Office of Clinical Pharmacology Review

NDA Number	219141
Link to EDR	\\CDSESUB1\evsprod\NDA219141\0001
Submission Date	28 Mar 2024
Submission Type	505(b)(2)
Brand Name	Inzirqo
Generic Name	Hydrochlorothiazide
Dosage Form and Strength	Powder for oral suspension (800 mg to be reconstituted with 66 mL water to form 80 mL suspension containing (b) (4) mg hydrochlorothiazide (b) (4)
Route of Administration	Oral
Proposed Indication	Treatment of hypertension and edema
Applicant	Novitium Pharma LLC
Associated IND	IND (b) (4)
OCP Review Team	Harisudhan Thanukrishnan, Ph.D. Doanh Tran, Ph.D.

Table of Contents

1. EXECUTIVE SUMMARY

In this New Drug Application (NDA), the Applicant is seeking approval for Inzirqo (hydrochlorothiazide Powder for Oral Suspension) via the 505 (b) (2) regulatory pathway relying on the FDA's previous finding of safety and effectiveness for Hydrodiuril (hydrochlorothiazide tablets, NDA 011835) as the reference listed drug (LD). Hydrodiuril is approved for the treatment of edema, as an adjunctive therapy in edema and for management of hypertension as the sole agent or to enhance effectiveness of other antihypertensives in the more severe forms of hypertension. The Applicant seeks approval of Inzirqo for the same indications and dosage regimen as the LD.

The Applicant's product is a different dosage form (powder for oral suspension-PFOS) compared to the LD (oral tablet). The Applicant claims that the PFOS dosage form can be preferred by patients with difficulty in swallowing (such as pediatric or geriatric populations) and will deliver an equivalent dose of hydrochlorothiazide and hence proposes to use the same dosing regimen as in the prescribing information for the LD.

To establish a scientific bridge between the proposed PFOS and the LD, the Applicant conducted the relative bioavailability study HYDR-21-047, which was a single dose cross-over study to compare the relative oral bioavailability (BA) and the effect of food on both the PFOS and the reference hydrochlorothiazide tablets. As Hydrodiuril was discontinued and no longer available, the Applicant used the 50 mg hydrochlorothiazide tablets of Teva Pharmaceuticals (ANDA 83177, designated reference standard (RS)) for this study. In addition to this main study, the Applicant also conducted a pilot relative bioavailability study HYDR-20-115 for a preliminary assessment of the relative BA between the PFOS and the RS.

The results of study HYDR-21-047 showed that following administration of 100 mg under fasted condition, the AUCs for both products were similar, as the geometric mean ratios (GMR) and 90% confidence intervals for AUC_{last} and AUC_{inf} were 1.03 (0.99-1.07) and 1.02 (0.98-1.06), respectively. The PFOS showed a higher C_{max} than the RS tablets and the GMR for C_{max} after administration in fasted state was 1.19 (1.10-1.28). This review focused (a) on the adequacy of the scientific bridging between the proposed PFOS product to the LD at the tested maximum dose of 100 mg under fasted condition and (b) on the dosing recommendation for the PFOS product based on the observed food effect.

A consult was sent to the Office of Study Integrity and Surveillance (OSIS) requesting biopharmaceutical inspections of the clinical and analytical sites for the pivotal relative bioavailability study HYDR-21-047. The OSIS determined that inspections were not needed for the requested sites based on previous inspection results available for the same sites and recommended that the study results be considered acceptable for review.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the information submitted to NDA 219141 and determined that a pharmacokinetic (PK) bridge has been established to support the approval of Inzirqo powder for oral suspension.

1.2 Summary of Labeling Recommendations

The content and format of labelling for hydrochlorothiazide was revised by the Applicant to conform with the PLR (Physician Labeling Rule) format. The summary of labeling recommendations provided by Office of Clinical Pharmacology review team is provided below:

Section 2.5- Important Administration Instructions

- INZIRQO may be administered with or without food

Section 7- Drug Interactions (Subsection 7.1)

- Include instruction to stagger the dose of INZIRQO by at least 4 hours before or 4 to 6 hours after the administration of anionic exchange resins.
- Remove text on (b) (4) from this section such as (b) (4) (b) (4) as recommended by the clinical safety review team.

Section 12.3- Pharmacokinetics

- Include the AUC and C_{max} of hydrochlorothiazide following the dosing of INZIRQO
- Include that T_{max} was delayed by 2 hours under "Effect of Food"

2. CLINICAL PHARMACOLOGY REVIEW

2.1 Overview of Clinical Studies

The NDA included three comparative bioavailability (BA) studies that were sequentially conducted in healthy adult volunteers. While the first two studies were pilot evaluations, the third study provided the final bioequivalence assessment between the proposed product and the reference formulation.

The first pilot BA study (HYDR-20-114) was a single-dose, open-label, randomized, balanced, three-period, three-sequence, three-treatment crossover study with two prototype formulations (T1 and T2) of hydrochlorothiazide powder for oral suspension (PFOS), both of which were not developed further. This study was conducted in 18 healthy adult male subjects with all treatments administered as a 50 mg dose under fasting conditions: (a) Test hydrochlorothiazide PFOS 50 mg/5 mL formulation T1 (b) Test hydrochlorothiazide PFOS 50 mg/5 mL formulation T2 and (c) Reference product hydrochlorothiazide tablets from Teva Pharmaceuticals. The information from Study HYDR-20-114 was included as supportive for safety data and is not reviewed further.

