CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-250s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Biopharmaceutics Review

NDA:	**22-250**
Submission Date:	**January 30, 2009**
Type of Submission:	**Original New Drug Application**
Product name	**Fampridine**
Dosage Form:	**Sustained-Release Tablet**
Dosage Strengths:	**10 mg**
Sponsor:	**Acorda Therapeutics**

Recommendation

The proposed in vitro dissolution method and specifications provided in NDA 22-250 are acceptable. The in vitro dissolution method is a USP Type II (rotating paddles) apparatus employed at (b) (4) rpm, using (b) (4) mL of USP (b) (4) (pH (b) (4)), and sampling times are: (b) (4), and (b) (4) hours. The proposed specifications for the percent fampridine released from 10 mg SR tablet are as follows:

1 Hour	4 Hours	8 Hours	12 Hours
(b) (4)			

Background

Fampridine is a potassium (K^+) channel blocker proposed as a treatment for walking ability in patients with Multiple Sclerosis (MS). Acorda Therapeutics is submitting this New Drug Application for fampridine formulated as 10 mg extended-release tablets intended for twice daily administration.

Early studies were performed using IR capsules. Clinical results using the IR capsule formulation showed fampridine to have a narrow therapeutic index. The doses of fampridine required to maintain therapeutic plasma concentration were linked to serious adverse events (most notably a tonic-clonic seizure at a plasma level >100 ng/mL). Thus, sustained or controlled release formulations that could reduce the peak and trough plasma concentration were developed.

(b) (4)

Subsequently, Elan identified a sustained release matrix tablet formulation (Fampridine-SR tablet, 10 mg) as an appropriate formulation for commercial use. In this formulation, Elan employed their MXDAS™ (Hydrophilic Matrix Drug Absorption System) technology which uses (b) (4)

the (b) (4) The rest of the review focuses on this formulation.

The composition of the Fampridine-SR tablet, 10 mg is listed in Table 1 below.

Table 1: Composition of Fampridine-SR Tablets, 10 mg, used in Pivotal Clinical Trials/Proposed Commercial Formulation

Ingredient	Amount
Fampridine	10.0 mg
Hydroxypropyl Methylcellulose, USP	(b) (4)
Microcrystalline Cellulose, USP	
Colloidal Silicon Dioxide, NF	
Magnesium Stearate, USP	
(b) (4)	
Total Weight	410.0 mg

Two API suppliers (b) (4) and (b) (4) have been evaluated during the course of product development. The API used to manufacture Fampridine SR tablets for all Phase 2 and 3 clinical studies have been supplied by (b) (4) and will remain the commercial supplier for the API.

Two commercial drug product manufacturers are proposed, Elan Pharmaceuticals Plc in Athlone, Ireland and (b) (4) in (b) (4) The sponsor submitted bioequivalence study to show that Fampridine SR, 10 mg tablet manufactured by these two manufacturers are bioequivalent.

DISSOLUTION:

Fampridine-SR tablets, 10 mg is an extended-release dosage form designed to release the drug substance in a controlled manner over a 12-hour period. The proposed in vitro dissolution method is a USP Type II (rotating paddles) apparatus employed at 50 rpm, using 900 mL of USP degassed phosphate buffer (pH 6.8), and sampling times are: 0.5, 1, 2, 4, 6, 8, 10, and 12 hours. Additional pH profiling studies performed in dissolution media ranging from pH 1.2 to 7.2 showed equivalent dissolution profiles as seen in Figure 1 below.

Figure 1: Comparison of Percent Fampridine Released from 10 mg Fampridine-SR Tablets in Media of Various pH

(b) (4)

Acorda's proposed specifications for dissolution is provided in Table 2, below.

Table 2: Proposed Specifications for Percent Fampridine Release from 10 mg SR Tablet

1 Hour	4 Hours	8 Hours	12 Hours

(b) (4)

The proposed dissolution specifications are based on the % fampridine dissolved values obtained and the ranges of these values. Since no IVIVC was established, the proposed dissolution range limits were set at (b) (4) % for each time point. Sampling time points were chosen at the start, middle and end of dissolution (i.e., 1, 4, 8, and 12 hours), to reflect the profile of the dissolution curve. The quantitative limits for the ranges at each time point take into account the dissolution profiles (at release) generated by the two bioequivalence batches, i.e., lot 0000022747 and lot R0105001, the mean of the values obtained form all pivotal batches, as well as the distribution of that data.

The following studies were undertaken to examine the in vitro dissolution release profiles for the drug product. These studies are summarized as follows:

Two Stage Acid/Neutral Dissolution Profile: This study was performed using fampridine-SR 10 mg tablets manufactured by Elan to simulate gastric conditions over a 12-hour period (0.1 N HCl up to 4 hours followed by phosphate buffer pH 6.8 for the remaining time). The dissolution profile generated for the fampridine-SR 10 mg tablet is provided in Figure 2 below.

Figure 2: Two Stage Dissolution Profile for 10 mg Fampridine-SR (Elan)

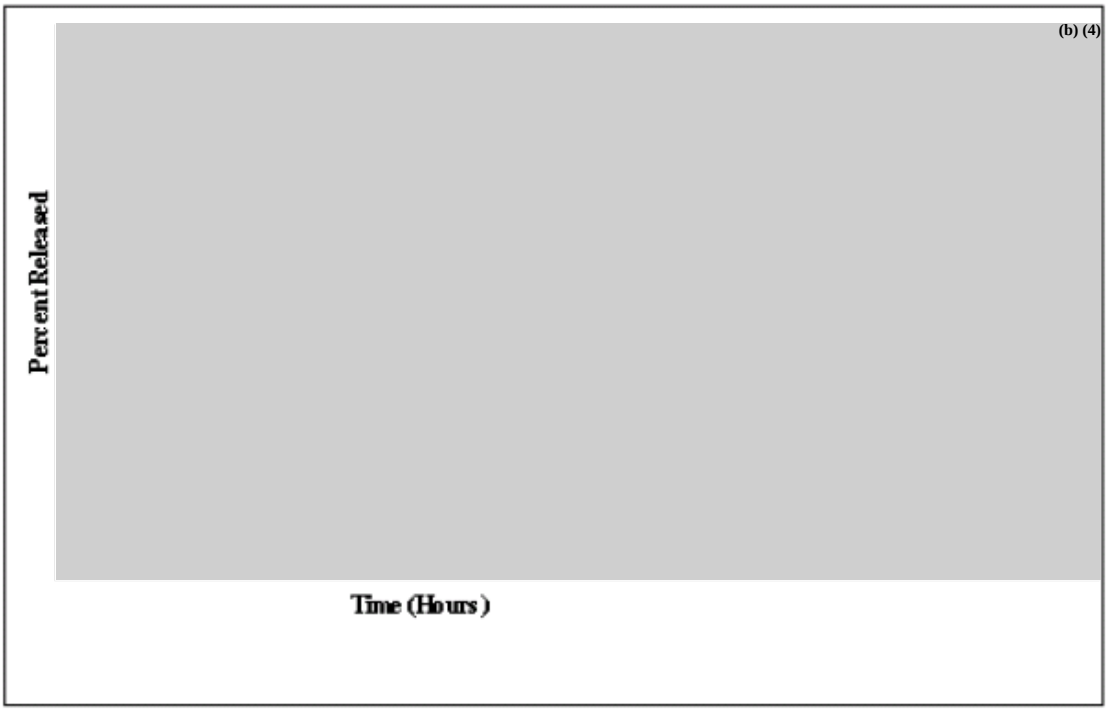
(b) (4)

Time (Hrs)

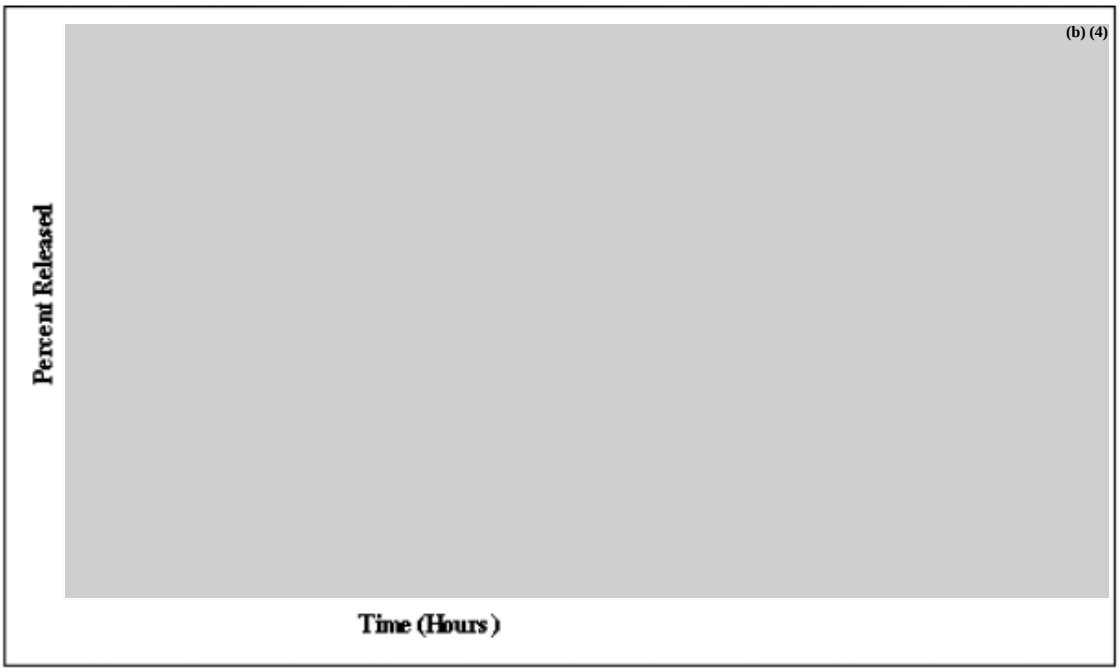
Alternative Drug Product Manufacturers: A study comparing dissolution profiles for Fampridine-SR tablets, 10 mg made at two different manufacturing sites (Elan and (b) (4) was performed in media at 4 different pH levels (1.2, 4.0, 6.8 and 7.2). This study also compared the effect of debossing on the resultant dissolution as the plain tablet (used for clinical blinding) was manufactured by Elan and the proposed commercial presentation (debossed with A10 on one side) was manufactured at (b) (4) Dissolution profiles at each pH (1.2, 4.0, 6.8 and 7.2) are presented in Figure 3 below.

Figure 3: Dissolution Profiles Comparing Alternative Drug Product Manufacturers (Elan and (b) (4) at four different pH levels (pH 1.2, 4.0, 6.8 and 7.2)

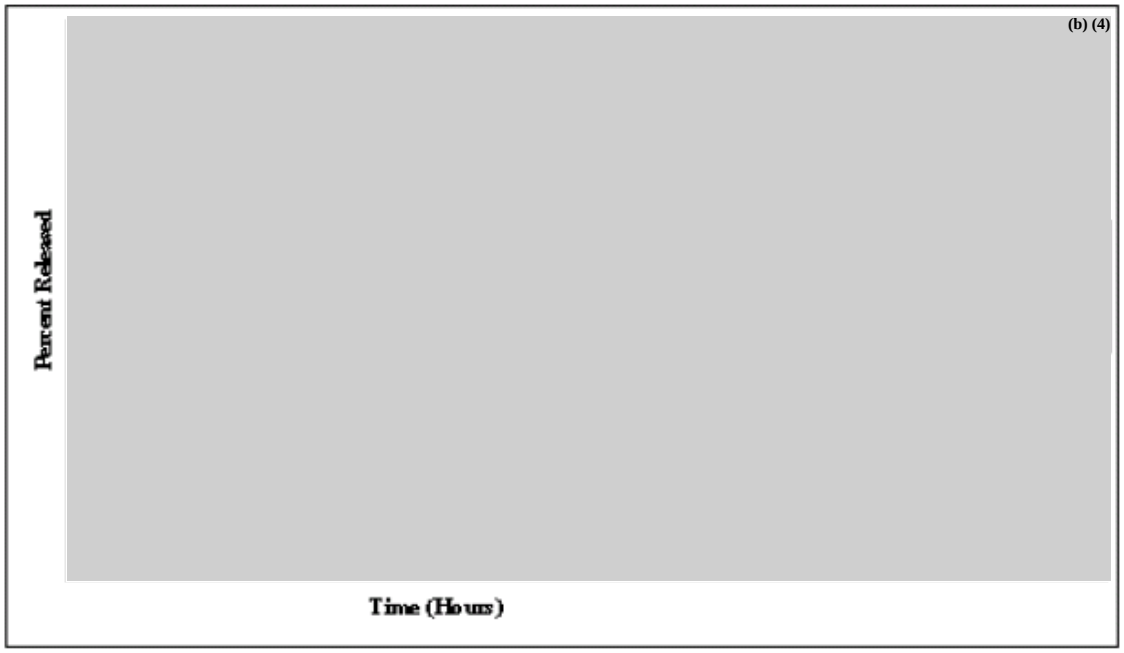
Dissolution at pH 1.2



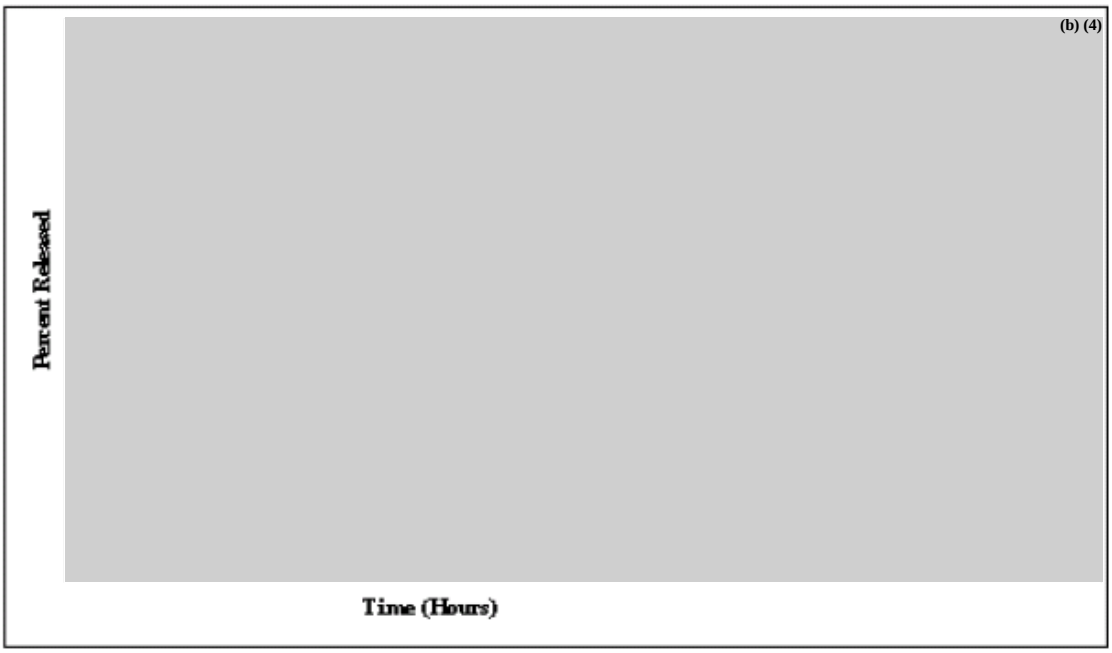
Dissolution at pH 4.0



Dissolution at pH 6.8



Dissolution at pH 7.2



f_2 analysis of the dissolution profiles demonstrates similarity of tablets manufactured at the two different sites, and similarity between debossed and non-debossed tablets as seen in Table 3 below.

