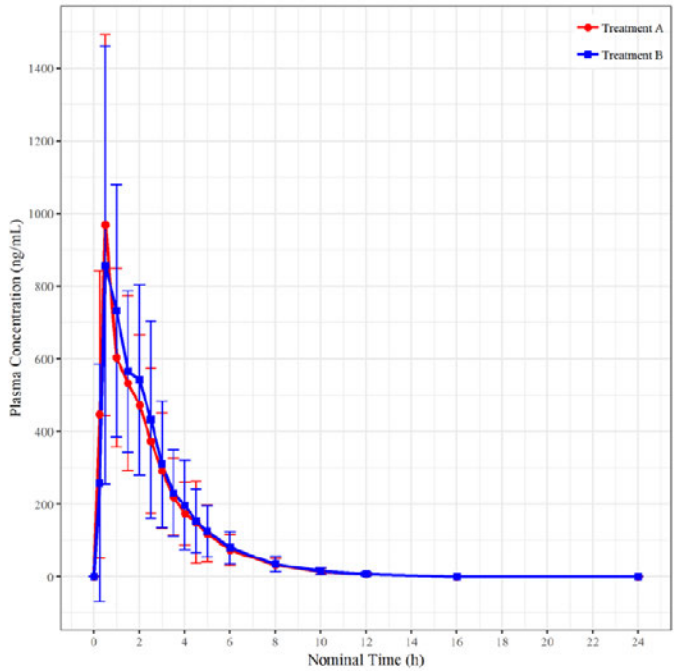
Analyte - Levodopa				
Parameter (units)	**Test**	**Reference**	**Geometric Mean Ratio (%)**	**90% Confidence Interval**
C_{max} (ng/mL)	1062.12	1129.95	94.0	85.02 - 103.92
AUC_{0-t} (ng*hr/mL)	2121.40	2213.45	95.84	93.50 - 98.25
AUC_{0-inf} (ng*hr/mL)	2144.04	2235.15	95.92	93.60 - 98.31
Analyte - Carbidopa				
Parameter (units)	**Test**	**Reference**	**Geometric Mean Ratio (%)**	**90% Confidence Interval**
C_{max} (ng/mL)	125.38	123.80	101.28	95.44 - 107.47
AUC_{0-t} (ng*hr/mL)	612.41	622.61	98.36	93.55 - 103.41
AUC_{0-inf} (ng*hr/mL)	616.82	626.89	98.39	93.62 - 103.41

(Source: Clinical Study Report 190051 pages 64, and 69 In-Text Table 11.4.2.3-2, Link \\CDSESUB1\evsprod\nda214869\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5312-compar-ba-be-stud-rep\190051\study-report\190051-csr-fr.pdf

The results of DHIVY IR tablet compared to the generic carbidopa/levodopa 25/100 mg IR tablet showed the ratio (test/reference) of the geometric means of C_{max}, AUC_{last}, and AUC_{0-inf} for LD to be 94%, 96%, and 96%, and for CD 101%, 98%, and 98%, respectively. The 90% confidence intervals of the LS Mean ratios are within the 80.0 to 125% range. The rate and extent of absorption of DHIVY and the generic CD/LD, 25/100 mg IR tablet were within the acceptable boundaries for bioequivalence.

The pharmacokinetic profiles for LD and CD resulting from a single dose administration of DHIVY IR tablet (Treatment A), and generic carbidopa/levodopa IR tablet (Treatment B) administered under fasted conditions are shown in **Figure 1** and **Figure 2**.

Figure 1. Mean (±SD) Levodopa Plasma Concentration for DHIVY (Treatment A), and Generic Carbidopa/Levodopa (Treatment B) (N = 45) - **Study 190051**



