CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212269Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA or BLA Number	212269
Link to EDR	\\CDSESUB1\evsprod\NDA212269\0001
Submission Date	07/19/2019
Submission Type	*Standard*
Proposed Brand Name	Ferriprox
Generic Name	Deferiprone
Dosage Form and Strength	1000 mg tablets
Route of Administration	Oral
Proposed Indication	for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate
Applicant	ApoPharma
Associated IND	*IND 045724*
OCP Review Team	*Oluseyi Adeniyi, Pharm.D., Ph.D.* *Olanrewaju Okusanya, Pharm.D., MS*
OCP Final Signatory	(b) (6)

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1. EXECUTIVE SUMMARY

ApoPharma submitted a 505(b)(1) application for 1000 mg deferiprone tablets proposed as a [redacted] (b) (4) formulation for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate. The new deferiprone formulation is proposed for administration two times day for a total daily dose range of 75 mg/ kg - 99 mg/ kg. This is in contrast to Ferriprox, immediate-release (IR) deferiprone (deferiprone IR) which is approved for the proposed indication for administration three times a day for the same total daily dose. The applicant did not conduct any efficacy studies. The evaluation of approvability relied on two bioavailability studies that evaluated the relative bioavailability of the new deferiprone formulation to deferiprone IR in single and multiple dose studies. Food effect and split-dose dumping of the new formulation were also evaluated.

Relative bioavailability studies demonstrated bioequivalence of the new deferiprone formulation to the IR formulation over a 24-hour dosing period under fed conditions and the pharmacokinetic profile of the new deferiprone formulation was comparable to deferiprone IR. Given the similarity in PK profile between the new deferiprone formulation and deferiprone IR, the bioavailability studies provide supportive evidence of effectiveness and adequacy of the proposed dosing interval. However, the studies do not show a clinically-meaningful delay in the release of deferiprone from the formulation. Additionally, the potential for alcohol-induced dose dumping, the absence of a GI tolerability advantage, and the recommendation to administer the new deferiprone formulation with food, are notable observations of the new formulation when compared to the deferiprone IR.

1.1 Recommendations

The office of Clinical Pharmacology finds the results of the bioavailability studies support approval of the new deferiprone formulation for the proposed indication. [redacted] (b) (4)

1.2 Post-Marketing Requirements and Commitments

No new PMR or PMC.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

The clinical pharmacology program includes two bioavailability studies; Study LA53-0116 was a single dose relative bioavailability and food effect study of the new deferiprone formulation and Study LA45-0116 was a comparative bioavailability of the new deferiprone formulation tablets versus Ferriprox IR tablets at steady state. Relative bioavailability in these studies was evaluated using the conventional criteria for demonstrating bioequivalence (i.e., the geometric mean ratio of the test to reference AUC and C_{max} and the corresponding 90% confidence intervals (CI) within 80.00–125.00%).

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The applicant has proposed administration of the new deferiprone formulation at an initial dose of 75 mg/kg/day to 99 mg/kg/day (rounded to the nearest 500 mg) divided into two doses approximately 12 hours apart with food. The establishment of the bioequivalent exposure between the new deferiprone formulation and deferiprone IR over a 24- hour period supports the proposed twice daily dosing regimen.

When taken with food, the new deferiprone formulation was shown to have similar exposure (GMR AUC (90% CI)) as that observed with deferiprone IR when evaluated either as a single dose (99.4 (97.1 - 102)) or at steady state (99.0 (95.3 - 102)).

The assessment found no clinically meaningful effect of food on the pharmacokinetics of the new deferiprone formulation; however, the new deferiprone formulation is proposed to be administered with food due to a concern about tolerability, and as such, bioequivalence assessment between the new deferiprone formulation and deferiprone IR was conducted under fed conditions and the new deferiprone formulation is labeled to be taken with food.

A relative bioavailability in Study LA53-0116 supported the splitting of the newly-formulated deferiprone tablets to make the appropriate dose to the nearest 500 mg.

The similarity of the PK profile between deferiprone IR and the new deferiprone formulation indicates that the proposed twice daily dosing regimen is adequate (b) (4)

2.2.2 Therapeutic individualization

No therapeutic individualization based on intrinsic or extrinsic factors is necessary.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

Labeling was negotiated with the applicant. The Office of Clinical Pharmacology provided recommendations in Sections 2, 7, and 12 of the labeling according to current guidances and labeling best practices for the communication of clinical pharmacology-related information.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

The new deferiprone formulation is proposed for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate. (b) (4)

The new formulation is intended to offer a more convenient dosing regimen compared to immediate release deferiprone approved for the same indication under NDA 021825.

3.2 General Pharmacological and Pharmacokinetic Characteristics

The new deferiprone formulation is a 1000 mg core tablet enclosed by an (b) (4) coat. The core tablet contains hypromellose acetate succinate (b) (4) and magnesium oxide (b) (4) In the pharmacokinetic studies, serum deferiprone and deferiprone 3-O-glucuronide concentrations were measured using a validated LC-MS/MS method (DFN-V3-523) which is detailed in Appendix 1.

3.3 Clinical Pharmacology Questions

3.3.1 Does the clinical pharmacology information provide supportive evidence of effectiveness?

Yes, based on data from LA53-0116 and LA45-0116, the applicant showed that the new deferiprone formulation resulted in a similar exposure as deferiprone IR when taken with food. As such, they were able to rely on the evidence of effectiveness for deferiprone IR as detailed in NDA 021825.

LA53-0116, was an open label, 4-period, 4-sequence, crossover study, conducted in 28 healthy subjects, that compared the PK of 1000 mg of the new deferiprone formulation to 1000 mg deferiprone IR (administered as two 500 mg deferiprone IR tablets) under a) fasting and b) fed conditions (a high-fat, high-calorie FDA-standardized breakfast (approximately 1000 calories, 14% protein, 53% fat, and 33% carbohydrate); and to compare the PK of 1000 mg of the new deferiprone formulation administered as whole tablets compared to two half tablets under fed conditions. A 7-day washout period separated each drug administration. This washout period is adequate given the half-life of approximately 2 hours for either formulation.

Of the 28 subjects randomized, 22 subjects completed the study and 19 subjects received all doses. In the 6 subjects who discontinued the study, the reasons for discontinuation were investigator decision (n=1), positive finding on drug screen (n=1), and withdrawal of consent (n=4). A plot of mean serum concentration-vs-time profile by treatment arm is shown in Figure 1 and a summary of the pharmacokinetic parameters by treatment arm is shown in Table 1.