Table 3: f_2 Statistics for Fampridine-SR Tablets: Site of Manufacture

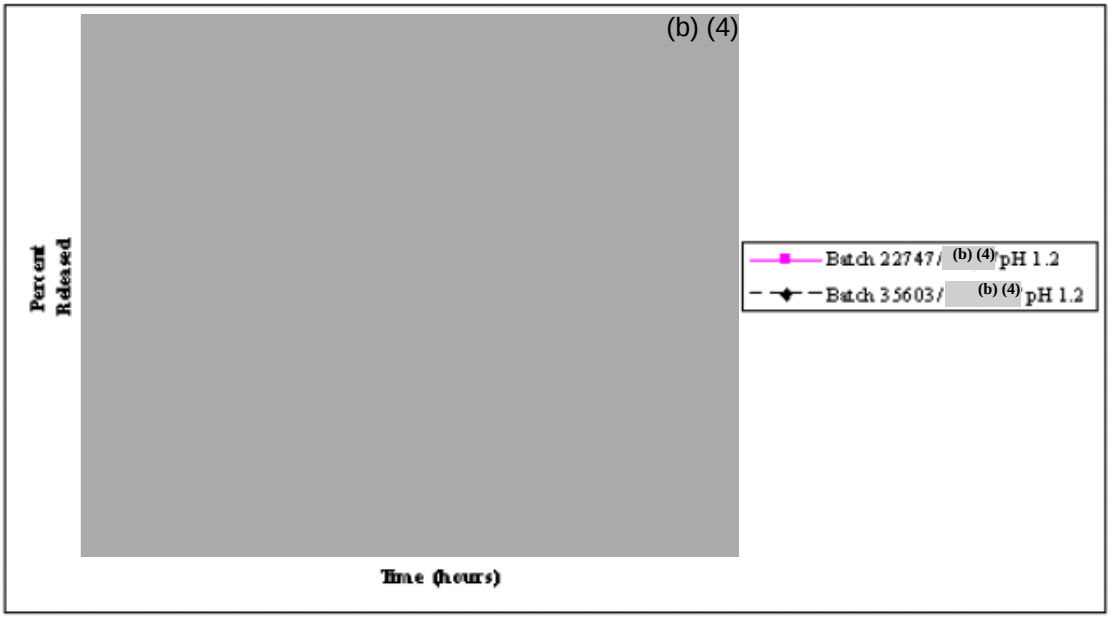
Media pH	Elan Batch No. (Strength)		(b) (4) Batch No. (Strength)	f_2 statistic
				(b) (4)

Alternative API Manufacturers: A study comparing dissolution profiles for fampridine-SR tablets, 10 mg manufactured using API from two different sources (b) (4) was performed in media at 4 different pH levels (1.2, 4.0, 6.8, and 7.2). This study also compared the effect of debossing on the resultant dissolution as the plain tablet (used for clinical blinding) was manufactured by Elan using (b) (4) and the proposed commercial presentation (debossed with A10 on one side) was manufactured by Elan using (b) (4). An f_2 of the dissolution profiles showed

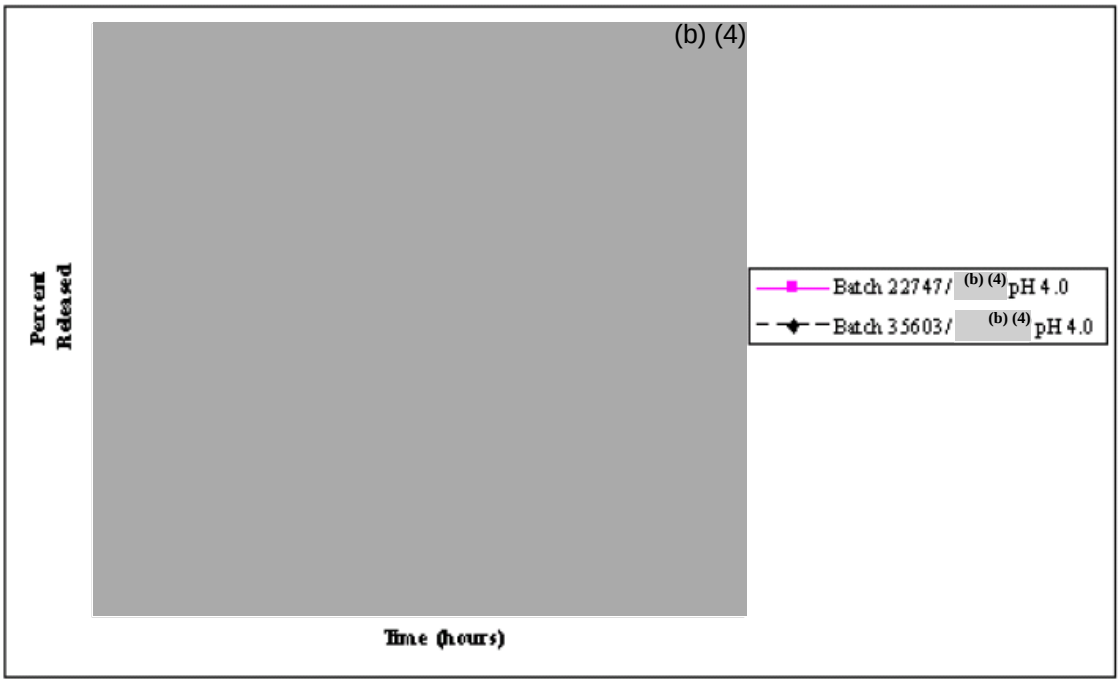
similarity of tablets manufactured from both sources of API. Dissolution profiles are shown in Figure 4 below.

Figure 4: Dissolution Profiles Comparing Alternative API Manufacturers (b) (4)

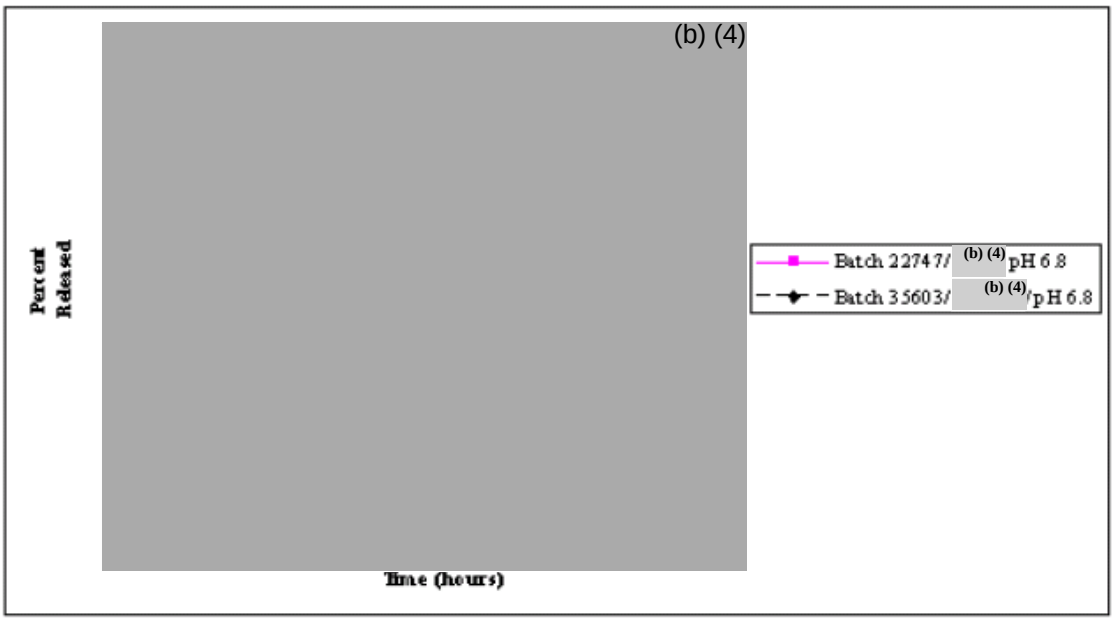
Dissolution at pH 1.2



Dissolution at pH 4.0



Dissolution at pH 6.8



Dissolution at pH 7.2

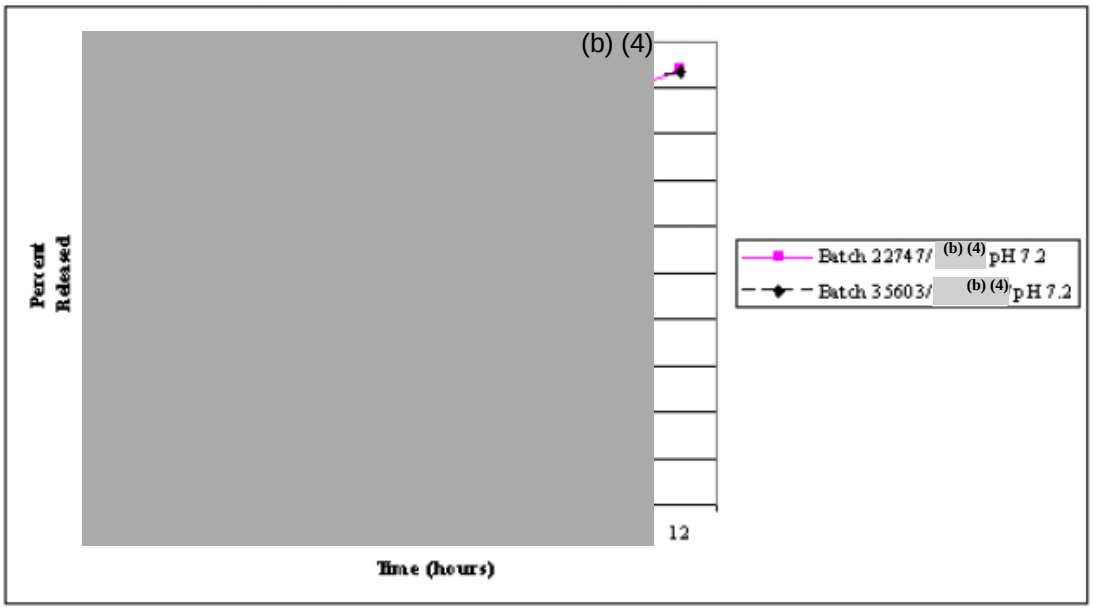


Table 4: Statistics for Fampridine-SR Tablets: Varying API Supplier

Media pH	(b) (4) Non-debossed Tablet Batch Tested with Media/Lot No.		(b) (4) Debossed Tablet Batch Tested with Media/Lot No.	f_2 Statistic (b) (4)
pH 1.2	0000022747 (10 mg Tablet)	*Vs.*	0000035603 (10 mg Tablet)	
pH 4.0	0000022747 (10 mg Tablet)	*Vs.*	0000035603 (10 mg Tablet)	
pH 6.8	0000022747 (10 mg Tablet)	*Vs.*	0000035603 (10 mg Tablet)	
pH 7.2	0000022747 (10 mg Tablet)	*vs.*	0000035603 (10 mg Tablet)	

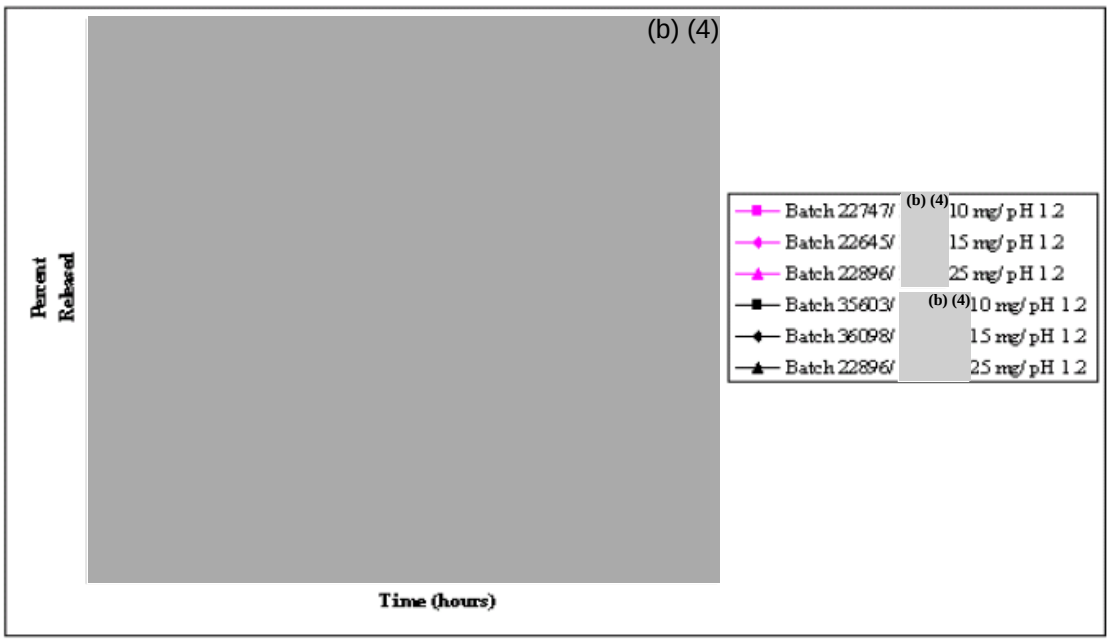
Effect of Tablet Strength on Drug Release: An additional dissolution profiling study was conducted, in media at 4 different pH levels (1.2, 4.0, 6.8, and 7.2), to demonstrate equivalence between three different dosage strengths (10 mg, 15 mg, and 25 mg) (b) (4) Tablets in this study were manufactured by Elan and were white and non-debossed. This information is being provided for information purposes. All three tablet strengths exhibit similar in vitro dissolution profile. f_2 values are provided in Table 5 and dissolution profiles at each pH (1.2, 4.0, 6.8, and 7.2) are presented in Figure 5 below .

Table 5: Statistics for Fampridine-SR Tablets: Varying Strengths

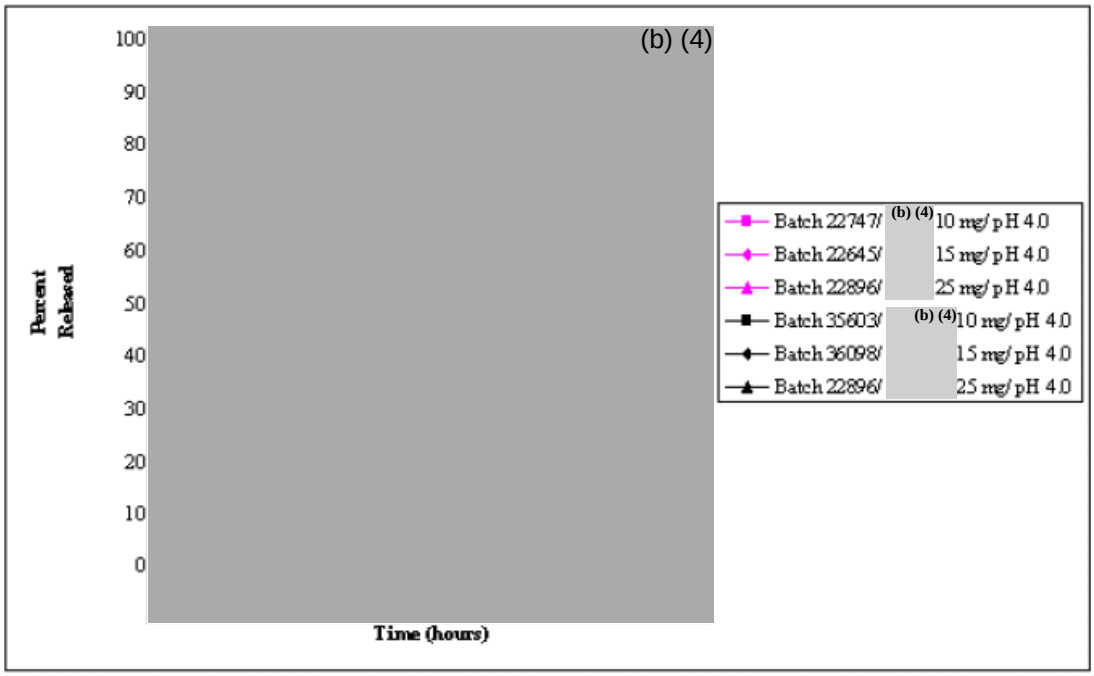
Media pH	Batch Nos. Compared Strength		f_2 Statistic
pH 1.2	22747 (10 mg Tablet)	22645 (15 mg Tablet)	(b) (4)
	22747 (10 mg Tablet)	22896 (25 mg Tablet)	
	22645 (15 mg Tablet)	22896 (25 mg Tablet)	
pH 4.0	22747 (10 mg Tablet)	22645 (15 mg Tablet)	
	22747 (10 mg Tablet)	22896 (25 mg Tablet)	
	22645 (15 mg Tablet)	22896 (25 mg Tablet)	
pH 6.8	22747 (10 mg Tablet)	22645 (15 mg Tablet)	
	22747 (10 mg Tablet)	22896 (25 mg Tablet)	
	22645 (15 mg Tablet)	22896 (25 mg Tablet)	
pH 7.2	22645 (15 mg Tablet)	22896 (25 mg Tablet)	
	22747 (10 mg Tablet)	22645 (15 mg Tablet)	
	22747 (10 mg Tablet)	22896 (25 mg Tablet)	