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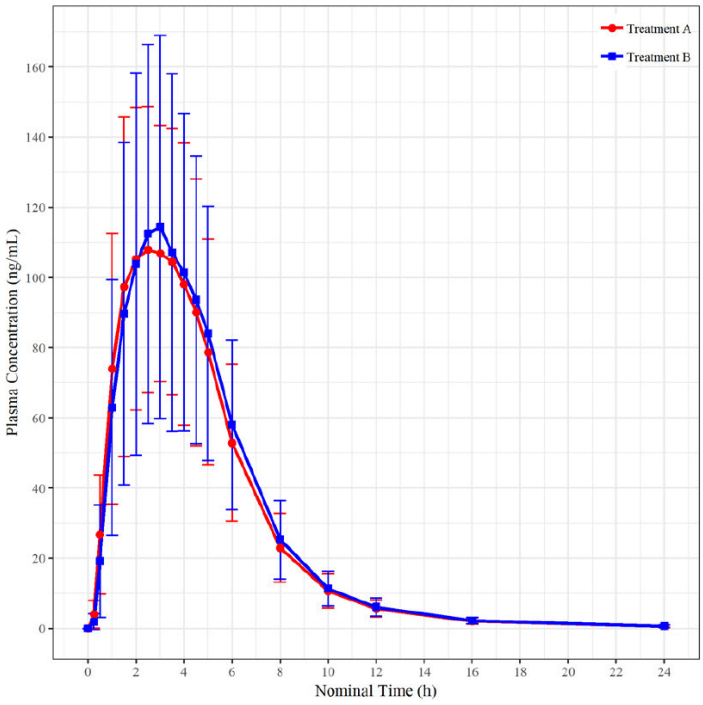
Administration of DHIVY IR tablet achieved a maximum concentration for LD in about 1.0 hrs., and for CD in about 2.84 hrs. Elimination half-life for LD was 1.9 hrs., and for CD approximately 3.45 hrs.

Figure 2. Mean (±SD) carbidopa Plasma Concentration for DHIVY (Treatment A), and Generic Carbidopa/Levodopa (Treatment B) (N = 45) **- Study 190051**



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Food Effect:
Food effect for DHIVY IR tablet was assessed after a high-fat meal as a part of the bioequivalence study. The results from food effect study for LD and CD are presented in the table below (**Table 2**). Administration of DHIVY after a high-fat meal did not affect total exposure (AUC) for LD. However, the peak exposure (Cmax) was decreased by approximately 24% and delayed by 30 minutes when DHIVY was administered with food. For CD, the total exposure and peak exposure decreased significantly (64% and 60%, respectively) and Tmax occurred 1.0 hour earlier in presence of food.
The effect of food on the exposure of CD/LD was not described in the USPI for SINEMET. The review team conducted a cross study comparison using the data from previously approved generic products (ANDAs 074260 and 090324) for SINEMET. In these products, the BE study was conducted under fed and fasted conditions which enabled the evaluation of SINEMET under fed and fasted conditions. Results showed the effect of food on CD/LD exposure in DHIVY was comparable to food effect observed for SINEMET in the BE studies conducted to support the approval of the generic products mentioned above and the SINEMET.
In addition, the decrease in exposure for carbidopa is not expected to have any impact on the efficacy of DHIVY, because levodopa total exposures were not affected by food intake. Carbidopa inhibits decarboxylation of peripheral levodopa and makes more levodopa available for transport to the brain. Further, carbidopa does not cross the blood-brain barrier and does not affect the metabolism of levodopa within the central nervous system. Its decarboxylase inhibiting activity is limited to the extracerebral tissues only.

The pharmacokinetic profiles showing food effect for levodopa (**Figure 3**), and carbidopa (**Figure 4**) resulting from a single dose administration of DHIVY in the fasted state (Treatment A), and the fed state (Treatment C) is shown below.

Table 2: Levodopa and Carbidopa Geometric mean ratios, and 90% confidence intervals of C_{max} and AUC, Food effect Study - Study number 190051

Analyte - Levodopa				
Parameter (units)	**DHIVY- fasting**	**DHIVY- fed**	**Geometric Mean Ratio (%)**	**90% Confidence Interval**
C_{max} (ng/mL)	1070.07	810.7	75.76	66.77 - 85.96
AUC_{0-t} (ng*hr/mL)	2121.43	2222.25	104.75	102.01 - 107.57
AUC_{0-inf} (ng*hr/mL)	2144.03	2243.80	104.65	101.94 - 107.44
Analyte - Carbidopa				
Parameter (units)	DHIVY- **fasting**	DHIVY- **fed**	**Geometric Mean Ratio (%)**	**90% Confidence Interval**
C_{max} (ng/mL)	125.50	50.07	39.98	36.56 - 43.72
AUC_{0-t} (ng*hr/mL)	612.32	221.55	36.18	33.79 - 38.74
AUC_{0-inf} (ng*hr/mL)	616.65	225.73	36.61	34.22 - 39.16