Figure 1: Linear plot of mean serum concentration versus time profile of treatments in LA53-0116

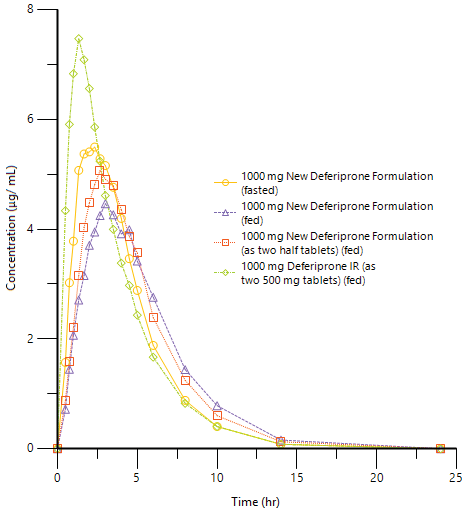

Table 1: Summary of Pharmacokinetic Parameters after Single Dose from Study LA53-0116

	New Deferiprone Formulation 1000 mg			Deferiprone IR 1000 mg
Parameter (Unit)	Fasting (n= 19)	Fed (n=21)	Fed given as half tablets (n=21)	Fed given as two 500 mg tablets) (n=20)
	Mean (CV%)			
C_{max} (µg/ mL)	6.17 (38.1)	6.09 (32.1)	6.84 (31)	9.52 (42.5)
AUC $_{(0-T)}$, (µg.h/ mL)	27.5 (29.3)	27.6 (25.6)	28.5 (25.9)	29.6 (27.4)
AUC $_{0-\infty}$ (µg.h/ mL)	27.9 (28.4)	28.4 (25.6)	29.1 (5.5)	30.2 (27.6)
T_{max} (hours)[a]	2.33 (1.33 - 4)	3 (2 - 8)	2.67 (1.33 - 6.03)	1.67 (0.5 - 8)
λ_z (hours $^{-1}$)	0.38 (11.3)	0.39 (15.5)	0.39 (15.7)	0.39 (13.9)
T_{half} (hours)	1.83 (11.4)	1.8 (16.5)	1.8 (16.5)	1.8 (14.8)
Vd /F (L)	102 (31.6)	97.3 (28.9)	94.7 (25.7)	90.7 (26.8)
CL/F (L)	39.1 (33.9)	38.1 (36.1)	37.3 (33.8)	35.9 (32.3)
T_{lag} (hours) [b]	0 (0 - 0)	0 (0 - 0.78)	0 (0 - 0.5)	0 (0 - 1)

[a, b] median (range)
Source: Reviewer's analysis of Study LA53-0116

The geometric mean ratio of the new deferiprone formulation to deferiprone IR for C_{max}, AUC $_{0-T}$ and AUC $_{0-\infty}$ are shown in Table 2. While the 90% confidence interval for the geometric mean ratio for AUC $_{0-T}$ and AUC $_{0-\infty}$ was within the 80 – 125% prespecified confidence bounds, the lower bound of the 90% confidence interval for the geometric mean ratio for C_{max} was outside the 80% pre-specified lower bound and was 34% lower for the new deferiprone formulation (Table 2). In addition, T_{max} for the new deferiprone formulation was delayed from 1.67 hours to 3 hours (Table 1).

The implication of the difference observed in the rate but not extent of absorption between the new deferiprone formulation and the IR formulation is further evaluated in a multiple-dose bioavailability study where the 90% confidence intervals for the geometric mean ratio for AUC and C_{max} were within the prespecified bounds (See Table 5). This difference in C_{max}, after a single dose, is not expected to be clinically meaningful for a medication that is taken for a long duration.

Table 2: Relative Bioavailability of 1000 mg New Deferiprone formulation compared to 1000 mg (2 x 500 mg) Deferiprone IR under Fed Conditions (Study LA53-0116)

Parameter (Unit)	Geometric Mean Ratio (%) (90% CI)
C_{max} (µg/ mL)	65.9 (58.5 - 74.4)
AUC $_{(0-T)}$, (µg.h/ mL)	92.6 (89.3 - 96)
AUC $_{0-\infty}$ (µg.h/ mL)	93.3 (90 - 96.6)

Source: Reviewer's analysis of Study LA53-0116

Although the delayed T_{max} indicates a slightly prolonged absorption period with the new deferiprone formulation, the clinical relevance is inconsequential to the comparative bioavailability. (b) (4)

(b) (4)

. The dissolution profiles of the new deferiprone formulation tablets in varying pH in vitro are detailed in the biopharmaceutics review.

Dose-Dumping:

Given that the new deferiprone formulation is expected to be split and administered as half tablets to make a 500 mg dose, the potential for dose dumping was assessed by evaluating the exposure of the tablets administered as half tablets compared to whole tablets under fed conditions.

As shown in Table 3, when administered as half tablets, the new deferiprone formulation resulted in no significant increase in AUC and a small increase in C_{max} (13%) compared to the whole tablet.

The directions of the dosage and administration accommodates small variations in each dose hence the small increase in C_{max} when administered as half tablets is not considered to be clinically relevant.

Table 3: Relative Bioavailability of New Deferiprone Formulation 1000 mg (as Half Tablets) compared to Whole Tablets under Fed Conditions (Study LA53-0116)

Parameter (Unit)	Geometric Mean Ratio (%) (90% CI)
C_{max} (µg/ mL)	113 (100 - 128)
AUC $_{(0-T)}$, (µg.h/ mL)	103 (99.3 - 107)
AUC $_{0-\infty}$ (µg.h/ mL)	102 (98.6 - 106)

Source: Reviewer's analysis of Study LA53-0116

3.3.2 Is the proposed general dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. The proposed dosage is 75 mg/kg to 99 mg/kg body weight, orally, divided into two doses, taken with meals is adequately supported by the data in the application. In addition to the data from Study LA 53-0116, the pharmacokinetics and safety of multiple doses of the new deferiprone formulation tablets versus Ferriprox IR tablets in healthy volunteers were evaluated in Study LA45-0116.

Study LA 45-0116 was a single-center, randomized, multiple-dose, open label, 2-period, 2-sequence, crossover study conducted under fed conditions. Subjects were randomized to receive six consecutive 1500 mg doses (One and half 1000 mg tablets) of the new deferiprone formulation administered two times daily (every 12 hours) for a total of 3000 mg daily for three consecutive days or nine consecutive 1000 mg doses (two 500 mg tablets) of deferiprone IR administered three times daily (every 8 hours), for a total of 3000 mg daily for three consecutive days.

On Day 3, after an overnight fast, subjects on the new deferiprone formulation received a standard high-fat, high-calorie meal (approximately 1000 calories, 14% protein, 53% fat, and 33% carbohydrate) completed within 30 minutes, and dose 5 was administered 30 minutes

after the start of the meal. On Day 3, after an overnight fast, subjects on deferiprone IR received a standardized high-fat, high-calorie meal (approximately 1000 calories, 14% protein, 53% fat, and 33% carbohydrate) 30 minutes before drug administration of Dose 7. Period 1 and 2 were separated by a minimum of 4 days for washout.