Figure 5: Dissolution Profiles Supporting Dose Proportionality of Various Fampridine Formulations

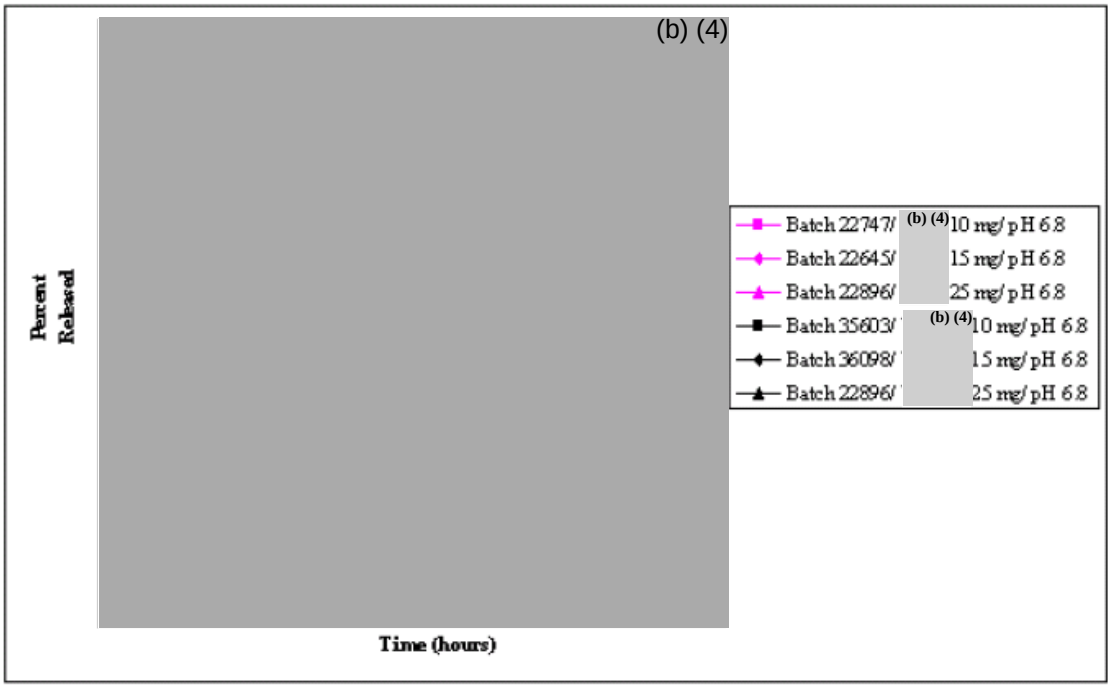
Dissolution at pH 1.2



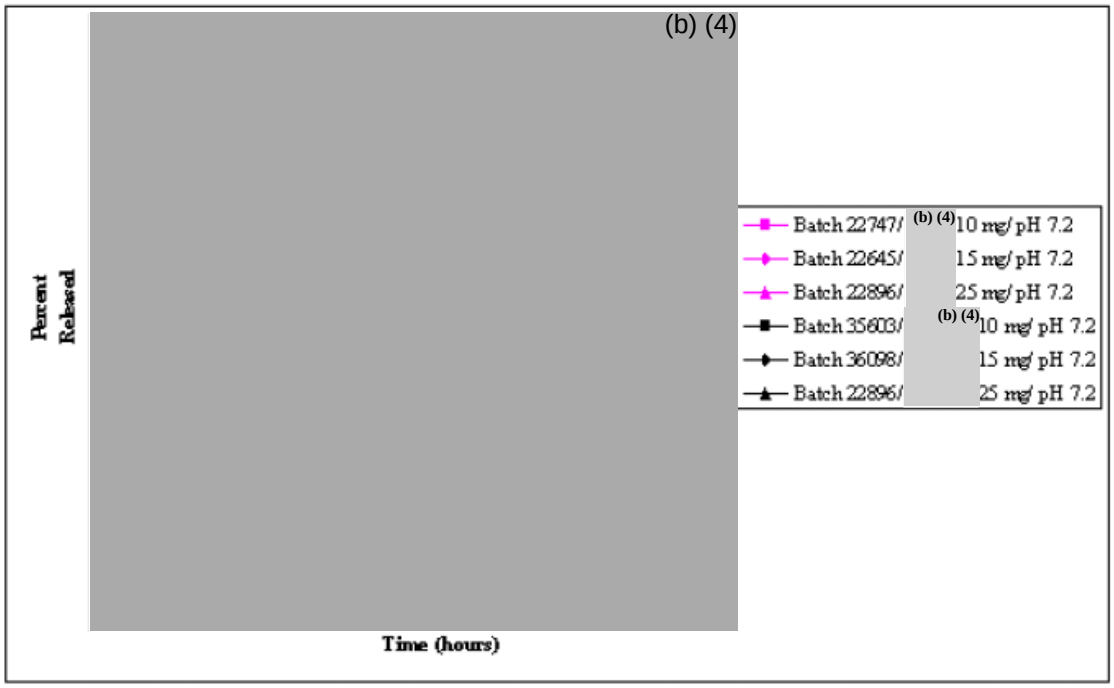
Dissolution at pH 4.0



Dissolution at pH 6.8



Dissolution at pH 7.2



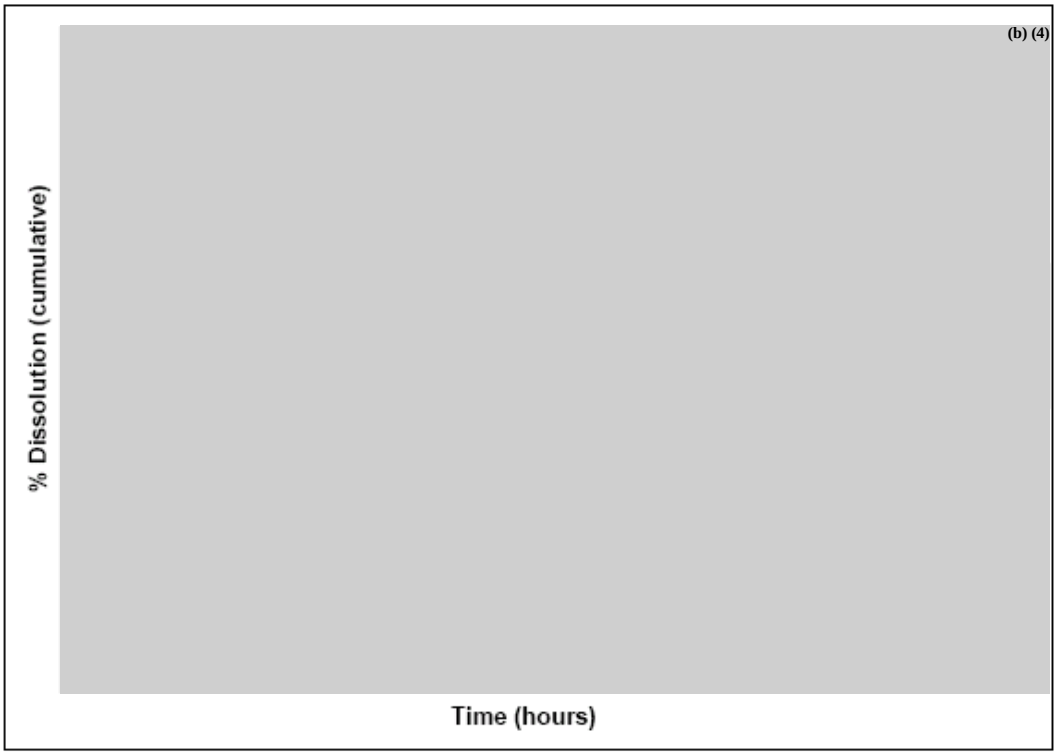
Effect of Alcohol: Acorda conducted a study to determine whether fampridine-SR tablets, 10 mg undergo "dose dumping" when combined with alcohol (ethanol). The study evaluated the impact of ethanol, for a 2-hour period at varying concentrations, on the dissolution profile of Fampridine-SR tablets, 10 mg. The study was performed as follows: 12 tablets (Elan Batch No. 0000051013) were placed in one of four dissolution vessels (baskets - USP Apparatus type 1) in 900-mL of 0.1N HCl media containing Alcohol (USP) at concentrations of 0%, 5%, 20%, or 40%. The apparatus was rotated at 100 rpm. The tablets were held in this acidic media for 2 hours in order to mimic human stomach residence time and conditions. After 2 hours, the samples were transferred to vessels (paddles - USP Apparatus type 2) containing 900 mL of the standard media (phosphate buffer at a pH of 6.8). The paddles were rotated at 50 rpm and dissolution testing was continued through to the standard 12-hour period.

Results of the study are presented in Table 6 and Figure 6 below. There is no evidence of dose dumping at any time-point, even under conditions of 40% alcohol. At the 4 hour time point, the results for 0% and 40% ethanol were marginally outside the release specification of (b) (4) This result is not surprising as the dissolution methodology necessary to perform this ethanol study (b) (4) is different than the validated dissolution method used in measuring specification values at release and on stability.

Table 6: Effect of Ethanol on Mean Dissolution Profiles of 10 mg Fampridine-SR Tablets (n=6)

	% Ethanol			
Time (Hours)	0%	5%	20%	40%
0.25	(b) (4)			
0.5				
0.75				
1				
1.25				
1.5				
1.75				
2				
4				
6				
8				
10				
12				

Figure 6: Dissolution Profile of 10 mg Fampridine-SR in Ethanol



In Vitro-In Vivo Correlation: Two studies were conducted to assess the potential of finding an in vitro-in vivo correlations during the fampridine development program, (b) (4) Since no in vitro/in vivo correlation was established no further discussion is presented here.

Analytical Method:

The initial dissolution method developed at Elan employed a (b) (4) method. This method was validated under Elan Validation Report (b) (4) Subsequently, Elan developed an (b) (4) for dissolution and performed a validation of this method under Elan Validation Report (b) (4) Elan will only be using the (b) (4) method.

During a technology transfer from Elan to (b) (4) for Fampridine-SR tablets, (b) (4) validated a (b) (4) dissolution method under (b) (4) Report (b) (4)

The fampridine dissolution method had previously been analyzed by a UV method using (b) (4). However, on validation of the UV method, it was determined that the (b) (4) tablet coating was responsible for a significant bias in the results. The current method uses HPLC with UV detection measured over a 235-290 nm wavelength range to eliminate this bias.

Elan Analytical Method:

Linearity, Accuracy, Bias and Range: The method was validated for testing Fampridine film coated 5, 10, 15, 20, 25, 30 and 40 mg SR tablets. The method is accurate and linear. The range covered was 5% of the lowest label claim to 125% of the highest label claim. The observed bias ranged from -0.3% to 1.5%. The linear regression analysis at all time points resulted in a R^2 value greater than 0.99 and the 95% CI about the intercept contains ± 0.5%. The 95% CI about the slope contains a value of 1 for all except the 8, 10 and 12-hour time-points. The values of the slope range from 0.98 to 0.99 in these instances which is considered acceptable.

Intra-Day Precision: The difference in the means of each dissolution profile obtained on the same day was compared for each analyst for each tablet strength. For the 10 mg and 40 mg strengths, practically all time points for the 95% CI contained a value of zero for each analyst, indicating no statistically significant difference in the profiles. For all time-points, the 95% CI contained a value of ± 3% which meets the predefined acceptance criteria of the study.

Inter-Day: The difference in the mean profiles obtained between days/analysts was compared for each strength. While the 95% CI did not contain zero for all time-points, it did contain a value of ± 4% for all time-points which meets the acceptance criteria of the study.

Solution Stability: The stock 4-Aminopyridine standard solutions are stable for 7 days when stored at room temperature.

(b) (4) **Analytical Method**: The following validation parameters were investigated: system suitability, specificity, linearity, accuracy, repeatability, intermediate precision, robustness and stability of standard and sample solutions.

Method Validation for 10 mg Tablet	
System suitability	% recovery of Fampridine ranged from 99.6% to 99.7%
Specificity	10 mg Tablet Placebo = 0.2%
Linearity	R= 1.0000, Slope = 1.007, Y-intercept = 0.1040
Accuracy	For 10% of 10 mg Tablet, the mean % Recovery =105.7%, RSD=4.8% For 100% of 10 mg Tablet, the mean % Recovery =101.4%, RSD = 0.5%
Repeatability	Mean % fampridine release at 0.5 h time Point = 18%, RSD 11.2% Mean % fampridine release at 1 h time Point = 26%, RSD 1.6% Mean % fampridine release at 10 h time Point =91%, RSD 0.6% Mean % fampridine release at 10 h time Point = 94%, RSD 0.4%
Intermediate precision	Mean % fampridine release at 0.5 h time Point =19%, RSD 2.1% Mean % fampridine release at 1 h time Point = 27%, RSD 2.3% Mean % fampridine release at 10 h time Point = 93%, RSD 1.5% Mean % fampridine release at 10 h time Point = 94%, RSD 0.4%
Robustness	93%-95%, RSD 0.6%
Stability	Fampridine in the working standard and Sample solution is stable for up to 3 days at room temperature and under refrigeration

Comments to Chemistry Reviewer

An f_2 analysis of the dissolution profiles demonstrated similarity of tablets manufactured at the two different sites (Elan and (b) (4)), similarity between the debossed and non-debossed tablets, and similarity of tablets manufactured from both sources of the API (b) (4) This confirms the similarity between clinical trial and proposed commercial formulations of Fampridine-SR tablet, 10 mg.

The proposed specification is acceptable and confirms the extended-release performance of the matrix tablet formulation.

There was a lack of effect of high concentrations of alcohol on fampridine-SR tablets, 10 mg which indicate that the tablet does not undergo “dose dumping” when combined with alcohol.