The second BA study (HYD-20-115) was conducted after reformulation of the hydrochlorothiazide test formulation T1 that was used in Study HYDR-20-114. T1 was reformulated into the putative final formulation by increasing the concentration of suspending agents to improve the viscosity and stability over time. This study was a pilot, single-dose, open-label, randomized, balanced, two-period, two-sequence, two-treatment crossover study in 13 healthy adult male subjects with the following treatments administered as a 50 mg dose under fasting conditions: (a) Test putative final formulation of hydrochlorothiazide PFOS 50 mg/5 mL (b) Reference product hydrochlorothiazide tablets from Teva Pharmaceuticals.

The third BA study (HYDR-21-047) was the pivotal, single-dose, open-label, randomized, balanced, five-period, five-sequence, two-treatment crossover BA study with the final formulation in 30 healthy adult male and female subjects. The test (hydrochlorothiazide PFOS) and reference (hydrochlorothiazide tablets from Teva Pharmaceuticals) products were both dosed at 100 mg in fasted and fed conditions in addition to the test product dosed at 50 mg in fasted condition. This study provided the comparative BA under fasted condition and evaluated food effect for both the test and reference products.

2.2 Clinical Pharmacology review questions

2.3.1 Is a bridge established between the proposed product (oral suspension) and listed drug product (tablets) to support the proposed dosing regimen?

Yes, to support bridging, the PK results for the powder for oral suspension (PFOS) was compared with the reference hydrochlorothiazide (HCT) tablets in both the pivotal (HYDR-21-047) and the pilot (HYD-20-115) comparative bioavailability (BA) studies. In addition, the pivotal HYDR-21-047 study also showed that the pharmacokinetics of HCT was dose proportional following 50 and

100 mg doses of the PFOS product. The comparative BA evaluations were done at the highest proposed dose of 100 mg in both the fasted and fed conditions.

Following a dose of 100 mg in fasted condition, the 90% confidence intervals and the geometric mean ratio of PFOS product to reference HCT tablets were within the pre-specified bioequivalence limits of 80% to 125% only for the ln-transformed AUC_{0-t} and $AUC_{0-\infty}$, but not for the C_{max}. The C_{max} was ~18% higher for the PFOS product and the upper bound of 90% CI exceeded the 125% limit, as shown in Table 1. A similar finding of bioequivalent AUCs and a higher C_{max} was also observed in the pilot study (~ 19% higher for PFOS; 90% CI 98% to 146%), although the pilot study was not powered for the statistical evaluation of the primary PK endpoints.

Table 1 Summary of statistical assessments of natural log-transformed PK parameters of hydrochlorothiazide compared between test (powder for oral suspension) and reference (tablets) products under fasted condition (N=29)

Parameter	Ratio (%) (Test/Reference)	Ratio % (90% CI)
AUC_{inf}	101.8	97.6-106.1
AUC_{last}	102.7	98.5-107.0
C_{max}	118.5	110.0-127.6

Source: Reviewer analysis using data of Study HYDR-21-047. One subject missed reference treatment period; AUC_{inf} = area under the curve from time 0 extrapolated to infinity; AUC_{last} = area under the plasma concentration versus time curve from time 0 (pre-dose) to time of last measurable non-zero plasma concentration; CI = confidence interval; C_{max} = maximum observed plasma concentration; Ratio = geometric least squares mean ratio

The median T_{max} for the PFOS was decreased by ~1 h in comparison to tablets (1.2 h vs to 2.3 h for the HCT tablets) after dosing in the fasted state.

As per the current approved product label for HCT tablets, dosing can be done without regard to timing of food intake. In addition to evaluation of food effect for the PFOS product, the current study also compared the bioavailability between the PFOS and HCT reference tablets in the fed condition. Following a dose of 100 mg in fed condition, the 90% confidence intervals and the geometric mean ratio of PFOS product to reference HCT tablets were within the pre-specified bioequivalence limits of 80% to 125% for the ln-transformed AUC_{0-t} and $AUC_{0-\infty}$, but not for the C_{max}. The C_{max} for the PFOS product was ~16% lower and the lower margin for 90% CI was slightly below the 80% limit after dosing under fed condition, as shown in Table 2.

Table 2 Summary of statistical assessments of natural log-transformed PK parameters of hydrochlorothiazide compared between test (powder for oral suspension) and reference (tablets) products under fed condition (N=28)

Parameter	Ratio (%) (Test/Reference)	Ratio % (90% CI)

AUC_{inf}	96.1	92.1-100.2
AUC_{last}	96.5	92.6-100.6
C_{max}	84.0	77.9-90.5

Source: Reviewer analysis using data of Study HYDR-21-047. One subject each missed either treatment period; AUC_{inf} = area under the curve from time 0 extrapolated to infinity; AUC_{last} = area under the plasma concentration versus time curve from time 0 (pre-dose) to time of last measurable non-zero plasma concentration; CI = confidence interval; C_{max} = maximum observed plasma concentration; Ratio = geometric least squares mean ratio

The median T_{max} for both formulations were comparable following dosing in the fed state (3.3 h for PFOS vs 3.7 h for HCT tablets).