Thirty-six subjects were randomized to the study, but only 34 subjects completed the study, all of whom received all doses. Of the 2 subjects that discontinued the study, adverse events (n=1) and withdrawal by subject (n=1) were the reasons for discontinuation. A summary of pharmacokinetic parameters over 24 hours at steady state by treatment arm is presented in Table 4.

Table 4: Summary of Pharmacokinetic Parameters at Steady State from Study LA 45-0116

	New Deferiprone Formulation 1500 mg BID (n =35)	**Deferiprone IR** 1000 mg TID (n=35)
Parameter (Unit)	Mean (CV%)	
$C_{24h, ss}$ (µg/ mL)	0.62 (58.7)	0.930 (47.3)
$C_{max,ss}$ (µg/ mL)	9.31 (23.3)	9.09 (31.7)
$C_{avg,ss}$ (µg/ mL)	3.55 (22.4)	3.54 (21.3)
$C_{min,ss}$ (µg/ mL)	0.49 (72.9)	0.79 (54.6)
$T_{max, ss}$ (hours)[a]	3 (1.5 - 6)	1.5 (1.5 - 4)
$AUC_{(0-24), ss}$ (µg.h/ mL)	83.6 (22.4)	83.4 (23.3)
Cl_{ss}/F (L/h)	36.7 (20.5)	36.8 (20.7)
Vd_{ss}/F (L)	109 (25.5)	108 (34.5)
Fluctuation (%)	251 (18.3)	239 (31.8)
Swing	24.7 (60.1)	13.7 (61.8)

[a] median (range) is reported
Source: Reviewer's analysis of Study LA45-0116

The 90% confidence interval of the geometric mean ratio for $C_{max, ss}$ and $AUC_{0-24, ss}$ are shown in Table 5 and both were within the prespecified 80 to 125% boundaries. When the new deferiprone formulation is administered as 1500 mg twice daily for a total of 3000 mg, it provided equivalent rate and extent of exposure as 1000 mg IR tablets administered three times daily (for a total of 3000 mg). The demonstration of comparability at steady state supports the proposed twice daily dosing regimen.

Table 5: Relative Bioavailability 1500 mg New Deferiprone Formulation BID compared to 1000 mg Deferiprone IR TID under Fed Conditions (Study LA45-0116)

Parameter (Unit)	**Geometric Mean Ratio (%) (90% CI)**
$C_{max,ss}$ (µg/ mL)	104 (95.8 - 112)
$AUC_{(0-24),ss}$ (µg.h/ mL)	99.4 (97.1 - 102)
$AUC_{(0-8),ss}/D$ (µg.h/ mL/ mg)	99.4 (91.5 - 108)

Source: Reviewer's analysis of Study LA45-0116

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3.3.3 Is an alternative dosing regimen and management strategy required for subpopulations based on intrinsic factors?

No. Studies performed under NDA 021825 (Ferriprox) (deferiprone IR) to evaluate the appropriateness of deferiprone based on organ function are applicable to the new deferiprone formulation.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Effect of Food

No. The effect of food was assessed in LA53-0116 between the new deferiprone formulation 1000 mg under fasted and fed conditions in healthy subjects. As shown in **Table 8**, food did not have an effect on the exposure of the new deferiprone formulation. The 90% confidence interval of the geometric mean ratio for C_{max}, AUC $_{0-T}$, AUC $_{0-\infty}$ were within the prespecified 80.00 to 125.00.% boundaries (Table 6).

Table 8: Relative Bioavailability of 1000 mg New Deferiprone Formulation under Fed to Fasting Conditions (Study LA53-0116)

Parameter (Unit)	Geometric Mean Ratio (%) (90% CI)
C_{max} (µg/ mL)	99.6 (87.9 - 113)
AUC $_{(0-T),}$ (µg.h/ mL)	99.0 (95.3 - 102)
AUC $_{0-\infty}$ (µg.h/ mL)	99.8 (96.3 - 103)

Source: Reviewer's analysis of Study LA53-0116

During the development of Ferriprox (NDA 021825), food decreased the rate of absorption of deferiprone (C_{max}) by 38%, while the overall extent of absorption (AUC) was not significantly affected (DARRTS: 03/27/2008 Office of Clinical Pharmacology Review). The effect of food on C_{max} was not considered to be clinically relevant and Ferriprox tablets were approved to be taken without regard to food. However, the bioequivalence of the new deferiprone formulation to the IR formulation was performed under fed conditions.

The applicant's rationale for conducting the study under fed conditions was derived from a pilot study using another [redacted] (b) (4) formulation that showed poor tolerability of the test the new deferiprone formulation, which differs from the to-be-marketed formulation under review.

The tolerability of the new deferiprone formulation was evaluated in two studies, LA58-0017 and LA60-0118, conducted in healthy subjects.

Study LA58-0117 was a single-center, randomized, open-label, parallel study to evaluate the gastrointestinal (GI) tolerability of the new deferiprone formulation tablets under fasting conditions. This study evaluated the highest recommended therapeutic dose with no titration under fasting conditions to assess tolerability under the conditions most likely to induce gastric distress. As such, subjects were randomized to receive the new deferiprone formulation (1000 mg tablets) 100 mg/kg/day as two divided doses (50 mg/kg every 12 hours) for three days or deferiprone IR (500 mg tablets) 100 mg/kg/day as three divided doses (33.3 mg/kg/dose every 8 hours).

Eighty-six subjects were planned, but the study was terminated after 39 subjects were enrolled. As shown in Table 7, 47.4% of subjects in the cohort who received the new deferiprone formulation experienced some type of GI-related AE vs. 30.0% in the IR cohort. Vomiting occurred in 26.3% of subjects taking the new deferiprone formulation compared to none taking the IR formulation. Three subjects voluntarily withdrew from the study due to vomiting (n=1) and headache, nausea, and dyspepsia (n=1) in the new deferiprone formulation cohort, and depressed mood, headache, and feeling hot (n=1) in the IR cohort.

Multiple doses of the new deferiprone formulation tablets administered twice daily at the maximum therapeutic dose under fasting conditions in healthy volunteers led to a higher than expected rate of GI events. As such, the study was terminated as the applicant determined that sufficient information on the GI tolerance of the new deferiprone formulation had been obtained.