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/s/

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5/4/2009 09:35:16 PM
BIOPHARMACEUTICS

Patrick Marroum
5/5/2009 09:24:00 AM
BIOPHARMACEUTICS

Clinical Pharmacology/Biopharmaceutics Review

PRODUCT (Generic Name):	Fampridine (4-Aminopyridine)
NDA:	22-250
SUBMISSION DATES:	4/22/09, 5/20/09, 5/28/09, 6/22/09, 6/24/09, 6/30/09, 7/14/09, 7/21/09, 7/22/09, 7/24/09, 8/4/09, 8/5/09, 8/12/09, 8/14/09, 8/20/09
PRODUCT (Brand Name):	Amaya™
DOSAGE FORM:	Extended Release Tablet
DOSAGE STRENGTH:	10 mg
INDICATION:	Treatment of patients with multiple sclerosis for the improvement of walking ability
NDA TYPE:	505 (b)(i)
SPONSOR:	Acorda Therapeutics, Inc.
OCP REVIEWER:	Jagan Mohan Parepally, Ph.D.
OCP TEAM LEADER:	Angela Men, M.D, Ph.D.
PM REVIEWER	Joo Yeon Lee, Ph.D
PM TEAM LEADER	Yaning Wang, Ph.D.
OCP DIVISION:	DCP-1, HFD-860
OND DIVISION:	Division of Neurology Drug Products, HFD-120

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY 4

1.1 RECOMMENDATION 4
1.2 PHASE IV REQUIREMENTS 5
1.3 OVERALL SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS 5

2.0 QUESTION BASED REVIEW 12

2.1 GENERAL ATTRIBUTES 12
2.1.1 What are the highlights of the chemistry and physicochemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review? 12
2.1.2 What is the proposed mechanism(s) of action and therapeutic indication(s)? 12
2.1.3 What is the proposed dosage(s) and route(s) of administration? 12
2.2 GENERAL CLINICAL PHARMACOLOGY 13
2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims? 13
2.2.2 Is there any significant exposure-response relationship? And does the relationship support the proposed dose (10mg bid)? 15
2.2.3 Does this drug prolong the QT or QTc interval? 17
2.2.4 What are the PK characteristics of the drug and its major metabolite? 18
2.2.5 What are the single dose and multiple dose PK parameters? 19
2.2.6 How does the PK of the drug in healthy volunteers compare to that in patients? 21
2.2.7 What are the characteristics of drug absorption? 22
2.2.8 What are the characteristics of drug distribution? 22
2.2.9 What are the characteristics of drug metabolism and elimination? 22
2.2.10 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship? 24
2.2.11 How do the PK parameters change with time following chronic dosing? 26
2.3 INTRINSIC FACTORS 26
2.3.1 What intrinsic factors (age, gender, race, weight, and organ dysfunction) influence exposure, and what is the impact of any differences in exposure on efficacy or safety responses? 26
2.3.2 Is there a need for dose adjustment in patients with mild and moderate renal impairment? What is the recommended dose in this special population? 28
2.3.3 Effect of Hepatic Impairment 28
2.3.4 Effect of Age 29
2.3.5 Effect of Gender 29
2.3.6 Effect of Race 30
2.3.7 Is there any information for the use of fampridine in pregnancy and lactation is in the application? 30
2.4 EXTRINSIC FACTORS 30
2.4.1 Is there an in vitro basis to suspect in vivo drug-drug interactions? 30
2.4.2 Is the drug an inhibitor and/or an inducer of CYP enzymes on an in vitro basis? 30
2.4.3 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes or other metabolic/transporter pathways? 31
2.4.4 Is there a change in PK profile of fampridine or any other concomitantly administered drugs commonly used in multiple sclerosis patients? 33
2.4.5 Is there a known mechanistic basis for renal elimination and drug-drug interactions related to this route of elimination? 34
2.4.6 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any? 35
2.5 GENERAL BIOPHARMACEUTICS 35
2.5.1 What is the composition of fampridine SR(b)(4) capsule formulations used in earlier clinical trials? 35
2.5.2 What is the composition of the to-be-marketed film-coated fampridine-SR tablet product? 36
2.5.3 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial? 36
2.5.4 What is the absolute bioavailability and relative bioavailability of the proposed to-be-marketed formulation to other dosage forms? 38
***2.5.5** What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types? 40*
2.5.6 What is the effect of alcohol intake on the bioavailability (BA) of the drug from the dosage form? 40
2.6 ANALYTICAL 41

2.6.1 Have the analytical methods been sufficiently validated? *41*

3.0 LABELING RECOMMENDATIONS **44**

4.0 APPENDIX **68**

4.1 INDIVIDUAL STUDY REVIEWS 68
4.1.1 PHARMACOKINETICS STUDIES *68*
4.1.2 INTRINSIC FACTORS *85*
4.1.3 EXTRINSIC FACTORS *94*
4.1.4 BIOPHARMACEUTIC STUDIES *108*
4.1.5 IN VITRO STUDIES *127*
4.2 PHARMACOMETRICS REVIEW 173
4.3 THOROUGH QT STUDY REVIEW

1.0 EXECUTIVE SUMMARY

The sponsor is seeking approval of Amaya™ (fampridine) as a treatment to improve walking ability in patients with multiple sclerosis (MS). Fampridine or 4-aminopyridine is a potassium channel blocker. Fampridine was primarily used as a research tool, in characterizing subtypes of potassium channel in laboratory. Fampridine has an orphan drug status in United States. To support the approval of the application, the clinical pharmacology and biopharmaceutics program for fampridine, consisted of 20 clinical studies, focusing on relative bioavailability of various oral formulations; pharmacokinetic characterization in healthy volunteers, MS patients and special populations; elucidation of the drug-drug interaction potential both *in vitro* and *in vivo* with commonly used concomitant medications; population pharmacokinetic and exposure-response analyses using data from various Phase 1, 2 studies and the pivotal clinical trials.

The proposed commercial dosage form is extended release film-coated tablet and the strength is 10 mg. The proposed dose for fampridine is 10 mg b.i.d.

Significant exposure response (the percent change from the baseline at the end of double blind phase in walking speed (ft/sec)) relationship was identified with placebo included in the exposure-response analysis. The relationship looked flat without placebo within the observed exposure range. At 10 mg bid dosing, exposure of fampridine for most of the patients appeared to be above 200 µg·hr/mL and few below 200 µg·hr/mL. The probability of having at least one CNS-related AE was steeply increased for patients with fampridine exposure above 200 µg·hr/mL. A lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

The dose has to be reduced to 7.5 mg b.i.d in patients with mild and moderate impairment based on exposure-response relationship of fampridine. Caution should be taken in prescribing 7.5 mg b.i.d fampridine in patients with moderate renal impairment. Renal function in moderately impaired patients should be closely monitored since higher exposure of fampridine may lead to CNS adverse events. Fampridine is not recommended in severe renal impairment.

1.1 RECOMMENDATION

The Office of Clinical Pharmacology (OCP/DCP I) has reviewed the clinical Pharmacology and Biopharmaceutics sections of the NDA 22-250. The submission is acceptable from a Clinical Pharmacology and Biopharmaceutics point of view provided the sponsor agrees with the Agency's labeling recommendations.

Labeling recommendations outlined in the Detailed Labeling Recommendations section of the review on page 43 should be conveyed to the sponsor.

<u>Comments to the Medical Officer</u>:

- For mild and moderate renal impairment patients, dose adjustment to 7.5 mg b.i.d is necessary based on the safety and efficacy profile of fampridine if 10 mg b.i.d dose is acceptable in normal MS patients. Fampridine is not recommended in severe renal impairment.

- A lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

1.2 PHASE IV REQUIREMENT

A new strength 7.5 mg fampridine-SR formulation is required for dose adjustment in patients with mild to moderate renal impairment.

1.3 OVERALL SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

Background:

Clinical pharmacology program included 20 clinical studies. To support the efficacy for multiple sclerosis of fampridine as a treatment to improve walking ability in patients with multiple sclerosis, this NDA contains results from 2 adequate and well-controlled trials (MS-F203 and MS-F204), one placebo-controlled, dose-ranging study (MS-F202), one early stage placebo controlled dose ranging study (MS-F201) and three long term open-label extension studies (MSF202EXT, MS-F203EXT and MS-F204EXT). To support the safety of fampridine data from clinical studies in spinal chord injury patients was also included along with the data from healthy subjects and multiple sclerosis patients. In addition, 5 *in vitro* studies were conducted to characterize the fampridine metabolism, plasma protein-binding, to identify the potential drug-drug interactions involving inhibition and induction, and to investigate the potential for involvement of transporters (P-gp). Important clinical pharmacology findings are summarized below.

General Pharmacokinetic Properties:

- The plasma C_{max} and AUC values of fampridine increased in a dose proportional manner following single-dose administration of 5-25 mg. Dose proportionality was also observed with 5-20 mg administered q12h as a tablet in the fasted state and further confirmed with fampridine SR 12.5 tablet following multiple-dose.
- The steady-state concentrations of fampridine in plasma are achieved within 4 days of q12h dosing. This is consistent with its apparent elimination half-life in plasma of approximately 5-6 h.

<u>Absorption:</u>

- Fampridine is rapidly and almost completely absorbed from gastrointestinal tract following oral administration.
- Relative bioavailability was 96% when compared to an aqueous oral solution. The sustained release tablet delays absorption of fampridine relative to the solution formulation characterized by lower Cmax concentration and delayed Tmax, with no effect on the extent of absorption.
- Food had relatively small impact on Cmax and AUC (a slight increase of approximately 17% and approximately 5% respectively) under fed conditions. Tmax values were delayed by approximately 2 hours. Since fampridine is indicated for chronic dosing, the delay in Tmax is not considered clinically significant. The results justify administration of fampridine-SR tablets with or without regard to food.

Distribution

- The apparent volume of distribution of fampridine is 2.6 L/kg.
- The mean protein binding was 1-3%, evaluated over a range of concentrations (5, 50, and 500 ng/mL) and pH (7.2-7.6). Fampridine was largely unbound and had a high free drug fraction at all three concentrations tested.

Metabolism and Elimination

- Fampridine is not extensively metabolized and mainly eliminated as unchanged drug in urine.
- The 2 major metabolites 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate were identified, both were inactive.
- *In vitro* studies with human liver microsomes indicate that CYP2E1 was the major enzyme responsible for the 3-hydroxylation of fampridine based on correlation analysis, chemical inhibition studies and incubations with recombinant human CYP enzymes. Several other CYP enzymes may be involved in playing a minor role in the 3-hydroxylation of fampridine.
- Radiolabeled mass-balance and metabolism study indicates that fampridine and metabolites are eliminated nearly completely after 24 hours with 96% of the dose recovered in the urine and 0.5% recovery in feces. Most of the excreted radioactivity in the 0-4 hour pooled urine was parent drug (90.3%). Two metabolites were identified: 3-hydroxy-4-aminopyridine (4.3%) and 3-hydroxy-4-aminopyridine sulfate (2.6%). These metabolites have been shown to have no pharmacologic activity on potassium channels.
- The elimination half-life of fampridine following administration of SR tablet formulation was 5.2 to 6.5 hours. The plasma half-life of the sulfate conjugate is approximately 7.6 hours and the half-life of 3-hydroxy-4-aminopyridine was not determined as concentrations for most subjects were close to or below the limit of quantitation.

- Overall renal clearance of fampridine was 22.2 L/hour (370 mL/min), suggests active tubular secretion since it is much higher than the glomerular filtration rate (GFR).

Relative Bioavailability and Bioequivalence

- The relative bioavailability of fampridine-SR 10 mg dose used in the pivotal Phase 3 studies MS-F203 and MS-F204 as compared to a 10 mg buffered aqueous oral solution (0.83 mg/mL) was assessed under fasting conditions. Fampridine-SR tablets, 25 mg were studied in the third arm. The peak concentrations following administration as a sustained release tablet were approximately 40% of those achieved with the oral solution and occur approximately 3.5 hours postdose. The plasma elimination half-life was prolonged, from 3.2 hours for the oral solution versus 5.4 to 5.5 hours on average for the sustained release tablets. The two sustained release tablets were bioequivalent.

- A bioavailability study to assess the bioequivalence of the 10 mg strength of Fampridine-SR tablets was conducted to support the substitution of the product (manufactured by Elan) by alternate manufacturer (b) (4) Single dose administration of 10 mg Fampridine-SR test formulation manufactured by (b) (4) was bioequivalent to reference formulation manufactured by Elan Pharmaceuticals (E10). The 90% CI of C_{max} and $AUC_{0\text{-}inf}$ fell within 80-125% acceptance criteria for BE.

Intrinsic Factors:

Body mass index and subject status (healthy volunteers vs. MS patients) did not appear to effect pharmacokinetics of fampridine according to population PK analysis.

Renal impairment: following single-dose administration of fampridine-SR in subjects with normal renal function, mild, moderate or severe renal impairment.

The mean Cmax and AUC(0-inf) of fampridine increased by 67% and 75% in mildly impaired subjects, by 60% and 105% in moderately impaired subjects, and by 100% and 299% in severely impaired subjects, respectively, when compared to normal subjects.

The mean C_{max} and $AUC_{(0\text{-}inf)}$ of 3-hydroxy-4-aminopyridine sulfate increased by 35% and 80% in mildly impaired subjects, by 123% and 216% in moderately impaired subjects, and by 8 fold and 26 fold in severely impaired subjects, respectively, when compared to normal subjects. The CL/F and CLr of fampridine showed significant relationship ($p < 0.0001$ for CL/F and $p = 0.0001$ for CLr) with creatinine clearance.

Hepatic Impairment: Fampridine has not been studied in patients with hepatic impairment. Since fampridine is primarily excreted unchanged in the urine, hepatic

insufficiency may not significantly affect fampridine pharmacokinetics or recommended dosing.

Age: A population pharmacokinetic analysis showed that fampridine clearance decreases with increasing age (49L/hr→39L/hr over 20 years to 80 years).

Gender: A population pharmacokinetic analysis showed that fampridine clearance was approximately 14.5% lower for females (36 L/hr) at the same age and CrCL. Also, the volume of distribution was found to be significantly lower in females as compared to males.

Race (Caucasian vs. Non-Caucasian): There were no effects of ethnicity observed on fampridine pharmacokinetics in the analyses. However, data from other races is limited according to the sponsor, although there were a small number of Black, Asian, Hispanic and Other subjects captured in the database and the disease is predominantly occurs in Caucasians.

Extrinsic Factors:

Drug-Drug Interaction with Baclofen

The study of single-dose 15 mg fampridine-SR coadministered with 10 mg Lioresal® (baclofen) in 12 healthy male volunteers did not show PK interaction. The geometric mean ratios for the plasma C_{max} and $AUC_{0-\infty}$ values ranged from 97.6-100.7% for fampridine, with and without concomitant administration of 15 mg fampridine, with 90%CI felling into 80-125%. These results were confirmed by population PK analysis of coadministration of fampridine-SR 10 mg tablet with baclofen showing no effect on PK parameters.

Drug-Drug Interaction with Beta-Interferon

The impact of 7.5 mg q8h of fampridine immediate release formulation coadministered with 8 million units of interferon beta-1b Betaseron® (baclofen) in 3 male and 6 female MS patients showed that fampridine kinetics were comparable following administration of fampridine alone (steady state Cmax of (b) (4) ng/mL and AUC_{0-8} of 216.0 ng·hr/mL) or following co-administration of interferon beta (steady state Cmax of (b) (4) ng/mL and AUC_{0-8} of 207.2 ng·hr/mL). No pharmacokinetic drug-drug interaction of interferon beta-1b was observed on fampridine plasma levels. These results were confirmed by population PK analysis of coadministration of fampridine-SR 10 mg tablet with betaseron, showed no effect on PK parameters.

Effect of Fampridine on PK of Coadministered Drugs

- *In vitro* data with human liver microsomes showed that fampridine was not a direct or time dependent inhibitor of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 at concentrations up to 30 µM. Which is approximately 10 times the average plasma fampridine concentration measured for the 10 mg tablet caused 12% inhibition of CYP2E1.

- Fampridine had had little or no potential to induce CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1 or CYP3A4/5 enzyme activities in human hepatocytes at concentrations upto 25 µM.

- *In vitro* studies indicated that fampridine is neither a substrate of the P-gp (MDR1-MDCK, MDCK cells), nor an inhibitor of digoxin (Caco-2 cells) transport activity.

Population PK analysis: Effect of Concomitant Medications

Population PK analysis evaluating the effect of most commonly used concomitant medications in MS patients indicated no change in fampridine plasma levels as result of coadministration of these concomitantly used medications. Common concomitant medications included baclofen, glatiramer acetate, interferon beta, tizanidine, renal transport inhibitors (including ACE inhibitors, nitrofurantoin, bactrim, amoxicillin, and trimethoprim) and diuretics (including HCTs and potassium sparing drugs).

Population Exposure-Response Relationship (Efficacy and Safety)

Yes, there is a significant exposure (AUC (µg·hr/mL))-response (the percent change from the baseline at the end of double blind phase in walking speed (ft/sec)) relationship when the placebo group was included in the analysis (reviewer's analysis). However, the relationship is flat without placebo within the observed exposure range.

A phase II study (MS-F202) and two phase III studies (MS-F203, MS-F204) were included in the sponsor's exposure-response analyses for both efficacy and safety. "Timed Walk Responder" was the primary efficacy endpoint, which was defined as a patient with a faster walking speed on timed 25-foot walk test for at least three of the four visits during the double-blind treatment period, as compared to the maximum walking speed for any of the four pre-treatment visits and the first posttreatment visit. Based on the primary endpoint, fampridine showed superior effectiveness (37.3% 10 mg bid.vs. 8.9% placebo for pooled data) in the efficacy analysis and the sponsor's exposure-response analysis also predicted similar results. However, a patient who may have no drug effect at the last visit of double blind treatment phase could be declared as a responder by definition of "timed walk responder". In addition, it is not possible to evaluate how much improvement a patient achieved in actual walking speed by the primary endpoint.

Hence, the reviewer performed the independent analysis using the percent change in walking speed from the baseline at the end of double blind phase as an endpoint.

Figure 8 (in appendix 4.2) presents the results from pooled data analysis. First the linear model was applied to the pooled data and linear regression showed the statistically significant relationship between AUC (µg·hr/mL) and the endpoint (p-value=0.015). The same model was fitted to the data after excluding placebo data and results are shown in the right panel (see Figure 8 in appendix 4.2). The flat relationship without placebo suggests that the response reached the plateau at 10 mg bid and a lower dose may be as efficacious as 10 mg bid. This observation is supported by the similar responder rate at each dose group.