Overall, the results for comparative BA indicated that the rate of absorption (C_{max} and T_{max}) for the PFOS was slightly higher and was also affected (decreased and delayed) by food in comparison to the approved HCT tablets. However, the total exposure (AUC) and elimination half-life (~10 h) did not differ between both formulations. As discussed below, the review team agrees with the Applicant's rationale that the approximately 19% higher C_{max} or the 16% lower C_{max} for PFOS in the fasted and fed state, respectively, are not expected to impact the clinical safety or efficacy of hydrochlorothiazide for treatment of hypertension or edema. The duration of diuretic effects following a single dose of hydrochlorothiazide approximately lasts for 12 hours which is beyond the observed T_{max} (or C_{max}) and suggests that under similar drug exposure (AUC), the effect of slightly higher C_{max} if any, is expected to be short lived. In general, considering the duration of diuretic effect for at least 12 hours post-dosing for HCT tablets, the observed C_{max} is ~480% higher than the plasma concentration of hydrochlorothiazide at 12 hours (C_{12}) and the slightly higher C_{max} (by ~19%) with a similar total exposure (AUC) is not expected to impact the clinical efficacy. On the other hand, during the 12 hours of diuretic effect for HCT tablets, the plasma hydrochlorothiazide concentration (C_{12}) is decreased to approximately ~80% lower than the C_{max}. Thereby, the ~16% lower C_{max} for PFOS under the fed state with the lower bound slightly falling below the 80 % and still showing a similar total exposure (AUC) is not expected to diminish the diuretic effects.

Clinical reviewer's assessment by Dr. Evan Fisher: As noted in the clinical pharmacology reviewer's assessment, although there were differences in C_{max} between the PFOS and HCT tablets, the total exposures (AUCs) were equivalent under both fasted and fed conditions. There were no clinically significant differences observed between the PFOS and HCT tablets in supine or standing systolic or diastolic blood pressures, adverse events, serum electrolytes and 24-hour continuous urinary electrolyte excretion. Thus, from a clinical perspective, we do not have safety concerns regarding the slightly higher C_{max} that was observed after dosing in the fasted state with the PFOS compared to the HCT tablets.

2.3.2 Is an alternate dosing recommendation required for the administration of the proposed product in relation to food intake?

No. The Applicant evaluated the bioavailability of hydrochlorothiazide at the highest dose of 100 mg in both the fasted and fed conditions (high-fat high calorie breakfast) for both the proposed (PFOS) and the reference HCT tablets. Results are summarized in Table 3 and details of study design are described under individual study review in Appendix.

Table 3 Summary of statistical assessments of natural log-transformed PK parameters of hydrochlorothiazide 100 mg under fed or fasted conditions for the test (powder for oral suspension; N=29) and reference (tablets; N=28) products

Treatment	Parameter	Ratio (%) (Fed/Fasted)	Ratio % (90% CI)
Test (PFOS)	AUC_{inf}	94.3	90.4-98.3
	AUC_{last}	93.8	90.0-97.7
	C_{max}	62.7	58.2-67.5
Reference (tablets)	AUC_{inf}	99.9	95.7-104.1
	AUC_{last}	99.8	95.7-104.0
	C_{max}	88.5	82.1-95.4

Source: Reviewer analysis using data of Study HYDR-21-047. AUC_{inf} = area under the curve from time 0 extrapolated to infinity; AUC_{last} = area under the plasma concentration versus time curve from time 0 (pre-dose) to time of last measurable non-zero plasma concentration; CI = confidence interval; C_{max} = maximum observed plasma concentration; Ratio = geometric least squares mean ratio

The current label for HCT tablets has no specific language regarding food effect. In the study HYDR-21-047, there was no food effect observed for the reference HCT tablets. The PK exposure of hydrochlorothiazide (both C_{max} and AUC) showed bioequivalence between fasted vs fed condition for the HCT tablets and food caused a slight delay in T_{max} by ~1 h (2.3 h to 3.7 h). In contrast, food effect was observed for the PFOS and under the fed condition, C_{max} was reduced by ~37% (90% CI was 58% to 68%) and T_{max} was delayed by ~2 h (1.2 h to 3.3 h). However, the AUCs were bioequivalent for the PFOS under fed and fasting conditions.

When compared with the reference HCT tablets under fed condition, the PFOS product showed equivalent AUCs with a slightly lower (~16%) C_{max}, as shown in Table 2. The T_{max} was also similar between HCT tablets (3.7 h) and PFOS (3.3 h) under fed condition. As discussed in section 2.3.1 above, the data suggests that this magnitude of food effect is not expected to have a clinically meaningful impact on clinical efficacy/safety of the proposed hydrochlorothiazide PFOS. The review team recommends that Inzirqo (hydrochlorothiazide PFOS) can be administered with or without food.