Table 9: Overall Summary of Adverse Events and Summary of GI-related Adverse Events in Study LA58-0117

Number of subjects with at least one:	**New Deferiprone Formulation 50 mg/ kg BID** (N=19) n (%)	**Deferiprone IR 33.3 mg/ kg TID** (N=20) n (%)
Treatment-emergent adverse event (TEAE)	15 (78.9)	10 (50.0)
All GI-related adverse events[1]	9 (47.4)	5 (25.0)
• Nausea	• 6 (31.6)	• 4 (20.0)

• Vomiting[2]	• 5 (26.3)	• 0 (0.0)
• Dyspepsia	• 2 (10.5)	• 1 (5.0)
• Abdominal Distension	• 1 (5.3)	• 0 (0.0)
• Abdominal Pain Upper	• 1 (5.3)	• 0 (0.0)
• Abdominal Pain	• 0 (0.0)	• 1 (5.0)
• Eructation	• 1 (5.3)	• 0 (0.0)
• Flatulence	• 1 (5.3)	• 0 (0.0)
• Decreased Appetite	• 1 (5.3%)	• 1 (5.0)
Serious TEAE	0 (0.0)	0 (0.0)
Severe TEAE	0 (0.0)	0 (0.0)
Drug-related TEAE	15 (78.9)	10 (50.0)
Number of subjects withdrawn due to a TEAE	0 (0.0)	0 (0.0)

[1] In the group that received the new deferiprone formulation, with the exception of Decreased Appetite, which was in the system organ class (SOC) of Metabolism and Nutrition disorders, all of the AEs listed here were in the SOC of Gastrointestinal disorders. In the IR group, 6 subjects had AEs in the SOC of Gastrointestinal disorders, but one of those events (Paraesthesia Oral) was not considered by the PI to be in the category of GI-related AEs as per protocol. [2] Only AE with significant difference, p-value = 0.02 without multiplicity adjustment; Fisher's Exact Test, one-sided. BID= two times daily; TID= three times daily
Source: Modified from applicant's analysis in Table 12.1 and Table 12.3 in Study Report LA58-0117

LA60-0118 was a single center, single arm, two-part study in healthy volunteers to evaluate the tolerability of the new deferiprone formulation 1000 mg tablets under fed conditions (standardized meals are given before each dose; calories, protein, fat and carbohydrate content are unspecified), with particular attention to gastrointestinal (GI) tolerability. Subjects were initially to receive the maximum therapeutic dosage of 100 mg/kg per day, i.e., 50 mg/kg twice daily, however, the study treatment was reduced mid-study to the usually starting dose 75 mg/kg/day i.e. 37.5 mg/kg twice daily due to a finding of elevated liver enzymes in half the subjects. Hence, subjects received one of the following:

- 1000 mg tablets of the new deferiprone formulation: 100 mg/kg/day (two 50 mg/kg doses, 12 hours apart, rounded to the nearest 500 mg half tablet) for three days (Part A). All doses were given upon completion of a standardized meal.
- 1000 mg tablets of the new deferiprone formulation: 75 mg/kg/day (two 37.5 mg/kg doses, 12 hours apart, rounded to the nearest 500 mg half tablet) for three days (Part B). All doses were given upon completion of a standardized meal.

Fifty subjects were enrolled in the study, 33 received the new deferiprone formulation at a dosage of 100 mg/kg/day (high dose) and 17 received it at a dosage of 75 mg/kg/day (low dose). Forty-seven subjects completed the study, and three subjects voluntarily withdrew from the study due to personal reasons (n=1), nausea, vomiting, headache, and fatigue (n=1) and nausea, vomiting, headache, and jaw disorders (n=1) in the high dose cohort.

Some type of GI-related AE was observed in 54.5% of subjects in the high-dose cohort vs. 11.8% in the low-dose cohort p= 0.0033. As shown in Table 9, nausea was observed in 42.4% of subjects in the high- dose cohort compared to none in the low-dose cohort, p= 0.0009 without multiplicity adjustment.

Table 10: Overall Summary of Adverse Events and Summary of GI-related Adverse Events in Study LA60-0118

	New Deferiprone Formulation	
Number of subjects with at least one:	**50 mg/kg BID** (N=33) n (%)	**37.5 mg/kg BID** (N=17) n (%)
Treatment-emergent adverse event (TEAE)	26 (78.8)	7 (41.2)
All GI-related adverse events[1]	18 (54.5)	2 (11.8)
• Nausea[2]	• 14 (42.4)	• 0 (0.0)
• Constipation	• 5 (15.2)	• 1 (5.9)
• Vomiting	• 4 (12.1)	• 0 (0.0)
• Abdominal Distension	• 2 (6.1)	• 0 (0.0)
• Abdominal Pain	• 1 (3.0)	• 0 (0.0)
• Diarrhoea	• 1 (3.0)	• 0 (0.0)
• Duodenogastric Reflux	• 1 (3.0)	• 0 (0.0)
• Dyspepsia	• 0 (0.0)	• 1 (5.9)
Serious TEAE	0 (0.0)	0 (0.0)
Severe TEAE	4 (3.4)	0 (0.0)
Drug-related TEAE	26 (78.8)	7 (41.2)
Number of subjects withdrawn due to a TEAE	0 (0.0)	0 (0.0)

AEs with significant difference [1]p-value = 0.0033 [2] p= 0.0009 p values without multiplicity adjustment; Fisher's Exact Test, one-sided BID= two times dailySource: Modified from applicant's analysis in Table 12.1 and Table 12.3 Study Report LA60-0118

While LA60-0118 showed multiple doses of the new deferiprone formulation tablets administered twice daily at the starting dose under fed conditions may reduce the occurrence of GI-related adverse events observed at the maximum dose, the similarity of occurrence in GI-related AEs at the maximum dose under fasting versus fed conditions, in study LA58-0117 and LA60-0118, indicate that the incidence of GI-related AEs was related to the high dose of deferiprone rather than the presence of food. The results underscored the potential for dose titration at the higher end of the dose recommendations to impact GI-tolerability. However, given that the bioequivalence of the new deferiprone formulation to the IR formulation was performed under fed conditions, and the IR formulation can be given without regard to food, the labelling will indicate that the new deferiprone formulation be administered with food. Of note, there was a wide discrepancy between incidence of gastrointestinal adverse events after the administration of the new deferiprone formulation under fed conditions in Study LA45-0116 (5.7%) and the cohort receiving maximum dose the new deferiprone formulation under fed conditions in Study LA60-0118 (54.5%). Adverse events were evaluated in similar-sized safety populations in these cohorts; n = 35 in Study LA45-0116 and n =33 in Study LA60-0118. The mean weight (SD) of subjects enrolled these cohorts was 77.3(8.6) kg in Study LA45-0116 and 75.5(7.2) kg in Study LA60-0118. The 3000 mg daily dose divided into two doses in Study LA45-0116, represents an average of 38.9 mg/kg for subjects enrolled in this cohort. As such, this discrepancy may represent a dose effect as well.

Effect of Alcohol

The applicant conducted in vitro evaluation of deferiprone release from the new deferiprone formulation tablets in 0.1N HCl with various concentrations (5-40% (v/v)) of alcohol. Dissolution studies showed no significant release of deferiprone from whole or half tablets the new deferiprone formulation in up to 10% (v/v) alcohol (Table 10). In 20% (v/v) alcohol, there was more than 50% release of deferiprone at 2 hours, and more than 80% release of deferiprone in 40% (v/v) alcohol at 2 hours). Refer to Biopharmaceutics Review for assessment of dissolution profiles.