Source: Clinical Study Report 190051 pages 74, and 79 In-Text Table 11.4.2.3-6, Link \\CDSESUB1\evsprod\nda214869\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5312-compar-ba-be-stud-rep\190051\study-report\190051-csr-fr.pdf

Figure 3. Mean (± SD) Levodopa Plasma Concentrations (DHIVY-fasting (treatment A) vs. DHIVY-fed (Treatment C))

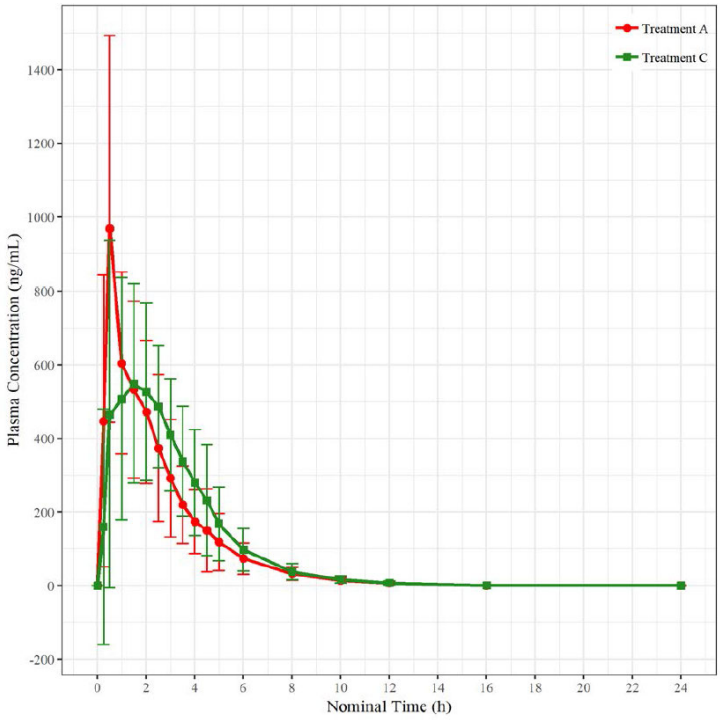

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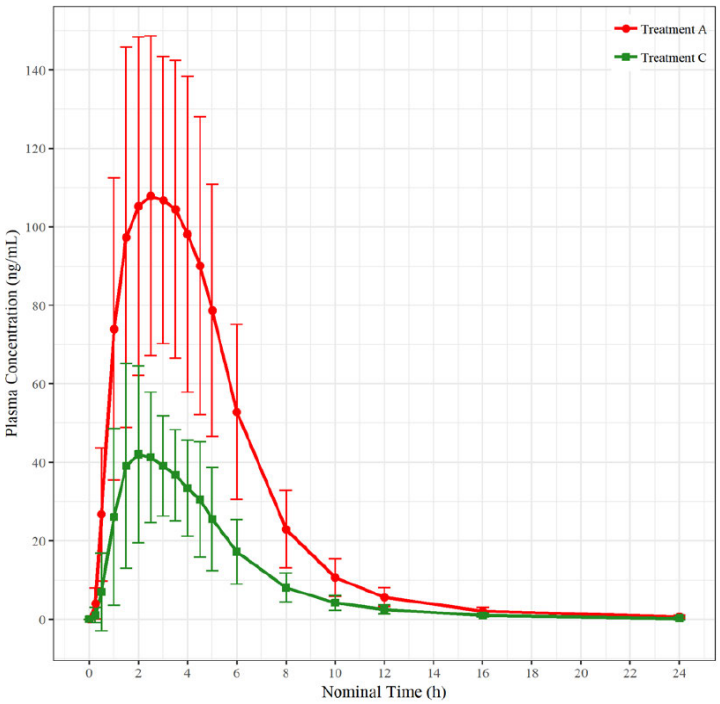
Figure 4. Mean (± SD) Carbidopa Plasma Concentrations (DHIVY-fasting (Treatment A) vs. DHIVY-fed (Treatment C))



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Parts II and III: Effect of Dose Interval on Plasma PK of LD and CD

The objective of Parts II and III was to evaluate the effect of the LD dose-interval on its plasma PK profile in healthy volunteers. The PK characteristics of CD/LD 12.5/50 mg given every 2 hours at 0, 2, 4, and 6 hours post-dose (Part III) were compared to the PK characteristics of CD/LD 25/100 mg given every 4 hours (Part II) over a period of 8.0 hrs., to simulate standard SINEMET dosing.