The safety of 10mg bid was evaluated using the probability of having at least one CNS-related AE as an endpoint. The sponsor claimed that CNS-related AE incidence was flat up to an AUC of 200 µg·hr/mL and 10 mg bid should be safe based on the model prediction.

In order to further evaluate the safety of 10 mg bid compared to placebo, the reviewer focused on the patients who were assigned to 10 mg bid or placebo. As observed in the sponsor's analysis, the incidence rate appeared to be similar to placebo up to the AUC of 200 µg·hr/mL and showed increasing trend for the range of AUC > 200 µg·hr/mL with a relatively flat but higher rate within 220 ug·hr/mL and 350 µg·hr/mL.

Overall, fampridine seems to show the effectiveness based on both primary endpoint (responder analysis) and the reviewer's endpoint (percent change from the baseline in walking speed (ft/sec)). However, the exposure-response relationship is flat within the studied exposure range and for most of the exposure range under 10 mg bid, the incidence rate of CNS-related AE is higher than that for the placebo group. Hence, a lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

_____ _____

Jagan Mohan Parepally, Ph.D. Date
Reviewer
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cc: HFD-120 NDA 22-250
HFD-860 Mehul Mehta, Ramana Uppoor, Angela Men, Jagan Parepally

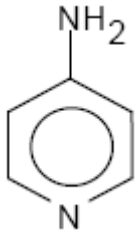
2.0 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 What are the highlights of the chemistry and physicochemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Fampridine, the active ingredient of AMAYA™, is chemically known as 4-aminopyridine. Its molecular formula is $C_5H_6N_2$ and the molecular weight is 94.12. (b) (4) The structure for fampridine drug substance is provided in Figure 1.

Figure 1. Chemical structure of fampridine



Fampridine is highly soluble in water (solubility, 50 mg/mL). It is soluble in methanol, acetone, tetrahydrofuran, isopropanol, acetonitrile, N, N-dimethylformamide, dimethylsulfoxide, and ethanol. At ambient conditions, fampridine exhibits solubilities in (b) (4) of less than (b) (4) and (b) (4) mg/mL, respectively.

2.1.2 What is the proposed mechanism(s) of action and therapeutic indication(s)?

Fampridine blocks multiple potassium channels including those found under the myelin sheath in nerve fibers of adult mammals, where they are located primarily in the paranodal and internodal membrane of the axon. In animal studies on demyelinated nerve fibers, fampridine has shown enhanced action potential conduction at concentrations of ~1 µM (94 ng/mL) with IC50 values in the range of 2–3 µM (188–282 ng/mL) recorded. Fampridine is indicated as a treatment to improve walking ability in patients with multiple sclerosis

2.1.3 What is the proposed dosage(s) and route(s) of administration?

The applicant proposes a recommended dose of AMAYA™ of 10 mg tablet twice daily taken orally with or without food. Dosage has to be adjusted to 7.5 mg tablet twice daily in patients with mild hepatic or moderate renal impairment. AMAYA™ is contraindicated in patients with severe renal impairment.

Drug/Drug Product Information:

Dosage Form/Strength: AMAYA™ is a sustained release tablet formulation of 4-aminopyridine. The composition of the proposed commercial formulation is shown in the following table.

Table 1: Composition of Fampridine-SR 10 mg Tablets

Ingredients	**Weight (mg/tablet)**
	Tablet Strength 10 mg
Fampridine (4-aminopyridine)	(b) (4)
Hydroxypropyl Methylcellulose (b) (4)), USP	
Microcrystalline Cellulose (b) (4) USP	
Colloidal Silicon Dioxide (b) (4) NF	
Magnesium Stearate, USP	
(b) (4) film coat	
Total Weight (mg)	410.0
Total Size (mm)	13 x 8

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The clinical development program for fampridine was designed to investigate and demonstrate its efficacy and safety for the treatment of patients with multiple sclerosis for the improvement of walking ability. The efficacy of fampridine-SR in patients was evaluated in two Phase 3 randomized, double-blind, parallel, placebo-controlled multi-center trials with 14 and 9 weeks duration in study MS-F203 and MS-F204, respectively. They were conducted using a primary endpoint and analysis, which had been in agreement with FDA in two Special Protocol Assessments. One additional Phase 2 placebo controlled trial (MS-F202) was also conducted to supports efficacy. All of these studies used the proposed dosing regimen of 10 mg b.i.d.

In addition, a series of clinical pharmacology and biopharmacuetics studies were conducted to characterize the pharmacokinetic and pharmacodynamic properties of fampridine.

- Individual studies to evaluate:
  - The relative bioavailability of various oral formulations of fampridine (5 studies)
  - The influence of food on the rate and extent of fampridine absorption (6 studies)
  - General pharmacokinetic properties of fampridine in healthy subjects (5 studies) and MS patients (7 studies)
- Analyses of population pharmacokinetics, pharmacokinetic-pharmacodynamics (PK/PD) were conducted to screen for drug interactions (common concomitant drugs) and to identify any key covariate relationships among age, race (Caucasian vs. Non-Caucasian), and gender. The studies included in this analyses were FeFa25F-SR11203; RD10F-SR012004; AN751-101; AN751-102; 0195-001US; MS-F201; MSF202; MS-F203; and MS-F204.
- A study to evaluate the potential effects of fampridine on QT intervals of the electrocardiogram, using a Clinical and a supratherapeutic dose compared to placebo and moxifloxacin (a positive control) in healthy subjects.
- A study to evaluate excretion of fampridine and its metabolites in renally impaired subjects.
- Studies to evaluate the potential effect of baclofen and betaseron on fampridine (2 studies).
- Mass balance and metabolite identification studies (2 studies).

Support of the efficacy and/or safety of fampridine for the treatment of walking disability in MS patients was based on results from one Phase 2 study and 2 pivotal Phase 3 studies (MS-F202, MS-F203, MS-F204). The study design and dosing information of these clinical trials are presented in the Table 2 below.

Table 2: The study design and dosing information of clinical trials

Study Title	**Design**	**Planned Doses Route Regimen**	**No. of Patients**	**Demographics Male, Female Mean Age (min, max) Race**
MS-F202: Double-Blind, Placebo- Controlled, 20-Week, Parallel Group Study to Evaluate Safety, Tolerability and Activity of Oral Fampridine-SR in Patients with Multiple Sclerosis	Double-blind, placebo-controlled, 20-week, parallel group	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR Placebo Oral doses administered b.i.d. for 15 weeks	206	75 (36%), 131 (64%) 49.8 yrs. (28, 69) 92.2% Caucasian 4.9% Black 1.5% Hispanic 1% Other 0.5% Asian/Pacific Islander
MS-F203: Double-Blind,	Double-	10 mg FAM-	300	95 (32%), 205

Placebo- Controlled, 21-week, Parallel Group Study to Evaluate Safety and Efficacy of Oral Fampridine-SR (10 mg b.i.d.) in Subjects with Multiple Sclerosis 33 MS Patients (Adequate/wellcontrolled study)	blind, randomized, placebo-controlled	SR Placebo Oral doses administered b.i.d. for 14 weeks		(68%) 51.4 yrs. (26, 70) 92.9% Caucasian 7.1% Non-Caucasian
MS-F204: Double-Blind, Placebo- Controlled, Parallel Group Study to Evaluate Safety and Efficacy of Oral Fampridine-SR (10 mg b.i.d.) in Patients with Multiple Sclerosis 35 MS Patients	Double-blind, randomized, placebo-controlled	10 mg FAM-SR Placebo Oral doses administered b.i.d. for 9 weeks	239	77 (32%), 162 (68%) 51.7 yrs. (24, 73) 91.2% Caucasian, 5.0% African-American, 1.7% Hispanic, 1.7% Other, 04% American Indian/Alaskan Native

2.2.2 Is there any significant exposure-response relationship? And does the relationship support the proposed dose (10mg bid)?

Yes, there is a significant exposure (AUC (µg·hr/L))-response(the percent change from the baseline at the end of double blind phase in walking speed (ft/sec)) relationship when the placebo group was included in the analysis (reviewer's analysis). However, the relationship is flat without placebo within the observed exposure range.
A phase II study (MS-F202) and two phase III studies (MS-F203, MS-F204) were included in the sponsor's exposure-response analyses for both efficacy and safety. "Timed Walk Responder" was the primary efficacy endpoint, which was defined as a patient with a faster walking speed on timed 25-foot walk test for at least three of the four visits during the double-blind treatment period, as compared to the maximum walking speed for any of the four pre-treatment visits and the first posttreatment visit. Based on the primary endpoint, fampridine showed superior effectiveness (37.3% 10mg bid.vs. 8.9% placebo for pooled data) in the efficacy analysis and the sponsor's exposure-response analysis also predicted similar results. However, a patient who may have no drug effect at the last visit of double blind treatment phase could be declared as a responder by definition of "timed walk responder". In addition, it is not possible to evaluate how much improvement a patient achieved in actual walking speed by the primary endpoint.

Hence, the reviewer performed the independent analysis using the percent change in walking speed from the baseline at the end of double blind phase as an endpoint.
Figure 2 presents the results from pooled data analysis. First the linear model was applied to the pooled data and linear regression showed the statistically significant relationship between AUC (ug·hr/mL) and the endpoint (p-value=0.015). The same model was fitted to the data after excluding placebo data and results are shown in the right panel in the The

flat relationship without placebo (Figure 2) suggests that the response reached the plateau at 10mg bid and a lower dose may be as efficacious as 10 mg bid. This observation is supported by the similar responder rate at each dose group (Table 3).

Figure 2: The pooled data analysis: the relationship between AUC (ug*hr/L) and the percent change from the baseline in walking speed (ft/sec) with placebo (left panel, p-value=0.015) and without placebo (right panel, p-value=0.935).

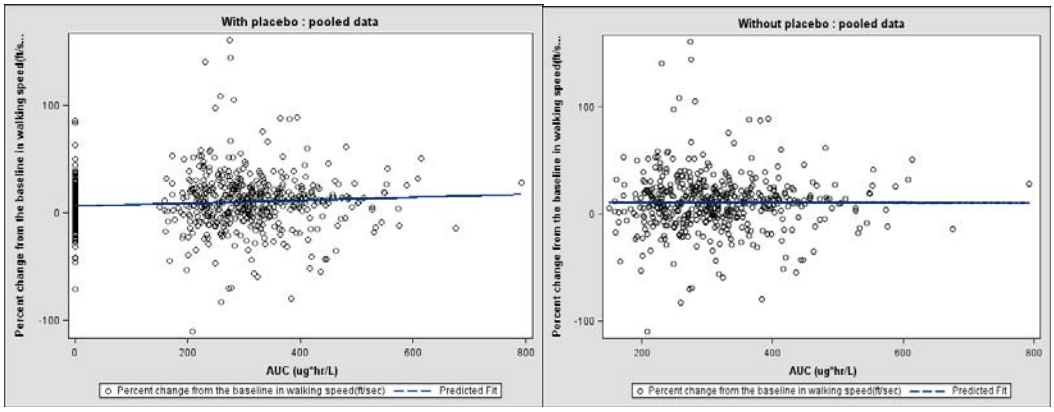


Table 3: Summary of Timed Walk Response for Studies MS-F202, MS-F203 and MS-F204 (ITT Population)

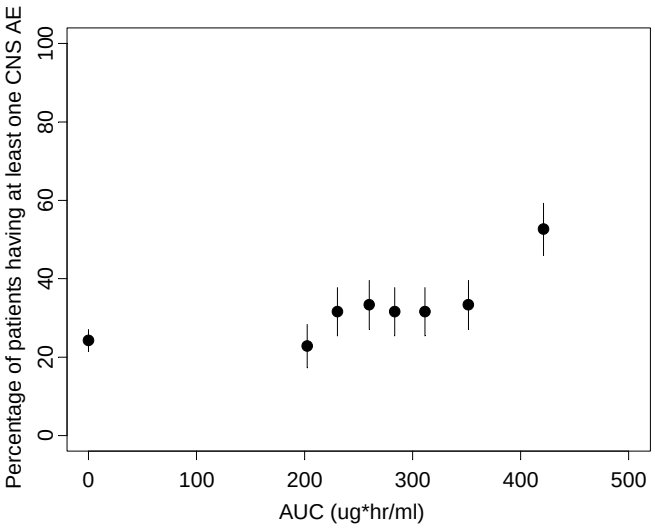
Characteristics	MS-F202	MS-F203	MS-F204
No. of subjects	205	296	237
Responder (N/Y)	143/62	212/84	175/62
Sample size by dose group (0/10/15/20 mg b.i.d.)	47/51/50/57	72/224/n.a./n.a.	118/119/n.a/n.a
Responder by dose group (0/10/15/20 mg b.i.d.)	4/18/18/22	6/78/n.a./n.a.	11/51/n.a/n.a
Response frequency by dose group (0/10/15/20 mg b.i.d.) (%)	8.5/35.3/36.0/38.6	8.3/34.8/n.a./n.a	9.3/42.9/n.a/n.a

The safety of 10mg bid was evaluated using the probability of having at least one CNS-related AE as an endpoint. The sponsor claimed that CNS-related AE incidence was flat up to an AUC of 200 µg·hr/L and 10 mg bid should be safe based on the model prediction.

In order to further evaluate the safety of 10 mg bid compared to placebo, the reviewer focused on the patients who were assigned to 10 mg bid or placebo. Figure 3 shows the observed CNS-related AE incidence at the octile of AUC at the dose of 10 mg bid and placebo. As observed in the sponsor's analysis, the incidence rate appeared to be similar to placebo up to the AUC of 200 µg·hr/L and showed increasing trend for the range of

AUC > 200 µg·hr/L with a relatively flat but higher rate within 220 ug·hr/mL and 350 ug·hr/mL.

Figure 3: The probability of having at least one CNS related AE by AUC at the dose of 10mg bid from the observed data. Each dot represents the proportion of having at least one CNS related AE at each octile of AUC and vertical bars indicate one standard error (±SE).



Overall, fampridine seems to show the effectiveness based on both primary endpoint (responder analysis) and the reviewer's endpoint (percent change from the baseline in walking speed (ft/sec)). However, the exposure-response relationship is flat within the studied exposure range and for most of the exposure range under 10 mg bid, the incidence rate of CNS-related AE is higher than that for the placebo group. Hence, a lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

2.2.3 Does this drug prolong the QT or QTc interval?

The review conducted by the Agency's Interdisciplinary Review Team (IRT) for TQT study indicates that Fampridine-SR (10 mg and 30 mg) has no significant QT prolongation effect. The largest upper bounds of the 2-sided 90% CI for the mean difference between fampridine SR (10 mg and 30 mg) and placebo were below 10 ms, the threshold for regulatory concern as described in ICH E14 guidance. In a double-blind, randomized, parallel study, 208 healthy subjects received 5 days dosing of fampridine SR 10 mg b.i.d., fampridine SR 30 mg b.i.d., placebo and moxifloxacin 400 mg. A total of 208 healthy subjects were enrolled and 198 subjects completed. Overall summary of findings is presented in Table 4 below (see TQT study review in appendix 4.3).

Table 4: The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for Fampridine SR (10 mg and 30 mg) and the Largest Lower Bound for Moxifloxacin

Treatment	Time (hour)	ΔΔQTcI (ms)	90% CI (ms)
Fampridine SR 10 mg	0.5	3.4	(-0.5, 7.2)
Fampridine SR 30 mg	0.5	2.3	(-1.5, 6.0)
Moxifloxacin 400 mg*	3	9.8	(5.9, 13.6)

2.2.4 What are the PK characteristics of the drug and its major metabolite?

General Pharmacokinetic Properties of Fampridine:

The plasma C_{max} and AUC values of fampridine increased in a dose proportional manner following single-dose administration of 5-25 mg. Dose proportionality was also observed with 5-20 mg administered q12h as a tablet in the fasted state and further confirmed with fampridine SR 12.5 tablet following multiple-dose. The steady-state concentrations of fampridine in plasma are achieved within 4 days of q12h dosing. This is consistent with its apparent elimination half-life in plasma of approximately 5-6 h.

The 2 major metabolites 3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine sulfate, both inactive, were analyzed in a study of fampridine PK in patients with renal impairment (RD10F-SR012004).