3. APPENDIX

3.1 Clinical Study #1 HYDR-21-047

A Single-Dose, Open-Label, Randomized, Two-Treatment, Five-Sequence, Five-Period Crossover Study in Healthy Adult Subjects to Assess the Relative Oral Bioavailability and Effect of Food on Hydrochlorothiazide Powder for Oral Suspension 50 mg/5 mL and 100 mg/10 mL with a Hydrochlorothiazide 100 mg Reference Standard Dose (50 mg x 2 Tablets) Under Fasted and Fed Conditions.

3.1.1 Study summary:

Study objective(s):

To assess the relative oral bioavailability of Inzirqo powder for oral suspension (manufactured by Novitium) with the reference product (Teva Pharmaceutical's hydrochlorothiazide tablets 100 mg (2 x 50 mg; ANDA083177) under fasted and fed conditions and to assess the dose proportional bioavailability of Inzirqo (50 mg and 100 mg) under fasted condition.

Study population: The study was conducted in 30 healthy subjects (17 male and 13 female), 18 to 45 years age, with a BMI of 18.5 kg/m^2 to 30.0 kg/m^2.

Study design: The study was a foreign clinical trial conducted under IND (b) (4) at Scitus Pharma Services Private Limited, Chennai, India. The Test Product (Treatment A, C and E) comprised of Inzirqo (hydrochlorothiazide powder for oral suspension (PFOS) 50 mg/5 mL and 100 mg/10 mL) while the Reference Product (Treatment B and D) was Hydrochlorothiazide (HCT tablets) USP 50 mg tablets. The PFOS was reconstituted in purified water and loaded into syringes for oral dosing following which syringes were first flushed with 60 mL of water and then with 180 mL of water to ensure complete dosing. HCT tablets (2 tablets x 50 mg) were administered with ~ 240 mL of water at ambient temperature.

- Relative BA fasted: Treatment A (Test Fasted) vs Treatment B (Reference Fasted)
- Relative BA fed: Treatment C (Test Fed) vs Treatment D (Reference Fed)
- Food effect Test: Treatment C (Test Fed) vs Treatment A (Test Fasted)
- Food effect Ref: Treatment D (Reference Fed) vs Treatment B (Reference Fasted)
- Proportionality: Treatment A (Test Fasted 100 mg/10 mL) vs Treatment E (Test Fasted 50 mg/5 mL)

For the fasted study periods, dosing followed an overnight fast of ≥ 10 hours while during the fed study periods, following an overnight fast of ≥ 10 hours, a high fat, high calorie breakfast was served 30 minutes prior to dosing. Standardized meals, including lunch, snacks and dinner were provided at 4-, 8- and 12-hours post-dose in each period.

A total of 24 blood samples for PK (~3 mL each) were obtained in each period at 0 h (pre-dose) and at 15, 30, 45, 60, 80, 100, 120, 140, 160, 180, 200 and 220 minutes and 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36 and 48 hours after study drug administration. Plasma was separated and stored at –70°C ± 15°C until quantification using a validated LC-MS/MS method. The assay had a lower limit of

quantification of 5 ng/mL of hydrochlorothiazide and concentrations below that were set to zero before PK and statistical data analysis.

Pharmacokinetic (PK) and statistical data analysis:
The Ln-transformed primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were analyzed for bioequivalence using an analysis of variance (ANOVA) model with the main effect of treatment, period, and sequence as fixed effects and subject nested within sequence as a random effect. The power of the ANOVA test to detect a 20% mean difference between treatments was calculated. Two one-sided tests were used to assess bioequivalence for each parameter.