The first CD/LD dose (time = 0) was administered in the morning after a fasting period of at least 10 hours and followed by a fasting period of at least 5 hours. Food was served approximately 5 hours after the first (0 hrs.) dose.
The results are presented in **Table 3** and **Figure 5** for Levodopa, and **Table 4** and **Figure 6** for Carbidopa, respectively.

The levodopa exposure in terms of AUC_{0-t} was similar after administration of Treatment DHIVY 12.5/50 mg q2hr) (administered at 0, 2, 4, and 6 hours post-first dose) compared to Treatment D (DHIVY 25/100 mg q4hr) (administered at 0 and 4 hours post-first dose). Time to peak concentration after 1 dose was also similar after administration of Treatment E (DHIVY 12.5/50 mg q2hr) compared to Treatment D (DHIVY 25/100 mg q4hr). When 12.5/50 mg every 2 hours dosing was compared to 25/100 mg every 4 hours dosing, peak levodopa exposure (C_{max}) declined by 44%.
The total exposure (AUC) and peak exposure (C_{max}) for carbidopa were 1.2-fold and 1.3-fold higher after administration of 25/100 mg dose every 4 hrs. compared to 12.5/50 mg dose administered every 2.0 hrs. This slight decrease in exposure of carbidopa after the fractionated dosing is partly attributed to the food intake administration after 5 hours of the initial dose. Food intake decreased the exposure of carbidopa with a decrease in bioavailability of the last of 12.5/50 mg dose after six hours. It was also noted that the PK profile was smoother with CD when it was administered after every two hours dosing interval than what would be expected after repeat dose administration. Specifically, there was no peak observed after the administration of the fourth dose of CD. Although it is expected that with the lower dose administered under fed conditions would result in a lower C_{max}, however, the complete disappearance of the peak was not clearly understood. This decrease in exposure is unlikely affects the efficacy and safety of DHIVY as explained above. Further, the dose proportionality for the lower dose of 12.5/50 can be established using in vitro dissolution profile.

DHIVY tablet design facilitates a more gradual and thus better-tolerated upward dose titration in accordance with recommendations in the labeling. The complications of nausea and vomiting, especially after treatment initiation, are thus expected to be reduced. The sponsor conducted dissolution studies with split tablets and whole tablets after long-term and accelerated stability storage. The split tablets ($1/4^{th}$ tablet, 6.25/25 mg CD/LD) and the whole tablet met the dissolution acceptance criteria. Splitting the tablet in to 4 fractions (i.e., 6.25/25 mg) and administration of the split tablet is expected to result in dose proportional exposures (AUC) to that of one whole tablet of 25/100 mg, CD/LD. Please refer to the biopharmaceutics review for more details[4]. The fractionated doses are meant for smaller dose adjustments, if required, at the same dosing frequency as whole tablet. It should be noted that the fractioned doses should not be used as an initial starting dose levels and should be used to facilitate the gradual upward titration only. It should also be noted that the sponsor has not conducted any efficacy and safety studies with fractionated dosing and/or a different dosing regimen. Results from parts II and III of the study can only support the exposure proportionality of split tablets.

[4] https://panorama.fda.gov/internal/document/preview?versionID=60f18abb00f0727e88bfc9a389de81d5&ID=60d62391000996f54c5ae18439295bda

Table 3: Summary of Pharmacokinetic Parameters for Levodopa: (25/100 mg q4hr vs. 12.5/50 mg q2hr)

Parameter (Unit)	DHIVY 25/100 q4hr				DHIVY 12.5/50 q2hr			
	Mean	Median	SD	CV%	Mean	Median	SD	CV%
AUC_{0-t} (h*ng/mL)	4975.61	4776.54	1006.04	20.22	5016.08	4675.32	1156.06	23.05
C_{max} (ng/mL)	1731.62	1635.4	538.94	31.12	1162.18	1179.41	356.27	30.66
T_{max} (h)	4.021	4.498	1.580	39.3	4.174	4.490	1.430	34.25