Absorption:

Fampridine is rapidly absorbed after oral administration. Absolute bioavailability of sustained-release tablets was not evaluated, but relative bioavailability was 96% when compared to an aqueous oral solution. Fampridine is absorbed largely as intact drug. The parent drug accounted for approximately 90% of total radioactivity in recovered from urine following dosing. When a single dose of 10 mg fampridine-SR was administered to healthy volunteers in a fasted state, peak concentrations ranging from 17.3 ng/mL to 21.6 ng/mL and occurred 3 to 4 hours post-administration. In comparison, the Cmax achieved with the same 10 mg dose of a fampridine oral solution was 42.7 ng/mL and occurred approximately 1.3 hours after dose administration. An average of 96% of a 15-mg radiolabeled dose of fampridine was recovered in the urine and 0.5% in faeces in 24 h following single-dose administration to 4 healthy males, indicative of a near complete absorption from the gastrointestinal tract. When fampridine was taken with food, Cmax was slightly increased (~17%) and approximately 5% decrease in AUC.

Table 5: Food-Effect Analysis of Fampridine SR 25 mg Tablet Formulation (study FeFa25F-SR112003)

Treatment	Parameter	Ratio	Lower	Upper

Comparisons			90% CI	90% CI
Fampridine SR 25 mg Fed/ Fampridine SR 25 mg Fasted	C_{max}	115	95.8	139
	$AUC_{0-\infty}$	96.9	81.1	115

Distribution:

Fampridine is largely unbound to plasma proteins (97–99%). The apparent volume of distribution is 2.6 L/kg. There was no apparent difference in pharmacokinetic parameter values following administration of fampridine-SR tablets to either healthy volunteers or patients with MS.

Metabolism and Elimination:

Fampridine and metabolites are eliminated nearly complete after 24 hours with 96% of the dose recovered in the urine and 0.5% recovery in feces. Most of the excreted radioactivity in the 0-4 hour pooled urine was parent drug (90.3%). Two metabolites were identified: 3-hydroxy-4-aminopyridine (4.3%) and 3-hydroxy-4-aminopyridine sulfate (2.6%). These metabolites have been shown to have no pharmacologic activity on potassium channels.

The elimination half-life of fampridine following administration of SR tablet formulation was 5.2 to 6.5 hours. The plasma half-life of the sulfate conjugate is approximately 7.6 hours and the half-life of 3-hydroxy-4-aminopyridine was not determined as concentrations for most subjects were close to or below the limit of quantitation. Overall renal clearance of 22.2 L/hour (370 mL/min) suggests active tubular secretion since it is much higher than the GFR.

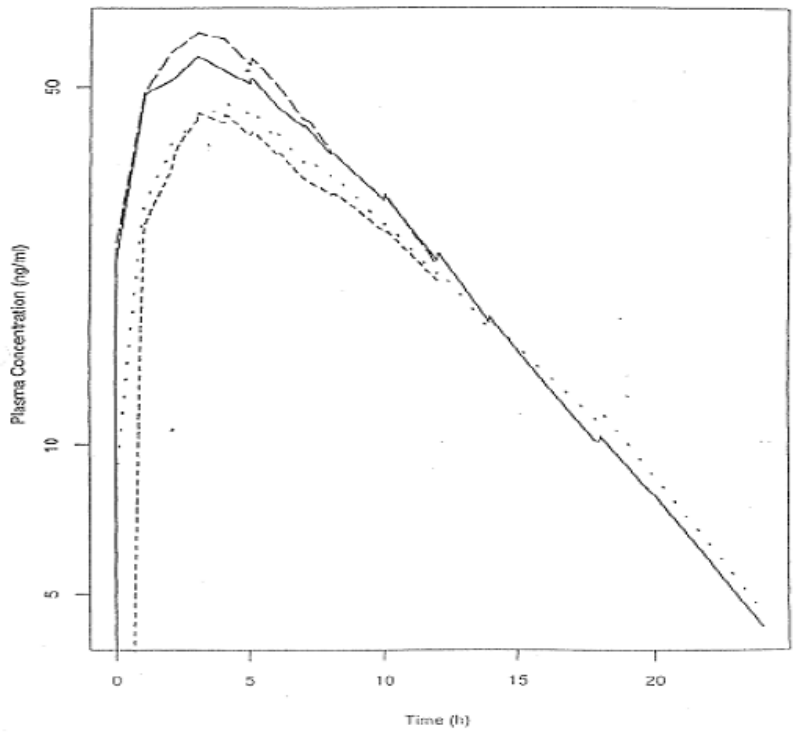
In vitro studies with human liver microsomes indicate that CYP2E1 was the major enzyme responsible for the 3-hydroxylation of fampridine based on correlation analysis, chemical inhibition studies and incubations with recombinant human CYP enzymes. Several other CYP enzymes including CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP3A5 and CYP4A11 appeared to play a minor role in the 3-hydroxylation of fampridine.

2.2.5 What are the single dose and multiple dose PK parameters?

The single- and multiple-dose pharmacokinetics of fampridine-SR in MS patients were compared in Study AN751-102. This was an open-label, multi-center, single treatment period, multiple-dose study of orally administered fampridine in patients with MS with 5, 10, 15 and 20 mg doses to assess the safety, tolerability, pharmacokinetics, of fampridine in 20 healthy male and female subjects.

The pharmacokinetic properties of fampridine were generally similar following single and multiple doses of the fampridine-SR tablets with an accumulation factor of approximately 1.4. The dose normalized mean concentration-time profiles of fampridine after multiple-dose administration of 5, 10, 15 and 20 mg are presented in the Figure 4 below. The mean PK parameters of fampridine following single- and multiple-dose are summarized in Tables 6 below. More details are available in the Individual study reports section.

Figure 4: Mean Plasma Fampridine Concentration *versus* Time Profile Following Multiple-Dose Administration of Fampridine-SR in MS Patients, N=20



dotted: single dose (20 mg normalized); short dashed: Day 1
long dashed: Day 8; solid: Day 15

Table 6: Mean (SD) Pharmacokinetic Parameter Values Following Multiple-Dose Administration of Fampridine-SR (N = 20) (AN751-102)

Parameter	Days on Drug (Fampridine-SR 20 mg b.i.d.)		
	Day 1	Day 8	Day 15
C_{max} (ng/mL)*	48.6 (13.3)	66.7 (18.6)	62.6 (14.6)
$AUC_{0\text{-}inf}$ *(ng·hr/mL)	NE	531.0 (159.2)	499.2 (113.8)
T_{max} (hr)	3.8 (1.17)	3.3 (1.08)	3.2 (1.30)
CL/F (mL/min)	NE	700.4 (288.8)	703.4 (175.5)
$t_{1/2}$ (hr)	NE	NE	5.8 (1.7)

2.2.6 How does the PK of the drug in healthy volunteers compare to that in patients?

The pharmacokinetic parameter values were generally similar following single dose administration of Fampridine-SR to healthy volunteers or patients with MS as shown in the Tables 7 and 8 below.

Table 7: Arithmetic Mean ± SD of Pharmacokinetic Parameter Values for Fampridine Following Administration of a Single Fampridine-SR Tablet, 10 mg in Subjects with Normal Renal Function (N = 5)

Parameter (unit)	Fampridine	3-Hydroxy-4-Aminopyridine	3-Hydroxy-4-Aminopyridine Sulfate
Plasma			
C_{max} (ng/mL)	21.6 ± 3.89	3.4 ± 1.13	7.7 ± 1.69
$AUC_{(0\text{-}t)}$ (ng·h/mL)	254.1 ± 35.02	3.0 (2.0, 5.0)	106.9 ± 32.53
$AUC_{(0\text{-}inf)}$ (ng·h/mL)	284.8 ± 31.78	26.3 ± 14.01	122.7 ± 19.67
T_{max} (hours)	3.0 (1.0, 5.0)	--	5.0 (4.0, 5.0)
$t_{½}$ (hours)	6.4 ± 1.31	Not Reported	7.6 ± 2.77
CL/F (L/hr)	35.5 ± 4.072	Not Reported	Not Reported
Urine			
$CumA_{e\ (0\text{-}36hr)}$ (mg)	5.7 ± 1.92	0.4 ± 0.15	0.8 ± 0.17
$A_{e\ (0\text{-}120hr)}$ (mg)	--	--	--
% Dose	56.8 ± 19.2	3.3 ± 1.1	3.2 ± 0.7
CL_{Renal} (L/hr)	22.2 ± 5.93	23.9 ± 17.49	7.61 ± 3.01

Table 8: Following are the PK parameters after a single dose of 10 mg fampridine-SR tablets in MS patients (AN751-101).

Parameter	Dose: 10 mg Tablet
C_{max} (ng/mL)	25.2 ± 1.4

Tmax	3.9 ± 0.3
AUC 0-t (ng.hr/mL)	252 ± 15.6
AUC 0-inf (ng.hr/mL)	283 ± 18
t1/2 (hr)	5.6 ± 0.4
CL/F (L/hr)	38.5 ± 2.3

2.2.7 What are the characteristics of drug absorption?

Fampridine is rapidly and almost completely absorbed from gastrointestinal tract following oral administration. Intravenous formulation is not available therefore absolute bioavailability of SR tablets (to-be-marketed formulation) has not been assessed. Relative bioavailability was 96% when compared to an aqueous oral solution. The sustained release tablet delays absorption of fampridine relative to the solution formulation characterized by lower Cmax concentration and delayed Tmax, with no effect on the extent of absorption. When a single 10 mg dose was administered to healthy volunteers while in a fasted state, peak concentrations ranging from 17.3 ng/mL to 21.6 ng/mL and occurred 3 to 4 hours post-administration (Tmax). In comparison, the Cmax achieved with the same 10 mg dose of a fampridine oral solution was 42.7 ng/mL and occurred approximately 1.3 hours after dose administration. *In vitro* testing for solubility showed that fampridine was highly soluble in water (b) (4)).

In vitro evaluation of fampridine as substrate and/or inhibitor of P-glycoprotein (P-gp) using bidirectional permeability assay in MDR1-MDCK cells and Caco-2 cells indicated that fampridine is neither a substrate nor an inhibitor of P-gp.

2.2.8 What are the characteristics of drug distribution?

The apparent volume of distribution of fampridine is 2.6 L/kg indicating large tissue distribution.

<u>Plasma protein binding:</u>

The *in vitro* protein binding of ^{14}C-fampridine was determined in human plasma by an equilibrium dialysis method. Mean protein binding was evaluated over a range of concentrations (5, 50, and 500 ng/mL) and pH (7.2-7.6). The mean protein binding was 1-3%. Fampridine was largely unbound and had a high free drug fraction at all three concentrations tested. Equilibrium was reached by a 4-hour dialysis period the free drug fraction ranged from 97 to 99%, depending on pH.

2.2.9 What are the characteristics of drug metabolism and elimination?

Data from non-clinical and clinical studies indicate that fampridine is not extensively metabolized and mainly eliminated as unchanged drug in urine.

Non-Clinical

In vitro studies with human liver microsomes indicate that CYP2E1 was the major enzyme responsible for the 3-hydroxylation of fampridine based on correlation analysis, chemical inhibition studies and incubations with recombinant human CYP enzymes. Several other CYP enzymes may be involved in playing a minor role in the 3-hydroxylation of fampridine including CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP3A5 and CYP4A11.

Clinical

Radiolabeled mass-balance and metabolism study 0496-002 indicates that fampridine and metabolites are eliminated nearly completely after 24 hours with 96% of the dose recovered in the urine and 0.5% recovery in feces. Most of the excreted radioactivity in the 0-4 hour pooled urine was parent drug (90.3%). Two metabolites were identified: 3-hydroxy-4-aminopyridine (4.3%) and 3-hydroxy-4-aminopyridine sulfate (2.6%). These metabolites have been shown to have no pharmacologic activity on potassium channels.

The elimination half-life of fampridine following administration of SR tablet formulation was 5.2 to 6.5 hours. The plasma half-life of the sulfate conjugate is approximately 7.6 hours and the half-life of 3-hydroxy-4-aminopyridine was not determined as concentrations for most subjects were close to or below the limit of quantitation.

The plasma concentration vs. time (PK) profiles of fampridine along with its 2 metabolites following a single oral dose 10 mg dose was investigated in renally impaired subjects in Study RD10F-SR012004 (figure 5).

Most of the drug was excreted in the urine as parent drug within 36 hours. Overall renal clearance of 22.2 L/hour (370 mL/min) suggests active tubular secretion since it is much higher than the glomerular filtration rate (GFR).

The plasma concentration-time profile of fampridine and the two primary metabolites in healthy volunteers with normal renal function are shown in Figure 5 below.

Figure 5: Mean Plasma Concentration of Fampridine, 3-Hydroxy-4-Aminopyridine, and 3-Hydroxy-4-Aminopyridine Following Fampridine-SR Tablets, 10 mg in Subjects with Normal Renal Function (N = 5) (RD10F-SR012004)

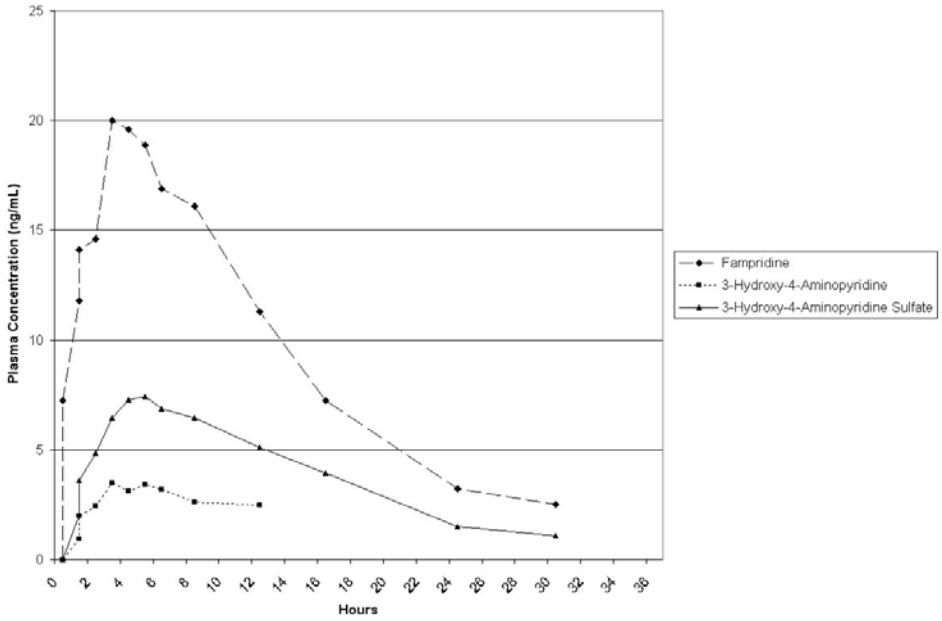


Table 9 represents the pharmacokinetic parameter values (arithmetic mean ± SD) for fampridine and its two primary metabolites obtained from study RD10F-SR012004.

Table 9:Arithmetic Mean ± SD of Pharmacokinetic Parameter Values for Fampridine Following Administration of a Single Fampridine-SR Tablet, 10 mg in Subjects with Normal Renal Function (N = 5)

Parameter (unit)	Fampridine	3-Hydroxy-4-Aminopyridine	3-Hydroxy-4-Aminopyridine Sulfate
Plasma			
C_{max} (ng/mL)	21.6 ± 3.89	3.4 ± 1.13	7.7 ± 1.69
$AUC_{(0-t)}$ (ng·h/mL)	254.1 ± 35.02	3.0 (2.0, 5.0)	106.9 ± 32.53
$AUC_{(0-inf)}$ (ng·h/mL)	284.8 ± 31.78	26.3 ± 14.01	122.7 ± 19.67
T_{max} (hours)	3.0 (1.0, 5.0)	--	5.0 (4.0, 5.0)
$t_{½}$ (hours)	6.4 ± 1.31	Not Reported	7.6 ± 2.77
CL/F (L/hr)	35.5 ± 4.072	Not Reported	Not Reported
Urine			
$CumA_{e\ (0-36hr)}$ (mg)	5.7 ± 1.92	0.4 ± 0.15	0.8 ± 0.17
$A_{e\ (0-120hr)}$ (mg)	--	--	--
% Dose	56.8 ± 19.2	3.3 ± 1.1	3.2 ± 0.7
CL_{Renal} (L/hr)	22.2 ± 5.93	23.9 ± 17.49	7.61 ± 3.01

2.2.10 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Dose proportionality of fampridine was demonstrated after administration of single doses ranging from 5-20 mg and after q12h administration of 5-20 mg of sustained release tablet formulations in MS patients (AN751-101, AN751-102). Additional evidence for dose-proportionality was obtained in healthy subjects who were administered 10, 15, 20 or 25 mg of single dose fampridine (ELA/G9101) and dose proportionality after multiple-dose administration of 10 and 30 mg q12h for 5days in study TQTc-FSR001.