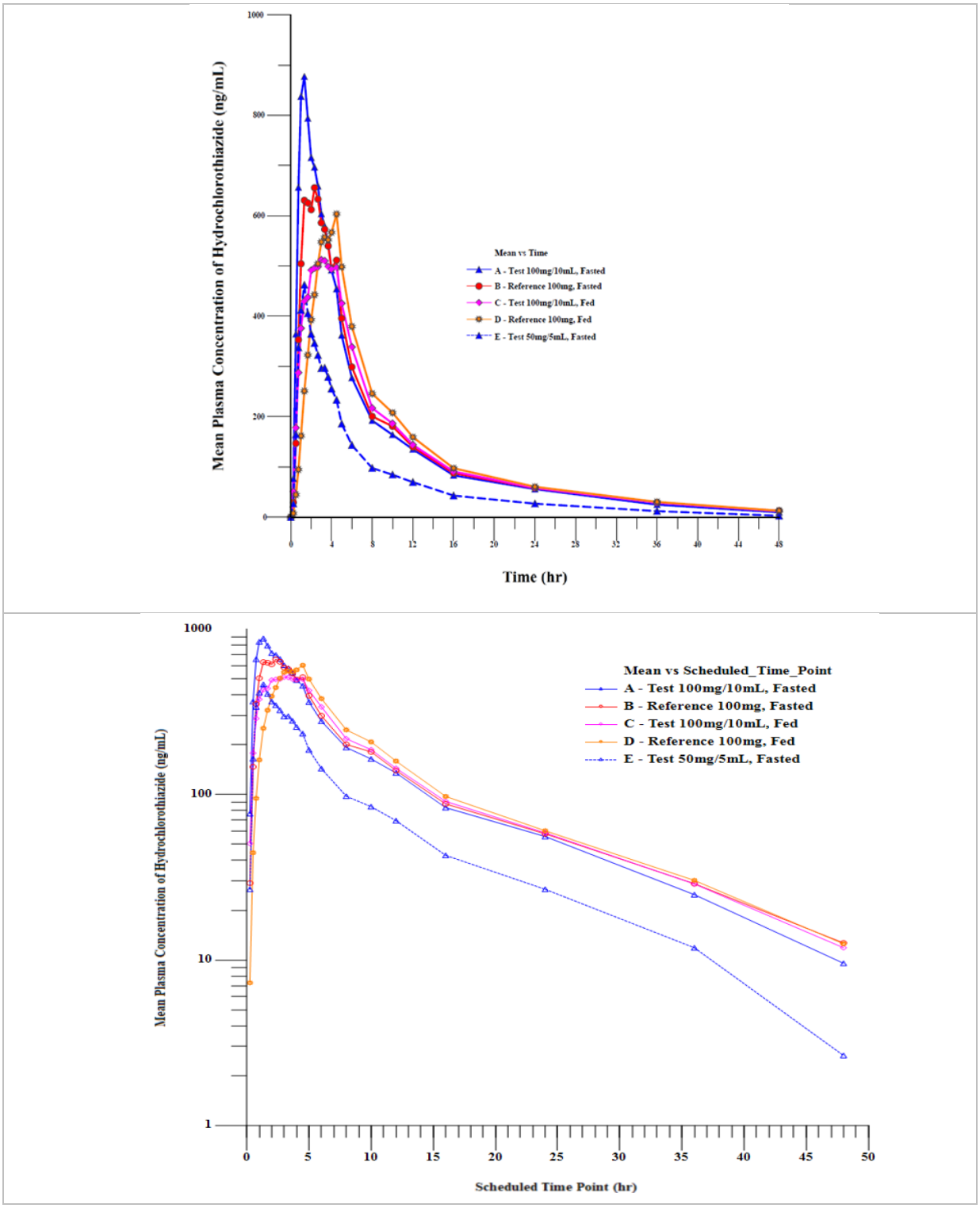
3.1.2 Study results:

A total of 30 subjects were dosed in Period 1, Period 2, Period 3 and Period 5 and 27 subjects were dosed in Period 4 (3 subjects did not report for check-in). The mean PK profiles and descriptive statistics for plasma PK parameters for hydrochlorothiazide are shown in Figure 1 and Table 4.

The following comparisons were assessed for the ln-transformed primary PK parameters:

PFOS vs tablets (fasted), PFOS vs tablets (fed), PFOS (fasted vs fed), tablets (fasted vs fed).
For all the above comparisons with exception of tablets (fasted vs fed), the geometric mean ratio and/or the 90% CI were outside of the 80% to 125% limits for only the C_{max} while the AUC_{0-t} and $AUC_{0-\infty}$ were within the 80% to 125% limits.

Figure 1 Mean plasma concentrations of hydrochlorothiazide vs time plots overlaid by treatments in study HYDR-21-047 (linear and semi-log scale)



Source: Clinical Study Report for Study HYDR-21-047

Table 4 Descriptive Statistics of the Pharmacokinetic parameters of hydrochlorothiazide by treatments in study HYDR-21-047

	Test (powder for oral suspension)			Reference (hydrochlorothiazide tablets)	
Parameters (Units)	**Treatment A (N=30) 100 mg/10 mL (Fasted)**	**Treatment C (N=29) 100 mg/10 mL (Fed)**	**Treatment E* (N=30) 50 mg/5 mL (Fasted)**	**Treatment B (N=29) 100 mg (2 x 50 mg Tablets) (Fasted)**	**Treatment D (N=29) 100 mg (2 x 50 mg Tablets) (Fed)**
C_{max} (ng/mL)	972 ± 260	591 ± 87	495 ± 140	808 ± 184	710 ± 124
AUC_{0-t} (hr*ng/mL)	5940 ± 1359	5545 ± 1198	2945 ± 682	5769 ± 1501	5708 ± 1103
$AUC_{0-\infty}$ (hr*ng/mL)	6099 ± 1377	5744 ± 1377	3066 ± 683	6000 ± 1734	5930 ± 1215
t_{max} (hr)#	1.2 (0.8 – 2.7)	3.3 (1.0 – 4.5)	1.3 (0.8 – 3.4)	2.3 (1.0 – 4.5)	3.7 (2.0 – 5.0)
$t_{½}$ (hr)	9.7 ± 1.6	9.8 ± 1.5	9.4 ± 1.3	10.3 ± 2.2	10.1 ± 2.0
K_{el} (1/hr)	0.074 ± 0.011	0.072 ± 0.010	0.075 ± 0.010	0.069 ± 0.013	0.071 ± 0.014
$AUC_{\%Extrap\_obs}$ (%)	2.7 ± 1.2	3.1 ± 1.9	4.1 ± 2.0	3.4 ± 2.5	3.6 ± 2.1

Source: Clinical Study Report for Study HYDR-21-047; Data are presented as mean ± standard deviation unless noted otherwise. *C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ in Treatment E are non-normalized after a test product dose of 50 mg (50 mg/5 mL); #t_{max} is presented as median (minimum and maximum)

Table 5 Summary of statistical assessments of natural log-transformed PK parameters of hydrochlorothiazide compared between test (powder for oral suspension) product doses of 50 mg (dose-normalized) and 100 mg.