Source: Clinical Study Report 190051 page 82, In-Text Table 11.4.2.3-9, Link \\CDSESUB1\evsprod\nda214869\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5312-compar-ba-be-stud-rep\190051\study-report\190051-csr-fr.pdf

Figure 5. Mean (± SD) Levodopa Plasma Concentrations (25/100 mg q4hr (Treatment D) vs. 12.5/50 mg q2hr (Treatment E))

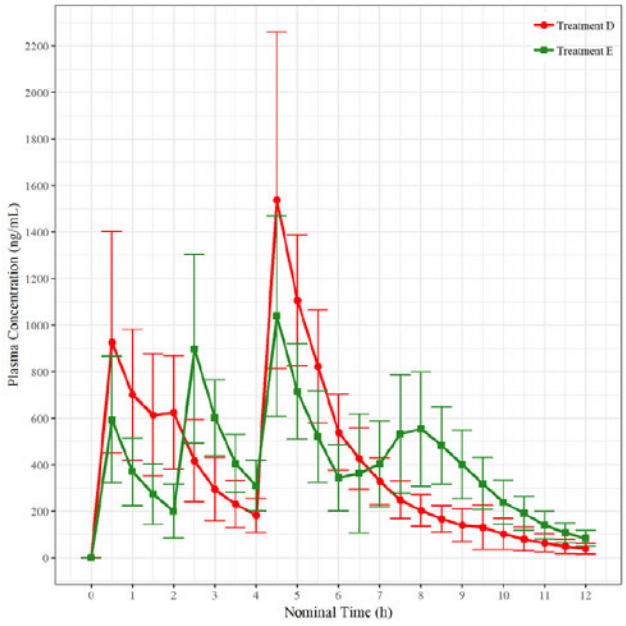

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Table 4: Summary of Pharmacokinetic Parameters for Carbidopa: (25/100 mg q4hr vs. 12.5/50 mg q2hr)

Parameter (Unit)	DHIVY 25/100 q4hr				DHIVY 12.5/50 q2hr			
	Mean	Median	SD	CV%	Mean	Median	SD	CV%
AUC_{0-t} (h*ng/mL)	1087.38	1063.87	342.24	31.47	895.7	813.76	271.93	30.36
C_{max} (ng/mL)	202.39	193.0	55.06	27.21	161.57	153.59	46.83	28.99
T_{max} (h)	5.091	5.489	1.354	26.61	5.408	5.505	0.528	9.77

Source: Clinical Study Report 190051 page 85, In-Text Table 11.4.2.3-10, Link
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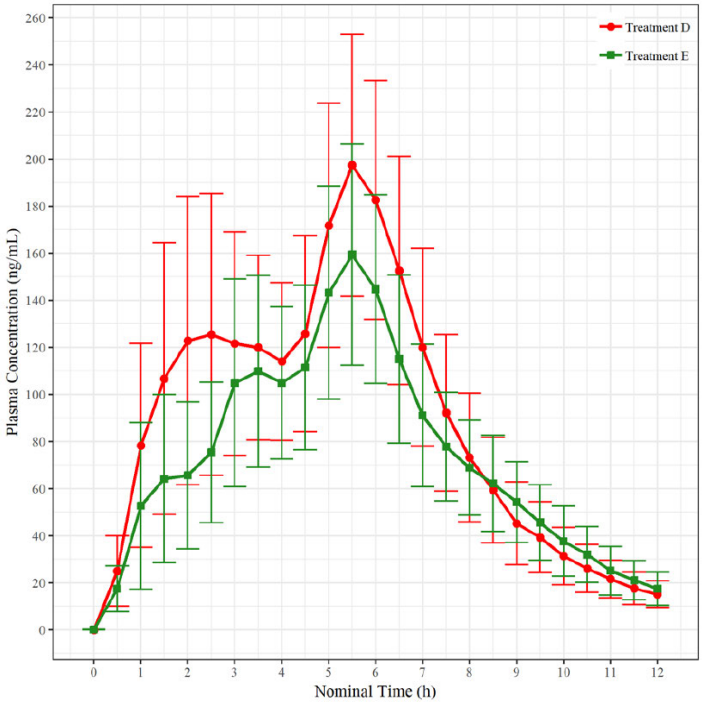
Figure 6. Mean (± SD) Carbidopa Plasma Concentrations (25/100 mg q4hr (Treatment D) vs. 12.5/50 mg q2hr (Treatment E))