Table 11: Mean Deferiprone Release from The New Deferiprone Formulation (1000 mg) in Varying Alcohol Content (v/v) in Dissolution Medium (0.1N HCl)

		0% alcohol (0.1N HCl)	5% alcohol	10% alcohol	20% alcohol	40% alcohol
Time (minutes)	Tablet form	Deferiprone (%)				
30	Whole	1	2	2	6	23
	Half	4	5	6	12	27
60	Whole	2	4	5	21	50
	Half	7	8	9	32	52
90	Whole	3	5	7	39	71
	Half	10	11	13	50	72
120	Whole	4	7	11	53	88
	Half	12	14	16	63	86

Source: Adapted from Applicant's Alcohol Dose Dumping Study Report Tables 1- 10

The applicant did not perform an in vivo bioavailability study co-administering the new deferiprone formulation with alcohol. The potential for alcohol to increase the rate of deferiprone release from the new deferiprone formulation will be indicated in the labelling.

4. APPENDICES

4.1 Bioanalytical Method Report

The Office of Clinical Pharmacology review team has assessed the adequacy and acceptability of the following bioanalytical methods used in clinical studies.

Method validation was acceptable to support studies LA 53-0116 and LA 45-0116. For both PK studies, serum deferiprone and deferiprone 3-O-glucuronide concentrations measured using a validated method (DFN-V3-523) was suitable for the evaluation of bioavailability. Both the method validation, " Validation of an HPLC Method using MS/MS Detection for the Determination of Deferiprone and Deferiprone 3-O-Glucuronide in Human Serum", and the sample analysis for LA53-0116 and LA45-0116 were performed at (b) (4) In this method, sample pre-treatment involved protein precipitation followed by extraction of deferiprone and deferiprone 3-O-glucuronide from 0.100 mL of human serum. Deferiprone-d3 and deferiprone-d3 3-O-ß-D-glucuronide were used as the internal standards (IS1 and IS2). deferiprone and deferiprone 3-O-glucuronide were identified and quantified using reversed-phase HPLC with MS/MS detection. Table 1 shows the summary of method performance of in the quantification of deferiprone and deferiprone 3-O-β-D glucuronide in human serum.

Table 1. Summary method performance of a bioanalytical method to measure deferiprone and deferiprone 3-O-β-D glucuronide in human serum

Bioanalytical method validation report name, amendments, and hyperlinks	VALIDATION REPORT N° DFN-V3-523 (R5) VALIDATION OF A HPLC METHOD USING MS/MS DETECTION FOR THE DETERMINATION OF DEFERIPRONE AND DEFERIPRONE 3-O-ß-D GLUCURONIDE IN HUMAN SERUM (see Module 5.3.1.4)
Method description	Protein precipitation Reversed-phase HPLC with MS/MS detection
Materials used for calibration curve & concentration	REFERENCE STANDARDS

Analyte Name	(b) (4) Reference Standard N°	Supplier	Purity
Deferiprone	RS-1486-A	ApoPharma Inc.	>99.8%
	RS-1486-B	ApoPharma Inc.	100.0% [1]
	RS-1486-D	ApoPharma Inc.	99.99%

[1]The Deferiprone reference standard, which had a purity of 100.0% and a potency of 99.1%, was re-certified during the course of the validation. The purity obtained following re-certification was >99.8% and the potency obtained following re-certification was 98.9%. Since the difference was less than 2% for both purities and potencies, the concentrations of stock solutions, calibrants and QC samples were not re-calculated.

Metabolite Name	(b) (4) Reference Standard N°	Supplier	Purity
Deferiprone 3-O-ß-D Glucuronide	RS-1487-A(F)	ApoPharma Inc. [2]	99.5%
Apo7339 [3]	RS-1487-B(F)	ApoPharma Inc.	99.6% [4]
Apo7339 [3]	RS-1487-C	ApoPharma Inc.	99.7%
			99.6%

[2] As the reference standard for Deferiprone 3-O-ß-D Glucuronide was certified by (b) (4) the supplier name mentioned above does not appear on the certificate of analysis.

[3] Apo7339 is the analyte name used in the certificate of analysis. This is an equivalent name for Deferiprone 3-O-β-D Glucuronide or Deferiprone 3-O-ß-D Glucuronide.

[4] Following recalculation of the water content (TGA), the potency was modified from 93.5% to 94.1%. Since the difference was less than 2%, the concentrations of stock solutions, calibrants and QC samples were not re-calculated.

Internal Standard 1 Name	(b) (4) Reference Standard N°	Supplier	Purity
Apo7114	RS-1501-A	ApoPharma Inc.	99.8%

Apo7114 is the analyte name used in the certificate of analysis. This is an equivalent name for Deferiprone-D3.

Internal Standard 2 Name	(b) (4) Reference Standard N°	Supplier	Purity
Deferiprone-D3 3-O-ß-D Glucuronide	RS-1488-A(F)	ApoPharma Inc.	99.6%
			99.4%

Preservative or Derivatizing agent: None

STOCK SOLUTIONS

Multiple stock solutions of Deferiprone and Deferiprone 3-O-ß-D Glucuronide were prepared at a concentration of 10.00 mg/mL in ACN:H2O 10:90% v/v and compared to verify the accuracy of reference standard weighing.

CALIBRANTS

A set of 11 calibrants samples containing Deferiprone and Deferiprone 3-O-ß-D Glucuronide was prepared with drug free human serum. Calibrant concentrations of Deferiprone ranged from 0.100 µg/mL to 50.000

<table>
<tr><td></td><td>µg/mL (0.100, 0.200, 0.500, 1.000, 2.500, 7.500, 12.500, 20.000, 35.000, 42.500, 50.000). Calibrant concentrations of Deferiprone 3-O-ß-D Glucuronide ranged from 0.100 µg/mL to 60.000 µg/mL (0.100, 0.200, 0.500, 1.200, 3.000, 9.000, 15.000, 24.000, 42.000, 51.000, 60.000).</td></tr>
<tr><td>Validated assay range</td><td>The validated assay range for deferiprone is from 0.100 µg/mL to 50.000 µg/mL. However, since the expected C_{max} is at 6.089 µg/mL, the truncated range of 0.100 µg/mL to 25.000 µg/mL was employed in order to have a range that properly covers subject profiles.