Parameter	Ratio (%) (100 mg/50 mg)	Ratio % (90% CI)
AUC_{inf}	99.3	95.6-103.2
AUC_{last}	100.8	97.0-104.9
C_{max}	98.3	91.1-106.1

Source: Clinical Study Report for Study HYDR-21-047

Proportionality in exposures (both C_{max} and AUC) to hydrochlorothiazide was assessed between the tested doses of 50 mg/5 mL and 100 mg/10 mL of PFOS in the fasted state. As shown in Table 5, the ratios and 90% CI for the dose-normalized ln-transformed PK parameters were within the 80% to 125% limits and indicated that the proposed PFOS product is dose proportional across the range of 50 mg to 100 mg.

3.1.3 Reviewer comments:

The study showed that the proposed PFOS product (Inzirqo) has equivalent extent of exposure (AUC) when compared to the reference HCT tablets under fasted and fed dosing conditions. However, Inzirqo showed a higher C_{max} (with a shorter T_{max}) and a slightly lower C_{max} (with same T_{max}) when compared to HCT tablets under fasted and fed dosing conditions, respectively. The Applicant was asked to justify the differences in C_{max} for Inzirqo, especially the higher C_{max} when dosed under fasted state. The details with further review of this justification can be referred to under the question-based review section. The review team agrees with the Applicant's assessment that the pharmacokinetics of hydrochlorothiazide are dose proportional between 50 mg and 100 mg of Inzirqo.

3.2 Clinical Study #2 HYDR-20-115

An Open Label, Balanced, Randomized, Two-Treatment, Two-Period, Two-Sequence, Two-Way Crossover, Single Dose, Oral Comparative Bioequivalence Study of Hydrochlorothiazide Powder for Oral Suspension 50mg/5mL (T), With That of Hydrochlorothiazide Tablets USP 50mg (R), in Healthy Adult Human Male Subjects Under Fasted Conditions.

3.2.1 Study summary:

Study objective(s): To compare and evaluate the oral bioavailability of Hydrochlorothiazide Powder for Oral Suspension 50mg/5ml (T), With That of Hydrochlorothiazide Tablets USP 50mg (R), In Healthy Adult Human Male Subjects Under Fasting Conditions.

Study population: The study was conducted in 14 healthy adult males, 18 to 45 years age, with a BMI of 18.5 kg/m^2 to 30.0 kg/m^2.