Figure 6: Plasma Concentration Time Profile Following Different Treatments (ELA/G9101)

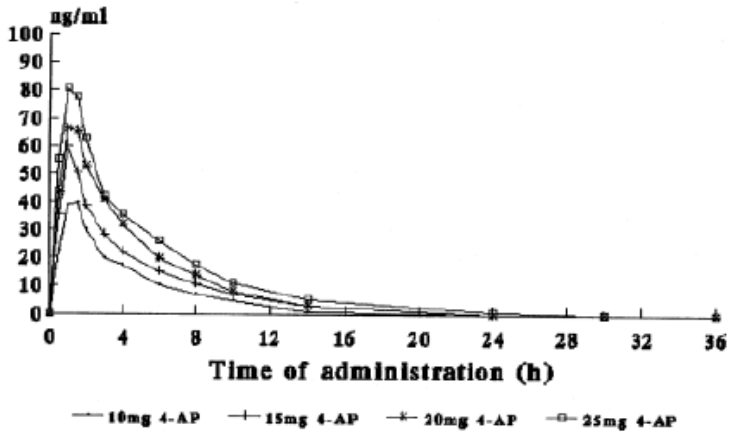


Figure 7: Pharmacokinetic Parameters Cmax and AUC following SD fampridine (5-20 mg) administration

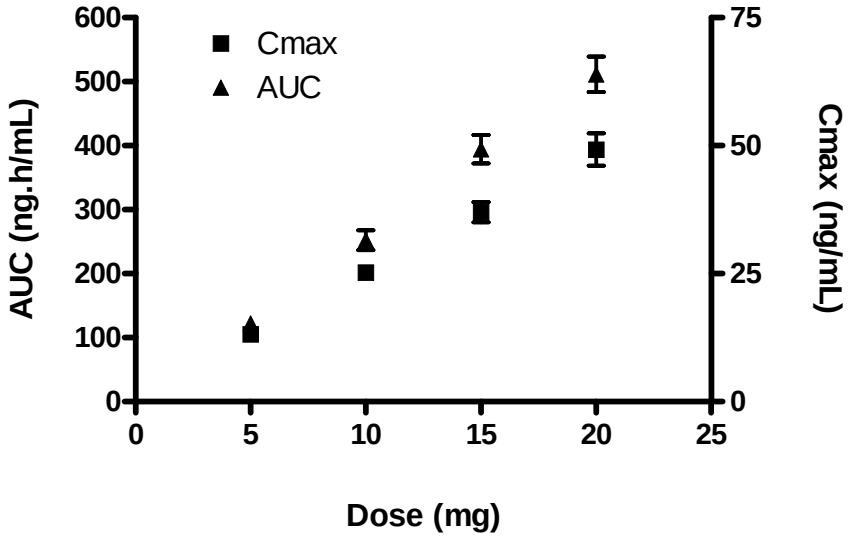


Table 10 below shows dose proportionality of PK parameters following multiple-dose administration of fampridine-SR 10 and 30 mg q12h for 5 days.

Table 10: Summary of Mean (±SD) Pharmacokinetic Parameters Following Administration of Multiple-Doses (Every 12 hours × 5 Days) of Fampridine-SR Tablet, 10 mg Dose, N=46 and 30 mg Dose, N=53 (TQTc-FSR001)

Parameter	Fampridine-SR Dose	
	10 mg b.i.d. (N = 46)	30 mg b.i.d. (N = 53)
$C_{max(ss)}$ (ng/mL)	25.3 (4.04)	75.0 (12.11)
T_{max} (hours)	3.85 (0.84)	3.98 (0.99)
$C_{min(ss)}$ (ng/mL)	9.34 (1.76)	28.22 (6.78)
$C_{av(ss)}$ (ng/mL)	16.71 (2.55)	50.32 (8.21)
$AUC_{0-\tau}$ (ng·h/mL)	200.5 (30.65)	603.09 (98.50)
Fluctuation Index	0.96 (0.15)	0.94 (0.19)
% Peak to Trough Fluctuation	15.97 (3.40)	46.77 (9.87)
Peak to Trough Swing	3.85 (0.84)	3.98 (0.99)

2.2.11 How do the PK parameters change with time following chronic dosing?

The mean plasma half life following administration of fampridine-SR tablet formulation was approximately 6 hours. Pharmacokinetic parameters measured at steady-state conditions in plasma on day 8 and 15 (Study AN751-102) after multiple-dose (q12h) administration were similar. The concentrations of fampridine were approximately 1.5 times relative to single-dose administration showing relatively small accumulation.

2.3 INTRINSIC FACTORS

2.3.1 What intrinsic factors (age, gender, race, weight, and organ dysfunction) influence exposure, and what is the impact of any differences in exposure on efficacy or safety responses?

Intrinsic factors, such as renal impairment, were studied in Phase 1 trials, as described in the following section. The potential significant covariates (age, gender and race) which may affect the fampridine PK were evaluated in population PK analysis (in Pharmacometrics Review in Section 4.2 of Appendix for details).

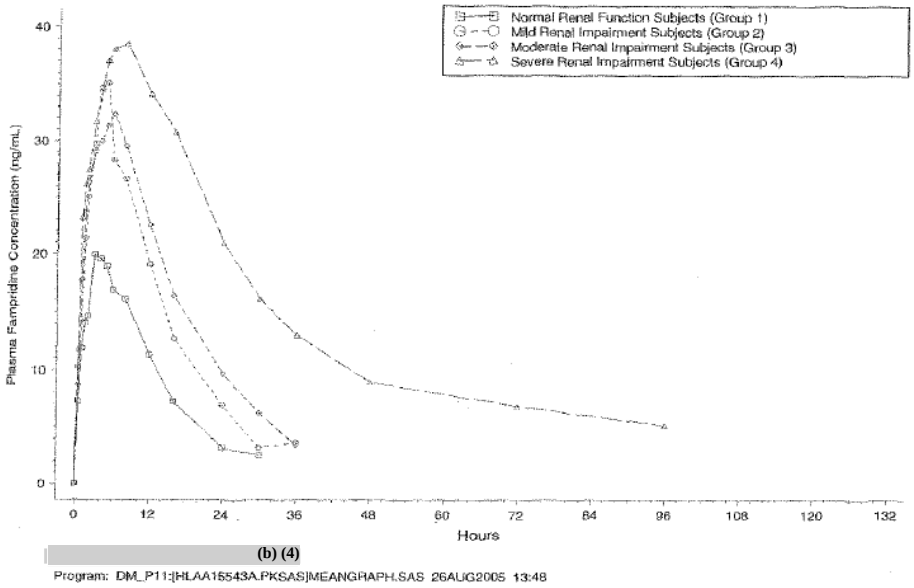
Renal impairment

A single-dose, open-label, two-stage study of fampridine-SR was conducted in subjects with normal renal function, mild, moderate or severe renal impairment. The CL/F and

CLr of fampridine showed significant relationship ($p < 0.0001$ for CL/F and $p = 0.0001$ for CLr) with creatinine clearance.

The mean percent fampridine recovered in urine was 56.8%, 60.0%, 61.8%, and 26.9 %, and mean renal clearance was 22.58 L/hr, 13.12 L/hr, 12.03 L/hr, and 2.86 L/hr in normal subjects, mildly impaired subjects, moderately impaired subjects, and severely impaired subjects, respectively. The mean Cmax and AUC(0-inf) of fampridine increased by 67% and 75% in mildly impaired subjects, by 60% and 105% in moderately impaired subjects, and by 100% and 299% in severely impaired subjects, respectively, when compared to normal subjects as shown in Figure 8 below . Increase in fampridine Cmax and AUC may subsequently increase in the incidence of CNS adverse events, most importantly seizure incidence. There was a linear and steep increase in CNS adverse events observed in exposure safety relationship (see section 4.2 for details).

Figure 8: Mean Fampridine Plasma Concentrations Versus Time in normal and renally impaired patients.

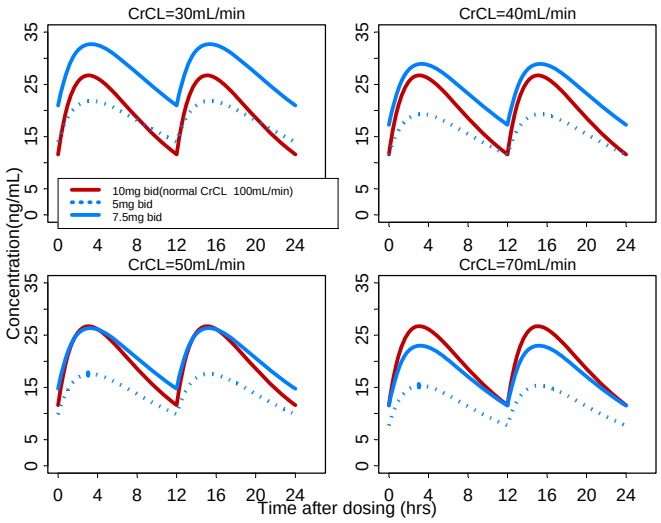


The mean Cmax and AUC(0-inf) of 3-hydroxy-4-aminopyridine sulfate increased by 35% and 80% in mildly impaired subjects, by 123% and 216% in moderately impaired subjects, and by 8 fold and 26 fold in severely impaired subjects, respectively, when compared to normal subjects.

2.3.2 Is there a need for dose adjustment in patients with mild and moderate renal impairment? What is the recommended dose in this special population?

Fampridine is contraindicated in severe renal impairment. However, sponsor did not propose any dose adjustment in mild to moderatre renal impairment. Fampridine dose has to be adjusted to 7.5 mg b.i.d in patients with mild and moderate impairment based on safety and efficacy of fampridine if 10 mg b.i.d dose is acceptable in normal MS patients. Caution should be taken in prescribing fampridine in patients with moderate renal impairment. Renal function in these patients should be closely monitored as the clinical situation warrants. Simulations were conducted to predict plasma concentrations of fampridine after dosing with fampridine-SR 5 mg and 7.5 mg twice daily and fampridine-SR 10 mg once daily (see Figure 9 below). However, peak concentrations at 5 mg bid were far below the expected peak concentration after 10 mg bid dosing in normal subjects and plasma concentrations at 10 mg once daily dosing resulted in greater peak concentration compared to normal subjects. Therefore depending on the acceptable safety and efficacy profile dose adjustment could be proposed or fampridine should be contraindicated in mild to moderate renal impairment.

Figure 9: Simulated fampridine plasma concentration vs. time profile in mild and moderate renal impairment patients for 5 mg, 7.5 mg bid and 10 mg qd



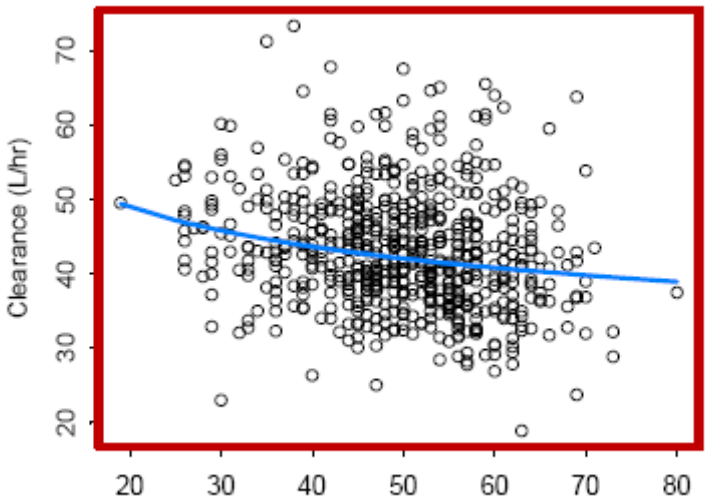
2.3.3 Effect of Hepatic Impairment

Fampridine has not been studied in patients with hepatic impairment. Since fampridine is primarily excreted unchanged in the urine, hepatic insufficiency may not significantly affect fampridine pharmacokinetics or recommended dosing.

2.3.4 Effect of Age

A population pharmacokinetic analysis showed that fampridine clearance slightly decreased with increasing age (see Figure 10 below). Dose adjustment is not necessary in elderly population.

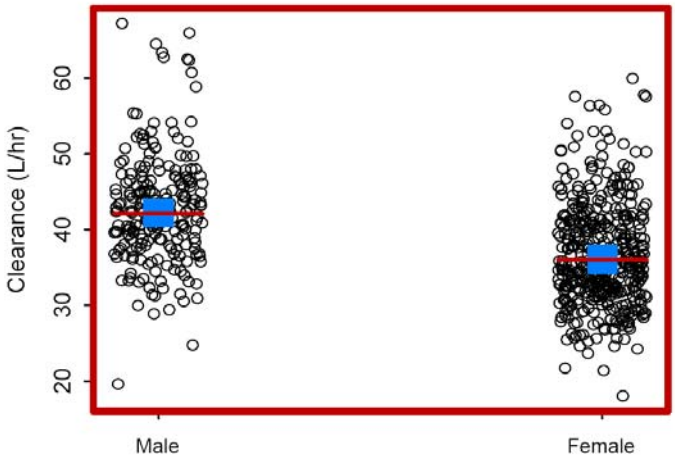
Figure 10: Pharmacokinetic parameter-covariate relationships for age



2.3.5 Effect of Gender

A population pharmacokinetic analysis showed slightly higher maximum fampridine plasma concentration than male patients (see Figure 11 below). Dose adjustment is not necessary.

Figure 11: Pharmacokinetic parameter-covariate relationships for gender



2.3.6 Effect of Race

There were no effects of ethnicity observed on fampridine pharmacokinetics in the analyses. However, data from other races is limited although there were a small number of Black, Asian, Hispanic and Other subjects captured in the database and the disease is predominantly occurs in Caucasians, so there is limited information according to the sponsor.

2.3.7 Is there any information for the use of fampridine in pregnancy and lactation is in the application?

Not studied

2.4 EXTRINSIC FACTORS

Drug-drug interactions

2.4.1 Is there an *in vitro* basis to suspect *in vivo* drug-drug interactions?

No. Fampridine is mostly eliminated unchanged in urine and largely unbound to plasma proteins.

Drug Interaction based on protein binding

Fampridine is largely unbound to plasma proteins (97-99%). The potential for drug interactions based upon protein binding have not been studied with fampridine.

2.4.2 Is the drug an inhibitor and/or an inducer of CYP enzymes on an *in vitro* basis?

Inhibition potential:

The inhibitory potential of fampridine towards the metabolism of CYP-specific substrates was determined in human liver microsomes pooled from adult males and females (from sixteen individuals). Fampridine at 30 µM concentration, which is approximately 10 times the average plasma fampridine concentration measured for the 10 mg tablet caused 12% inhibition of CYP2E1. *In vitro* data with human liver microsomes showed that fampridine was not a direct or time dependent inhibitor of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 at concentrations up to 30 µM.

Induction potential:
Cultured human hepatocytes from 3 donors were utilized for this study. The potential for 4-aminopyridine to induce human hepatocytes at 0.025 µM, 0.25 µM, 2.5 µM and 25 µM fampridine had little or no effect on CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1 or CYP3A4/5 enzyme activities.

2.4.3 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes or other metabolic/transporter pathways?

In vitro studies indicated that fampridine is neither a substrate of the P-gp (MDR1-MDCK, MDCK cells), nor an inhibitor of digoxin (Caco-2 cells) transport activity. Therefore, clinically significant interactions with drugs that are substrates or inhibitors of these transporters are unlikely.

<u>P-gp Substrate Assessment</u> (using MDR1-MDCK and MDCK cells)

The transport of 4-aminopyridine at 0.7, 7, and 70 µM in apical (AP) to basolateral (BL) and BL to AP directions was measured in both MDR1-MDCK and MDCK cells. In parallel, bidirectional permeability results for digoxin, a P-gp substrate, was also measured with the same cell batch. Fampridine was determined not to be a P-gp substrate since the efflux ratio was ~1 in cells expressing P-gp and MDR1-MDCK cells (see Table 11).