The validated assay range for Deferiprone 3-O-β-D Glucuronide is from 0.100 µg/mL to 60.000 µg/mL. The range was truncated to 0.100 µg/mL to 30.000 µg/mL for Deferiprone 3-O-ß-D Glucuronide</td></tr>
<tr><td>Material used for QCs & concentration</td><td>REFERENCE STANDARDS

<table>
<tr><th>Analyte Name</th><th>(b) (4) Reference Standard N°</th><th>Supplier</th><th>Purity</th></tr>
<tr><td rowspan="3">Deferiprone</td><td>RS-1486-A</td><td>ApoPharma Inc.</td><td>>99.8%</td></tr>
<tr><td>RS-1486-B</td><td>ApoPharma Inc.</td><td>100.0% [1]</td></tr>
<tr><td>RS-1486-D</td><td>ApoPharma Inc.</td><td>99.99%</td></tr>
</table>
[1]The Deferiprone reference standard, which had a purity of 100.0% and a potency of 99.1%, was re-certified during the course of the validation. The purity obtained following re-certification was >99.8% and the potency obtained following re-certification was 98.9%. Since the difference was less than 2% for both purities and potencies, the concentrations of stock solutions, calibrants and QC samples were not re-calculated.

<table>
<tr><th>Metabolite Name</th><th>(b) (4) Reference Standard N°</th><th>Supplier</th><th>Purity</th></tr>
<tr><td>Deferiprone 3-O-ß-D Glucuronide</td><td>RS-1487-A(F)</td><td>ApoPharma Inc. [2]</td><td>99.5%</td></tr>
<tr><td>Apo7339 [3]</td><td>RS-1487-B(F)</td><td>ApoPharma Inc.</td><td>99.6% [4]</td></tr>
<tr><td rowspan="2">Apo7339 [3]</td><td rowspan="2">RS-1487-C</td><td rowspan="2">ApoPharma Inc.</td><td>99.7%</td></tr>
<tr><td>99.6%</td></tr>
</table>
[2] As the reference standard for Deferiprone 3-O-ß-D Glucuronide was certified by (b) (4) the supplier name mentioned above does not appear on the certificate of analysis.

[3] Apo7339 is the analyte name used in the certificate of analysis. This is an equivalent name for Deferiprone 3-O-β-D Glucuronide or Deferiprone 3-O-ß-D Glucuronide.

[4] Following recalculation of the water content (TGA), the potency was modified from 93.5% to 94.1%. Since the difference was less than 2%, the concentrations of stock solutions, calibrants and QC samples were not re-calculated.</td></tr>
</table>

	<table><tr><th>Internal Standard 1 Name</th><th>(b) (4) Reference Standard N°</th><th>Supplier</th><th>Purity</th></tr><tr><td>Apo7114</td><td>RS-1501-A</td><td>ApoPharma Inc.</td><td>99.8%</td></tr></table> Apo7114 is the analyte name used in the certificate of analysis. This is an equivalent name for Deferiprone-D3. <table><tr><th>Internal Standard 2 Name</th><th>(b) (4) Reference Standard N°</th><th>Supplier</th><th>Purity</th></tr><tr><td>Deferiprone-D3 3-O-ß-D Glucuronide</td><td>RS-1488-A(F)</td><td>ApoPharma Inc.</td><td>99.6% 99.4%</td></tr></table> STOCK SOLUTIONS Multiple stock solutions of Deferiprone and Deferiprone 3-O-ß-D Glucuronide were prepared at a concentration of 10.00 mg/mL in ACN:H2O 10:90% v/v and compared to verify the accuracy of reference standard weighing. QUALITY CONTROL SAMPLES A set of 5 levels of QC samples containing Deferiprone and Deferiprone 3-O-ß-D Glucuronide was prepared with drug free human serum. QC sample concentrations of Deferiprone were 0.100 µg/mL, 0.300 µg/mL, 5.000 µg/mL, 25.000 µg/mL and 37.500 µg/mL. QC sample concentrations of Deferiprone 3-O-ß-D Glucuronide were 0.100 µg/mL, 0.300 µg/mL, 6.000 µg/mL, 30.000 µg/mL and 45.000 µg/mL.	
Minimum required dilutions (MRDs)	NA		
Source & lot of reagents (LBA)	NA		
Regression model & weighting	Least squares regression analysis employing a weighted ($1/x^2$) linear (y=mx+b) for Deferiprone and Deferiprone 3-O-ß-D Glucuronide		
Validation parameters	Method validation summary		**Acceptability**
Standard calibration curve performance during accuracy & precision	Number of standard calibrators from LLOQ to ULOQ	11	Yes
	Cumulative accuracy (%bias) from LLOQ to ULOQ Deferiprone Deferiprone 3-O-ß-D Glucuronide	 92.7 to 105.5% 96.4 to 103.1%	Yes
	Cumulative precision (%CV) from LLOQ to ULOQ Deferiprone	 ≤ 9.0% ≤ 5.9%	Yes

	Deferiprone 3-O-ß-D Glucuronide		
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs QCs: Deferiprone Deferiprone 3-O-ß-D Glucuronide	 86.0 to 106.5% 96.1 to 108.3%	Yes
	Inter-batch %CV QCs: Deferiprone Deferiprone 3-O-ß-D Glucuronide	 ≤ 13.6% ≤ 6.4%	Yes
	Total Error (TE) QCs:	NA	Yes
Selectivity & matrix effect	Matrix effect was assessed by employing 6 independent lots of analyte-free matrix for the preparation of each of the low and high QC samples. Deferiprone: Accuracy (% nominal): 97.3% for Low QC and 99.9% for High QC. Precision: 6.4% for Low QC and 2.9% for High QC. Deferiprone 3-O-ß-D Glucuronide: Accuracy (% nominal): 105.3% for Low QC and 101.8% for High QC. Precision: 2.9% for Low QC and 2.1% for High QC.		Yes
Interference & specificity	No significant interference observed in the 10 blank matrix lots screened compared to the LOQ of 0.100 µg/mL for Deferiprone and for Deferiprone 3-O-ß-D Glucuronide, at the retention times and mass transitions of Deferiprone, Deferiprone 3-O-ß-D Glucuronide, IS1 or IS2. Assay specificity in the presence of concomitantly administered compounds was also assessed by adding the compounds to a lot of blank matrix demonstrated to have insignificant interference, as well as to low and high QC samples. The blank matrix was assayed with and without IS, and each QC concentration level was assayed in 6 replicates. Assay specificity in the presence of the		Yes