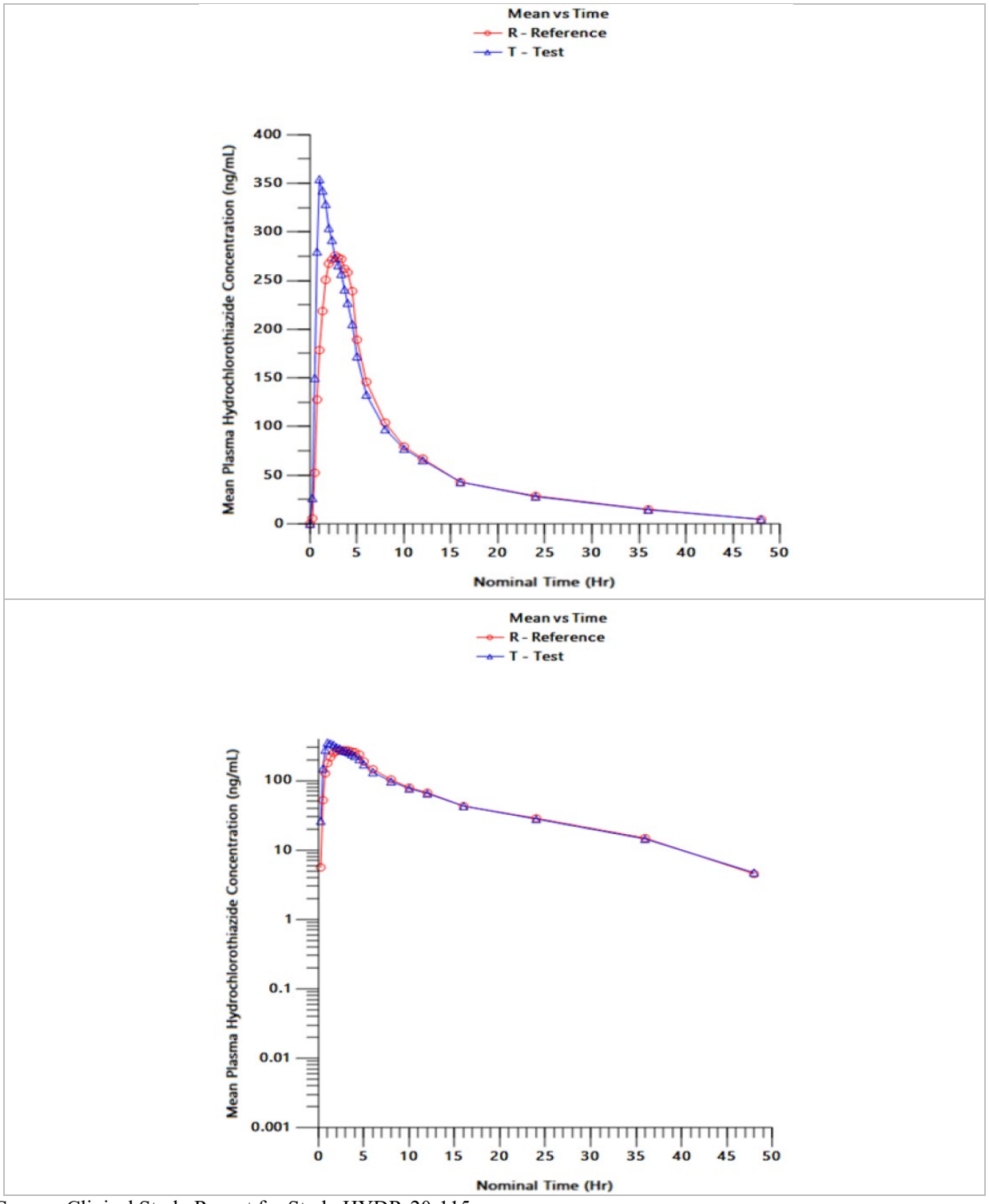
Study design:
The study was a foreign clinical trial conducted without an IND at Scitus Pharma Services Private Limited, Chennai, India. The Test Product (T) comprised of the putative final formulation for the hydrochlorothiazide powder for oral suspension (PFOS) 50 mg/5 mL manufactured by Novitium Pharma, NJ, USA. The Reference Product (R) was Hydrochlorothiazide (HCT tablets) USP 50 mg tablets manufactured by Teva Pharmaceuticals (ANDA 083177). The test product was to be reconstituted in 120 mL of purified water; however, in period 1 the reconstitution was done in ~66 mL of purified water by error and for consistency, the same volume (~66 mL) was used for reconstitution in period 2 as well. The PFOS was reconstituted in purified water and loaded into syringes for oral dosing following which syringes were first flushed with 60 mL of water and then with 180 mL of water to ensure complete dosing. HCT tablets (1 tablet x 50 mg) was administered with ~ 240 mL of water at ambient temperature. The dosing in both periods followed an overnight fast of ≥ 10 hours.
A total of 24 blood samples for PK (~3 mL each) were obtained in each period at 0 h (pre-dose) and at 15, 30, 45, 60, 80, 100, 120, 140, 160, 180, 200 and 220 minutes and 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36 and 48 hours after study drug administration. Plasma was separated and stored at –70°C ± 15°C until quantification using a validated LC-MS/MS method. The assay had a lower limit of quantification of 5 ng/mL of hydrochlorothiazide and concentrations below that were set to zero before PK and statistical data analysis.

Pharmacokinetic (PK) and statistical data analysis:
The Ln-transformed primary PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were analyzed for bioequivalence using an analysis of variance (ANOVA) model with the main effect of treatment, period, and sequence as fixed effects and subject nested within sequence as a random effect. The power of the ANOVA test to detect a 20% mean difference between treatments was calculated. Two one-sided tests were used to assess bioequivalence for each parameter.

3.2.2 Study results:

A total of 14 subjects were dosed in Period 1 and 13 subjects were dosed in Period 2 (1 subject did not report for check-in). The study was a pilot study, and the final study power was estimated to be 50% for C_{max} and 70% for the $AUC_{0-\infty}$. The mean PK profiles, descriptive statistics and statistical results for plasma PK parameters for hydrochlorothiazide are shown in Figure 2, Table 6 and Table 7.

Figure 2 Mean plasma concentrations of hydrochlorothiazide vs time plots overlaid by treatments in study HYDR-20-115 (linear and semi-log scale)



Source: Clinical Study Report for Study HYDR-20-115

Table 6 Descriptive Statistics of the Pharmacokinetic parameters of hydrochlorothiazide for the test and reference treatments in study HYDR-20-115 (N=13)

Parameters (Units)	Hydrochlorothiazide (Mean ± SD)	
	Test (T)	Reference (R)
C_{max} (ng/mL)	375 ± 97	315 ± 91
AUC_{0-t} (hr*ng/mL)	2761 ± 777	2659 ± 912
$AUC_{0-\infty}$ (hr*ng/mL)	2901 ± 793	2811 ± 957
T_{max} (hr) #	1.3 (1.0 – 1.7)	2.7 (1.7 – 4.5)
$t_{1/2}$ (hr)	11.1 ± 1.3	10.6 ± 2.1
K_{el} (1/hr)	0.06 ± 0.007	0.07 ± 0.013
AUC_%Extrap_obs	5.0 ± 1.2	5.5 ± 1.7

Source: Clinical Study Report for Study HYDR-20-115; (#) Tmax is presented as Median (Minimum and Maximum)