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Conclusions:

The study 190051 demonstrated bioequivalence of DHIVY IR tablet with the generic carbidopa/levodopa IR tablet in the fasted state based on C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. DHIVY facilitates a gradual and upward dose titration in accordance with recommendations in the labeling. It should be noted that the dosing and administration of SINEMET applies to DHIVY with no changes to starting dose or max dose. The fractioned dose should not be used as an initial starting dose levels but should be used to facilitate the gradual upward dose titration. All labeling pertaining to intrinsic and extrinsic factors will be same as in the label for the listed drug (SINEMET IR Tablet).

4. Bioanalytical Method Validation

For the quantitation of Levodopa and Carbidopa in human EDTA K2 plasma over the ranges of 5 to 7500 ng/mL for levodopa and 0.5 to 750 ng/mL for carbidopa, the applicant used a validated Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) method TM.2380. The method was developed and validated by (b) (4). The method for the determination of levodopa and carbidopa in human plasma was in compliance with the standards established by the FDA Bioanalytical Method Validation Guidance (2018) and was shown to be precise, accurate, sensitive, and selective over the validated range. The validation parameters are summarized in **Table** 5.

Table 5. Bioanalytical Method Validation Summary

Parameter	Value
Analyte	Levodopa and Carbidopa
Internal standard (IS)	Levodopa-d3 and carbidopa-d5
Limit of quantitation (pg/mL)	5 (Levodopa), 0.5 (Carbidopa)
Calibration curve ranges	5.0 – 7500 ng/mL (Levodopa) 0.5 – 750 ng/mL (Carbidopa)
Average recovery of drugs (%)	91.84 – 88.57 (Levodopa) 98.72 – 88.44 (Carbidopa)
Average recovery of IS (%)	89.79 (Levodopa), 92.37 (Carbidopa)
Standard curve concentrations (ng/mL)	5, 10, 100, 375, 750, 1500, 3000, 3750 (Levodopa) 0.5, 1.0, 10, 37.5, 75, 150, 300, 375 (Carbidopa)
QC concentrations (ng/mL)	LLQC: 5, QC1: 15, QC2: 1875, QC3: 2812.5 (Levodopa) LLQC: 0.5, QC1: 1.5, QC2: 187.5, QC3: 281.25 (Carbidopa)
QC Intraday precision range (%)	0.57 - -8.90 (Levodopa); -1.0 - 3.67 (Carbidopa)
QC Intraday accuracy range (%)	2.41 - 6.13 (Levodopa), 1.08 - 4.38 (Carbidopa)
QC Inter-day precision range (%)	-0.21 - 5.0 (Levodopa), -0.06 - -0.11 (Carbidopa)
QC Inter-day accuracy range (%)	0.65 - 5.09 (Levodopa), 1.7 - 8.17 (Carbidopa)
Matrix Selectivity including hyperlipemic and hemolyzed	No significant interference for both analytes and corresponding internal standards (n=8)
Bench-top stability (hrs.) (equivalent to short- term stability of analyte in matrix)	24h at room temperature
Reinjection reproducibility	67h 37min at 4ºC (Levodopa) 73h45min at 4ºC (Carbidopa)
Stock stability (days) (equivalent to long-term stability of analyte or internal standard in solution)	71d at -80ºC (Carbidopa and Levodopa)
Processed stability (hrs.) (equivalent to post-preparative stability)	95h 23min at 4ºC (Carbidopa and Levodopa)
Freeze-thaw stability (cycles)	4 cycles at -20ºC and 5 cycles at -80ºC
Long-term storage stability (days) (equivalent to long-term stability of analyte in matrix)	7 and 64d at -20°C and 7, 64 and 104d at -80ºC (Levodopa) 7d at -20ºC; 7, 64 and 102d at -80ºC (Carbidopa)

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/s/

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