<table>
<tr><td></td><td>following concomitantly administered compounds was assessed:
<table><tr><th>Concomitantly Administered Compound Name</th><th>Concentration</th></tr><tr><td>Acetaminophen</td><td>50.00 µg/mL</td></tr><tr><td>Acetylsalicylic Acid</td><td>80.00 µg/mL</td></tr><tr><td>Caffeine</td><td>10.00 µg/mL</td></tr><tr><td>Ibuprofen</td><td>20.00 µg/mL</td></tr><tr><td>Nicotine</td><td>80.00 ng/mL</td></tr><tr><td>Cotinine</td><td>0.15 µg/mL</td></tr><tr><td>Dextromethorphan</td><td>0.80 µg/mL</td></tr><tr><td>Pseudoephedrine</td><td>2.00 µg/mL</td></tr><tr><td>Cyproterone Acetate</td><td>50.00 ng/mL</td></tr><tr><td>Drospirenone</td><td>0.15 µg/mL</td></tr><tr><td>Ethinyl Estradiol</td><td>0.25 ng/mL</td></tr><tr><td>3-Keto Desogestrel[1]</td><td>7.80 ng/mL</td></tr><tr><td>Levonorgestrel[2]</td><td>7.20 ng/mL</td></tr><tr><td>Norelgestromin[3]</td><td>5.20 ng/mL</td></tr><tr><td>Norethindrone</td><td>15.00 ng/mL</td></tr><tr><td>Acetylsalicylic Acid</td><td>5000.00 ng/mL</td></tr><tr><td>Salicylic Acid</td><td>20000.0 ng/mL</td></tr><tr><td>Isoniazid</td><td>16.00 µg/mL</td></tr></table>
No significant interference observed. Observed bias range is from 97.1 to 103.5% for Deferiprone, and from 93.3 to 106.2% for Deferiprone 3-O-ß-D Glucuronide.</td><td></td></tr>
<tr><td>Hemolysis effect</td><td>The impact of hemolysis was also assessed as part of the matrix effect by employing an additional lot of analyte-free hemolysed matrix for the preparation of each of the low and high QC samples. The hemolysed matrix tested consisted of blank matrix with the addition of a volume of thawed blood representing 5.0% of the total volume of blank matrix, which results in a heavily hemolysed sample. Each of the low and high QC sample concentration levels were assayed for this matrix lot. Acceptance criteria are met for matrix effect in hemolysed matrix. At least 67% (2/3) of the replicates at each of the low and high QC samples prepared were within ± 15.0% of their nominal concentrations.</td><td>Yes</td></tr>
<tr><td>Lipemic effect</td><td>The impact of the presence of lipids was also assessed as part of the matrix effect by employing an additional lot of analyte-free lipemic matrix for the preparation of each of the low and high QC samples. Lipemic matrix consists of blank matrix obtained from human donors with a high level of triglycerides, i.e. above 300 mg/dL (above 3.4 mM). Each of the low and high QC sample concentration levels were assayed for this matrix lot. Acceptance criteria are met for matrix effect in hemolysed matrix. At least 67% (2/3) of the replicates at each of the low and</td><td>Yes</td></tr>
</table>

	high QC samples prepared were within ± 15.0% of their nominal concentrations.	
Dilution linearity & hook effect	A QC pool was prepared in human serum at a concentration twice the ULQ concentration for Deferiprone (100 µg/mL) and Deferiprone 3-O-ß-D Glucuronide (120 µg/mL) to assess dilution integrity. Deferiprone: 100.000 µg/mL diluted 5-fold Accuracy (% nominal): 96.0% Precision 7.9% Deferiprone 3-O-ß-D Glucuronide: 120.000 µg/mL diluted 5-fold Accuracy (% nominal): 100.5% Precision 5.2%	Yes
Bench-top/process stability	The short-term stability of Deferiprone and Deferiprone 3-O-ß-D Glucuronide in human serum was successfully assessed by comparing 6 replicates of stability samples, maintained at 22°C nominal, versus 6 replicates of freshly prepared comparison samples at both low and high QC concentrations. Stability confirmed up to 19.6 hours at 22°C nominal. Percentage deviation from comparison samples range from 94.4 to 100.8% for Deferiprone, and from 96% to 98.4% for Deferiprone 3-O-ß-D Glucuronide. Percentage CV were ≤15.0% and %Nominal between 85.0%-115.0%. Autosampler storage stability of Deferiprone and Deferiprone 3-O-ß-D Glucuronide was successfully assessed by extracting, injecting and re-injecting QC samples at low and high concentrations. Re-injection was performed following storage at 4°C nominal. Concentrations were determined from a freshly extracted calibration curve assayed with both stability and comparison QC samples. Stability confirmed up to 142.7 hours at 4°C. Percentage deviation from comparison samples range from 98.9 to 103.7% for Deferiprone, and from 101.9 to 103.8% for Deferiprone 3-O-ß-D Glucuronide. Percentage CV were ≤15.0% and %Nominal between 85.0%-115.0%.	Yes
Freeze-Thaw stability	The freeze-thaw stability of Deferiprone and Deferiprone 3-O-ß-D Glucuronide in human serum was successfully	Yes

	assessed by comparing 6 replicates of stability samples, previously frozen at -20°C or -80°C nominal and thawed at 22°C nominal, over 3 cycles, versus 6 replicates of freshly prepared comparison samples, both at low and high QC concentrations. Percentage deviation from comparison samples range from 93.1 to 102.1% for Deferiprone, and from 94.9 to 96.6% for Deferiprone 3-O-ß-D Glucuronide. Percentage CV were ≤15.0% and %Nominal between 85.0%-115.0%.	
Long-term storage	The long-term stability of Deferiprone in human serum was successfully assessed by comparing 6 replicates of stability samples, stored unextracted at -80°C nominal, versus 6 replicates of freshly prepared comparison samples at both low and high QC concentrations. Stability confirmed up to 394 days at -20°C nominal. Percentage deviation from comparison samples range from 97.9 to 104.1% for Deferiprone, and from 109.3% to 112.2% for Deferiprone 3-O-ß-D Glucuronide. Percentage CV were ≤15.0% and %Nominal between 85.0%-115.0%. Stability confirmed up to 370 days at -80°C nominal. Percentage deviation from comparison samples range from 98.4 to 103.9% for Deferiprone, and from 101.6% to 106.8% for Deferiprone 3-O-ß-D Glucuronide. Percentage CV were ≤15.0% and %Nominal between 85.0%-115.0%.	Yes
Parallelism	Not applicable	N/A
Carry over	As potential significant carryover is expected with this analytical method, samples were injected using ordered (i.e. non-randomized) sequences, where the order in which the samples are injected is chosen such that the impact can be minimized. The presence or absence of carryover was evaluated prior to the injection of every batch using the injection of a high concentration sample (equivalent to the ULQ concentration) followed by analyte-free samples, as well as the injection of a LOQ sample. To start the injection of each batch, carryover must be deemed not significant, i.e. the response ratio of the applicable analyte-free sample must be ≤20.0% of the	Yes