Table 7 Summary of statistical assessments of natural log-transformed PK parameters of hydrochlorothiazide compared between test and reference products in the pilot study HYDR-20-115 (N=13)

Parameter	Ratio (%) (T/R)	Ratio % (90% CI)	~~Power (%)~~	~~Intra-subject variability (%)~~
AUC_{inf}	105.9	90.7-123.6	~~69.5~~	~~22.2~~
AUC_{last}	106.4	90.9-124.5	~~68.3~~	~~22.5~~
C_{max}	119.7	98.1-145.9	~~50.6~~	~~28.7~~

Source: Clinical Study Report for Study HYDR-20-115

3.2.3 Reviewer comments:

The pilot study results using the putative final formulation of the powder for oral suspension showed an early T_{max} with a ~20% higher C_{max} and an equivalent extent of absorption (AUC) in comparison to the reference HCT tablets. The results of the pilot study were similar to the main study.

3.3 Summary of Bioanalytical Method Validation and Performance

Plasma concentrations of hydrochlorothiazide (HCT) were determined by a validated liquid chromatography (UHPLC) with tandem mass spectrometry (MS/MS) method. A liquid/liquid method was used for extraction of HCT from plasma (dipotassium EDTA) samples and HCT D2 was used as the internal standard. Extracted samples were analyzed using reverse-phase liquid chromatography and analytes were detected using tandem mass spectrophotometry (Xevo TQ-S MS/MS system). The lower limit of quantitation (LLQ) was 5 ng/mL, and the upper limit of quantitation was 500 ng/mL. The method validation parameters are summarized in Table 8.

Table 8 Summary of bioanalytical method validation for quantification of hydrochlorothiazide in human plasma

Bioanalytical method validation and report number	Quantification of Hydrochlorothiazide in Human Plasma by UPLC-MS/MS Spectrometry detection Method No. HT-AM/ Report No. (b) (4)
Method description	Liquid-Liquid Extraction Method using LC coupled with Tandem Mass Spectrometry detection
Matrix	Human plasma with K_2EDTA anticoagulant
Standard calibration curve and concentration	Calibrator concentrations: 5, 10, 25, 50, 100, 200, 300, 400 and 500 ng/mL
Validated assay range	LLOQ: 5.0 ng/mL ULOQ: 500 ng/mL
Material used for QCs and concentration	QC concentrations: 15, 60, 165 and 360 ng/mL
Minimum required dilutions	NA
Validation Parameters	Method Validation Summary
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ 9 Cumulative accuracy (%bias) from LLOQ to ULOQ from all runs: -6.6% to 4.5% Cumulative precision (%CV) from LLOQ to ULOQ from all runs: 2.6% to 9.6%
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 4 QCs -3.6% to 2.7% Inter-batch %CV in 4 QCs 4.4% to 7.9%
Dilution linearity	Diluted 1 to 2 and 1 to 4 Six replicates of the dilution QC (750 ng/mL) were diluted 2x and 4x prior to analysis with bias and CV being <1.4% and <3.1%, respectively.
Bench-top/process stability	20 hours at Room Temperature
Freeze-thaw stability	QC samples were taken through 5 freeze-thaw cycles. (-70°C to room temperature).
Long-term storage	Stability of hydrochlorothiazide established at -20°C and -70°C for 76 days prior to analysis

Study HYDR-21-047:

The concentrations used for quality control (QC) samples were 15, 65, 175, and 380 ng/mL. The study samples were analyzed by 43 days, within the validated stability period of 76 days (when stored at -20°C or -70°C). The accuracy (% deviation) and precision (%CV) of the calibration runs and quality control samples for the 38 analytical runs were ≤15% which is acceptable. Incurred sample reproducibility was assessed by re-assaying 294 out of the 3525 patient samples (8.3% of

the sample size), and 98.98% of the re-assayed patient samples results were within 20%, thus meeting the acceptance criteria. The bioanalytical method performance to quantify HCT in the study plasma samples was found to be acceptable.

Study HYDR-20-115:

The concentrations used for quality control (QC) samples were 15, 61, 166, and 360 ng/mL. The study samples were analyzed by 17 days, within the validated stability period of 76 days (when stored at -20°C or -70°C). The accuracy (% deviation) and precision (%CV) of the calibration runs and quality control samples for the 7 analytical runs had maximum values of -5.7% and 5.8%, respectively, which is acceptable (acceptance limit of (b) (4) %). Overall, the bioanalytical method performance to quantify HCT in the study plasma samples was found to be acceptable.

3.4 OSIS inspections

Clinical and Analytical site inspection

Biopharmaceutical inspections were requested for the clinical and analytical sites for the pivotal study HYDR-21-047. The office of Study Integrity and Surveillance (OSIS) determined that inspections were not needed for the sites based on prior inspection results for the clinical and analytical sites (DARRTS 06/20/2024; Reference ID: 5401024).

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

HARISUDHAN THANUKRISHNAN
01/21/2025 09:15:04 AM

REKHA KAMBHAMPATI
01/21/2025 09:52:40 AM

DOANH C TRAN
01/21/2025 11:05:02 AM