	response ratio of the LOQ. Furthermore, carryover was continuously monitored within every batch when applicable, using the results of the analyte-free samples fortified with IS in each batch, which were always injected following a ULQ calibrant, both at the beginning and end of the injection sequence. The validation results that are reported in the present validation report met all batch and validation acceptance criteria and are thus free of significant carryover.	
Method performance in study number LA45-0116 BIOANALYTICAL REPORT N° APM-P9-463 SPONSOR PROJECT N° LA45-0116 STUDY FOR THE DETERMINATION OF DEFERIPRONE IN HUMAN SERUM USING HPLC WITH MS/MS DETECTION		
Assay passing rate	Of 2523 analyzable study samples received, 2523 samples were successfully assayed at (b) (4) Sixteen batches were analyzed during this study. Out of these, calibration curves were accepted in 12 batches and 2 were not evaluable due to a bioanalytical issue (error occurred during sample preparation). Analyses were repeated on 455 samples (including not evaluable batches and samples coded due to analytical reasons) which represents 18% of total analyzed samples.	Yes
Standard curve performance	• Cumulative bias range: 97.6 to 101.8% • Cumulative precision: ≤ 5.1% CV	Yes
QC performance	• Cumulative bias range: 96.8 to 103.4% • Cumulative precision: ≤ 4.6% CV The precision and accuracy criteria were met after excluding one value, significantly outside its nominal concentration, showing a concentration of 7.453 µg/mL and a percent deviation of 2384.2%. During review of the results, no specific problem arising from the processing of this sample was noted and only this sample appeared as potentially impacted. When this value is excluded from statistics, the precision and accuracy acceptance criteria would be met, with 4.6% for the precision and 101.2% for the accuracy. The results of precision (%C.V.) of the low QC sample concentration without excluding	Yes

	this value didn't meet acceptance criteria, i.e. %C.V. at 241.8%. • Cumulative bias range: 96.8 to 186.3*% • Cumulative precision: ≤ 241.8% CV During review of the results, no specific problem arising from the processing of this sample was noted and only this sample appeared as potentially impacted. This has no impact on the reported study samples concentrations since the method precision and accuracy was demonstrated.	
Method reproducibility	At least 10% of the total analyzable study samples were re-assayed and compared to the original values. For Deferiprone, 254 samples have been re-assayed as ISR, and on the 254 evaluable samples re-assayed, 250 samples (98.4 %) have met the percent difference criterion of ≤20.0%. At least 2/3 of the total samples selected for ISR evaluation have met the percent difference criteria of ≤20.0% between original and reassayed concentrations	Yes
Study sample analysis/ stability	Study samples, along with low and high QC samples, were stored under conditions described at -20 °C nominal. Study samples and calibrants/QC samples were stored for up to 35 days and 5 months, respectively prior to analysis.	

Method performance in study number LA53-0116 **BIOANALYTICAL REPORT N° APM-P7-564 SPONSOR PROJECT NO LA53-0116** **STUDY FOR THE DETERMINATION OF DEFERIPRONE IN HUMAN SERUM USING HPLC WITH MS/MS DETECTION**		
Assay passing rate	Of 1786 analyzable study samples received, 1786 samples were successfully assayed at (b) (4) . Seventeen batches were analyzed during this study and all were accepted. Analyses were repeated on 13 samples which represents 0.7% of total analyzed samples.	Yes
Standard curve performance	• Cumulative bias range: 96.6 to 104.2% • Cumulative precision: ≤ 7.6% CV	Yes
QC performance	• Cumulative bias range: 96.8 to 103.0%	Yes

	• Cumulative precision: ≤ 5.6% CV	
Method reproducibility	At least 10% of the total analyzable study samples were re-assayed and compared to the original values. For Deferiprone, 183 samples have been re-assayed as ISR, and on the 183 evaluable samples re-assayed, 178 samples (97.3%) have met the percent difference criterion of ≤20.0%. At least 2/3 of the total samples selected for ISR evaluation have met the percent difference criteria of ≤20.0% between original and re-assayed concentrations	Yes
Study sample analysis/ stability	Study samples, along with low and high QC samples, were stored under conditions described at -20 °C nominal. Study samples and calibrants/QC samples were stored for up to 43 days prior to analysis.	

Bioanalytical method validation report name and hyperlink	VALIDATION REPORT N° DFN-V3-523 (R5) VALIDATION OF A HPLC METHOD USING MS/MS DETECTION FOR THE DETERMINATION OF DEFERIPRONE AND DEFERIPRONE 3-O-ß-D GLUCURONIDE IN HUMAN SERUM Appendix 3		
Changes in method	Truncated concentration range		
New validated assay range if any	Deferiprone: 0.100 µg/mL to 35.000 µg/mL Deferiprone 3-O-ß-D Glucuronide: 0.100 µg/mL to 30.000 µg/mL		
Validation parameters	**Cross-validation performance**		**Acceptability**
Standard calibration curve performance during accuracy & precision	Cumulative accuracy (%bias) in standard calibrators from LLOQ to ULOQ Deferiprone Deferiprone 3-O-ß-D Glucuronide	 96.5 to 103.4% 96.1 to 102.0%	Yes
	Cumulative precision (%CV) from LLOQ to ULOQ Deferiprone Deferiprone 3-O-ß-D Glucuronide	 ≤ 8.1% ≤ 4.4%	Yes

QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs Deferiprone Deferiprone 3-O-ß-D Glucuronide	 96.3 to 104.3% 99.2 to 104.1%	Yes
	Inter-batch %CV Deferiprone Deferiprone 3-O-ß-D Glucuronide	 ≤ 7.5% ≤ 3.7%	Yes
	Percent total error (TE)	NA	
Cross-validation	Numbers of spiked or incurred samples analyzed and result	NA	

For studies LA53-0116 and LA45-0116, serum deferiprone and deferiprone 3-O-glucuronide concentrations were determined using a truncated concentration range than in DFN-V3-523 at [redacted] (b) (4) The table below describes modification and cross-validation performance

Bioanalytical method validation report name and hyperlink	VALIDATION REPORT N° DFN-V3-523 (R5) VALIDATION OF A HPLC METHOD USING MS/MS DETECTION FOR THE DETERMINATION OF DEFERIPRONE AND DEFERIPRONE 3-O-ß-D GLUCURONIDE IN HUMAN SERUM Appendix 3		
Changes in method	Truncated concentration range		
New validated assay range if any	Deferiprone: 0.100 µg/mL to 35.000 µg/mL Deferiprone 3-O-ß-D Glucuronide: 0.100 µg/mL to 30.000 µg/mL		
Validation parameters	**Cross-validation performance**		**Acceptability**
Standard calibration curve performance during accuracy & precision	Cumulative accuracy (%bias) in standard calibrators from LLOQ to ULOQ Deferiprone Deferiprone 3-O-ß-D Glucuronide	 96.5 to 103.4% 96.1 to 102.0%	Yes
	Cumulative precision (%CV) from LLOQ to ULOQ Deferiprone Deferiprone 3-O-ß-D Glucuronide	 ≤ 8.1% ≤ 4.4%	Yes

QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs Deferiprone Deferiprone 3-O-ß-D Glucuronide	 96.3 to 104.3% 99.2 to 104.1%	Yes
	Inter-batch %CV Deferiprone Deferiprone 3-O-ß-D Glucuronide	 ≤ 7.5% ≤ 3.7%	Yes
	Percent total error (TE)	NA	
Cross-validation	Numbers of spiked or incurred samples analyzed and result	NA	

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/s/

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