Table 11: Permeability and Recovery of 4-Aminopyridine and Digoxin in MDR1-MDCK Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
4-aminopyridine (0.7 μM)	P_{app} ($\times 10^{-6}$cm/s)	3.1	3.7	2.4	3.1 ± 0.6	3.0	3.0	3.0	3.0 ± 0.0	1.0
	% Recovery	78.8	76.7	73.8	76.4 ± 2.5	79.6	79.5	79.6	79.6 ± 0.1	
4-aminopyridine (7 μM)	P_{app} ($\times 10^{-6}$cm/s)	3.7	3.7	3.6	3.7 ± 0.0	3.1	3.2	2.9	3.0 ± 0.1	0.8
	% Recovery	99.6	97.6	97.9	98.4 ± 1.1	103	91.2	101	98.3 ± 6.2	
4-aminopyridine (70 μM)	P_{app} ($\times 10^{-6}$cm/s)	4.0	3.4	3.9	3.8 ± 0.3	3.6	3.8	3.9	3.7 ± 0.1	1.0
	% Recovery	111	105	109	108 ± 2.8	110	109	108	109 ± 1.0	
Digoxin (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	1.9*	0.1	0.1	0.1 ± 0.0	14.1	12.7	16.8	14.5 ± 2.1	123
	% Recovery	89.4*	87.2	89.7	88.4 ± 1.8	92.5	89.4	92.2	91.4 ± 1.7	

*The measured concentration was unusually high and its value was excluded from calculations.

<u>P-gp Inhibitor Assessment</u> (Using Caco-2 cells)

Fampridine was determined not to be a P-gp inhibitor since permeation of digoxin, a known P-gp substrate, was unaffected in the presence of fampridine. P-gp inhibitors, cyclosporine (CsA) and ketoconazole were used as positive controls (see Table 12). The bi-directional transport of digoxin was measured in the absence and presence of 4-aminopyridine, CsA, and ketoconazole in Caco-2 cells. A 15-minute pre-incubation with 100 μM 4-aminopyridine, 10 μM CsA, or 10 μM ketoconazole solution was performed to inhibit the functions of P-gp.

Table 12: Permeability and Recovery of Digoxin in Caco-2 Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
Digoxin (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	0.9	0.9	0.9	0.9 ± 0.0	14.4	13.4	14.9	14.2 ± 0.8	16.0
	% Recovery	101	102	102	102 ± 0.7	103	99.3	114	106 ± 7.7	
Digoxin + 4-aminopyridine (100 μM)	P_{app} ($\times 10^{-6}$cm/s)	1.1	0.9	1.2	1.0 ± 0.2	16.6	19.1	21.9	19.2 ± 2.7	18.3
	% Recovery	88.4	94.5	100	94.3 ± 5.7	105	102	104	104 ± 1.3	
Digoxin + CsA (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	2.8*	3.2*	3.3	3.3	2.7	3.0	3.0	2.9 ± 0.2	0.9
	% Recovery	104*	120*	121	121	110	105	103	106 ± 4.1	
Digoxin + Ketoconazole (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	1.3	2.3	2.3	2.0 ± 0.6	4.6	4.5	5.3	4.8 ± 0.4	2.5
	% Recovery	100	101	109	103 ± 4.7	107	102	100	103 ± 3.1	

*The cell monolayer failed post-experiment LY criteria; thus their values were excluded from average calculation.

2.4.4 Is there a change in PK profile of fampridine or any other concomitantly administered drugs commonly used in multiple sclerosis patients?

In order to evaluate the clinically relevant effects of co-administration of fampridine (10 mg b.i.d) with other drugs commonly used in MS patients, the sponsor conducted drug-drug interaction studies with baclofen and betaseron.

Sponsor also conducted a population PK analysis evaluating the effect of most commonly used concomitant medications. Common concomitant medications included baclofen, glatiramer acetate, interferon beta, tizanidine, renal transport inhibitors (including ACE inhibitors, nitrofurantoin, bactrim, amoxicillin, and trimethoprim) and diuretics (including HCTs and potassium sparing drugs). There was no change in fampridine plasma levels as result of coadministration of these concomitantly used medications.

Baclofen: Study 0194-002 evaluated the impact of single-dose 15 mg of fampridine-SR coadministered with 10 mg Lioresal® (baclofen) in 12 healthy male volunteers. The PK results of the study 0194-002 showed an insignificant PK interaction based on that the geometric mean ratios for the plasma Cmax and AUC∞ values, ranging from 97.6-100.7% for fampridine, with and without concomitant administration of 15 mg fampridine (see Tables 13 and 14). Population PK analysis of effect of coadministration of fampridine-SR 10 mg tablet with baclofen showed no effect on PK parameters.

Table 13: Effect of baclofen on fampridine: The mean PK data and 90% CIs for these treatments

Parameter	Fampridine	Fampridine + Baclofen	Ratios	Lower 90% CI	Upper 90% CI
$AUC_{0-\infty}$ (ng.hr/mL)	399.8 ± 78.3	402.4 ± 99.0	97.9	91.9	109.4
C_{max} (ng/mL)	47.2 ± 6.1	46.2 ± 8.1	100.5	90.1	105.5
T_{max} (hrs)	4.3 ± 0.6	4.2 ± 0.9			

Table 14: Effect of fampridine on baclofen: The mean PK data and 90% CIs for these treatments

Parameter	Baclofen	Baclofen + Fampridine	Ratio	Lower 90% CI	Upper 90% CI
$AUC_{0-\infty}$ (ng.hr/mL)	1055 ± 206	1024 ± 245	97.06	87.2	106.9
C_{max} (ng/mL)	199.5 ± 39.3	201.0 ± 41.1	100.7	87.3	114.2
T_{max} (hrs)	1.02 ± 0.8	0.81 ± 0.3			

Beta-Interferon: Study 1194-001 evaluated the impact of 7.5 mg q8h of fampridine immediate release formulation coadministered with 8 million units of interferon beta-1b Betaseron® (baclofen) in 3 male and 6 female MS patients. Fampridine kinetics were comparable following administration of fampridine alone (steady state Cmax of 56.7 ng/mL and AUC_{0-8} of 216.0 ng·hr/mL) or following co-administration of fampridine (steady state Cmax of 50.1 ng/mL and AUC_{0-8} of 207.2 ng·hr/mL). No pharmacokinetic drug-drug interaction of interferon beta-1b was observed on fampridine plasma levels (Tables 15&16). Population PK analysis of effect of coadministration of fampridine-SR 10 mg tablet with betaseron showed no effect on PK parameters.

Table 15: Effect of betaseron on fampridine: The mean PK data and 90% CIs for these treatments

Parameter	Fampridine With Betaseron®	Fampridine	90% CIs
Cmax	40.98 (1.36)	41.73 (1.29)	74.66 - 129.17
AUC(0-12)	167.49 (1.39)	173.11 (1.40)	89.23 - 104.92
AUC(0-inf)	191.05 (1.39)	198.96 (1.42)	86.61 - 106.45

Table 16: Effect of fampridine on betaseron: The mean PK data and 90% CIs for these treatments

Parameter	**Fampridine + Betaseron® (day 43)**	**Fampridine (day 3)**	**Lower 90% CI**	**Upper 90% CI**
AUCss (0-8)	199.0 ± 1.3	206.1 ± 1.4	88.3	105.6
$C_{max,ss}$	49.33 ± 1.2	54.4 ± 1.3	74.0	111.1

2.4.5 Is there a known mechanistic basis for renal elimination and drug-drug interactions related to this route of elimination?

Renal clearance of fampridine 22.2 L/hour (Clr=370 mL/min) suggests active tubular secretion, since it is much higher than the GFR. Sponsor did not conduct individual studies evaluating drug-drug interaction potential due to renal secretion. However, population PK analysis evaluating the effect of concomitant medications such as renal transport inhibitors (including ACE inhibitors, nitrofurantoin, bactrim, amoxicillin, and trimethoprim) and diuretics (including HCTs and potassium sparing drugs) did not result in significant change in fampridine clearance. Therefore, atleast these drugs most commonly used in MS patients may not have impact on fampridine plasma concentrations.

2.4.6 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

Not studied

2.5 GENERAL BIOPHARMACEUTICS

2.5.1 What is the composition of fampridine SR/CR capsule formulations used in earlier clinical trials?

During early clinical development several formulations were used including fampridine immediate release capsules, fampridine sustained release (SR) capsules and fampridine (b) (4) capsules. Extended release formulations were developed due to high peak plasma concentration obtained from IR formulation. However, difference between SR capsule and (b) (4) capsule formulation was not clear. The sponsor provided more information upon request. According to the sponsor the terms SR and (b) (4) were used interchangeably for capsule and tablet formulations used in the early development history of the product. The term SR was adopted in the later stages of development of the proposed commercial matrix tablet formulation, though early stage matrix tablets were sometimes referred to as (b) (4)

Table 17: Composition of Fampridine SR/(b) (4) Capsule Formulations 1 and 2

(b) (4)

Two SR capsule formulations (1 and 2) that have been studied are presented in the Table 17. There were two basic SR capsule formulations. Formulation 1 was based on (b) (4)

Formulation 2 was developed by the sponsor to overcome differences in the dissolution profile obtained from the (b) (4) versus the (b) (4) The second SR capsule series of formulations of fampridine (Formulation 2) were based on (b) (4) as the (b) (4) in combination with other excipients. However, formulation 2 demonstrated instability and its development was discontinued.

Finally, SR matrix tablet formulation (proposed final commercial product) using MXDAS™ (Hydrophilic Matrix Drug Absorption System) technology was developed to improve stability profile of SR capsule formulation.

2.5.2 What is the composition of the to-be-marketed film-coated fampridine-SR tablet product?

Amaya is a SR tablet formulation of 4-aminopyridine. The composition of the proposed commercial formulation is shown in the following Table 18.

Table 18: Composition of Fampridine-SR 10 mg Tablets

Ingredients	**Weight (mg/tablet)**
	Tablet Strength 10 mg
Fampridine (4-aminopyridine)	10.0
Hydroxypropyl Methylcellulose (b) (4)), USP	(b) (4)
Microcrystalline Cellulose (b) (4) USP	
Colloidal Silicon Dioxide (b) (4) NF	
Magnesium Stearate, USP	
(b) (4) film coat	
Total Weight (mg)	410.0
Total Size (mm)	13 x 8

2.5.3 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

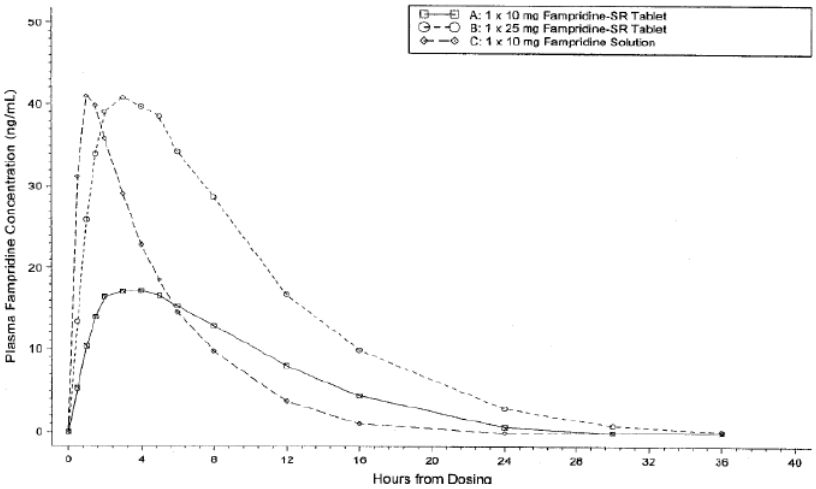
The final coated tablet formulation (Stage 3) was the formulation used in Phase 2 and Phase 3 clinical studies. The proposed commercial drug product, Fampridine-SR tablet, 10 mg, is identical to the Stage 3 formulation used in clinical studies. The composition of the 10 mg strength tablet used in pivotal clinical trials is the same as that proposed for commercial production. Following bioequivalence studies (Study BE10.25F-SR10OS122003 and Study BE10F-SR022004) were conducted using proposed commercial drug product.

Pivotal Biopharmaceutic Studies of Fampridine-SR

Relative Bioavailability: Study BE10.25F-SR10OS122003

Study BE10.25F-SR10OS122003 was a randomized, open-label, single-dose, three-way crossover relative bioavailability study of Fampridine-SR tablets, 10 mg as compared to a 10 mg buffered aqueous oral solution (0.83 mg/mL) under fasting conditions. Fampridine-SR tablets, 25 mg were studied in the third arm. Mean peak plasma concentrations of 42.7 ng/mL achieved approximately 1 hour after 10 mg oral solution of fampridine administration. The peak concentrations following administration as a sustained release tablet were approximately 40% of those achieved with the oral solution and occur approximately 3.5 hours postdose. The plasma elimination half-life was prolonged, from 3.2 hours for the oral solution versus 5.4 to 5.5 hours on average for the sustained release tablets. The two sustained release tablets were bioequivalent (figure 12).

Figure 12: Mean Plasma Fampridine Concentration *versus* Time Profiles for the 10 mg and 25 mg Fampridine-SR Tablet and the 10 mg Fampridine Oral Solution Dose Administered to Healthy Volunteers (BE10.25F-SR10OS122003)



Study BE10F-SR022004 is a Single-Dose, Randomized, Open-label, Two-Way Crossover, Bioequivalence Study of 2 Commercial 10-mg Fampridine-SR Tablets (E10 and P10) in Healthy Adult Volunteers. The objective of this study was to assess the bioequivalence of the 10 mg strength of Fampridine-SR tablets manufactured by Elan and Patheon Pharmaceuticals. Single dose administration of 10 mg Fampridine-SR test formulation manufactured by Patheon Pharmaceutical (P10) is bioequivalent to reference formulation manufactured by Elan Pharmaceuticals (E10). Following table shows 90% CIs calculated for C_{max} and $AUC_{0\text{-inf}}$.

Table 19: Analysis of Bioequivalence between Fampridine Formulations [Treatment B (P10) and Treaiment A (E10)]

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine P10 (B)/ E10 (A)	C_{max}	103.8	97.9	109.9
	$AUC_{0-\infty}$	96.7	92.7	101.0

2.5.4 What is the absolute bioavailability and relative bioavailability of the proposed to-be-marketed formulation to other dosage forms?

Absolute Bioavailability

Sponsor did not conduct absolute bioavailability studies as fampridine intravenous formulation is not available. However, absolute bioavailability IR formulation was reported to be 95%, by Uges et al., 1982. Relative bioavailability compared to aqueous oral solution was also found to be 95%.

Relative Bioavailability

During early clinical development several formulations were used including fampridine immediate release capsules and fampridine sustained release (SR) capsules. However final commercial product (to-be marketed formulation) is fampridine-SR tablet (10 mg). Relative bioavailability of immediate release and sustained release capsule formulations was assessed in Study 0494-006 and Study 1194-002.

Study 0494-006: Study to compare the bioavailability of an immediate release and a slow release formulation of 4-aminopyridine and the bioavailability of the slow release formulation in the fed and fasted state. In this study, extent of Fampridine exposure (AUC) was comparable for Fampridine IR 12.5 mg capsules and Fampridine SR 12.5 mg tablets. Peak plasma concentration of Fampridine SR 12.5 mg tablets was 23%-41% of Fampridine IR 12.5 mg capsules and Tmax was prolonged from approximately 1 hr for IR capsules to 3 hrs for SR tablets.

Study 1194-002: A Study to Compare the Bioavailability of An Immediate Release Capsule with Each of Two Slow Release Formulations of 4- Aminopyridine and to confirm that the Slow Release Formulations are Bioequivalent. In this study, the total extent (AUC) of fampridine exposure was similar between the IR capsule formulation and SR formulations (tablet and capsule). The 4-AP matrix tablet formulation was equivalent to the slow release capsule formulation in terms of AUC comparison (see table 20). Cmax for sustained release tablet was significantly lower than the sustained release capsule as shown in the Figure 13 below.

Figure 13, PK profiles of SR tablet, SR capsule and IR capsule

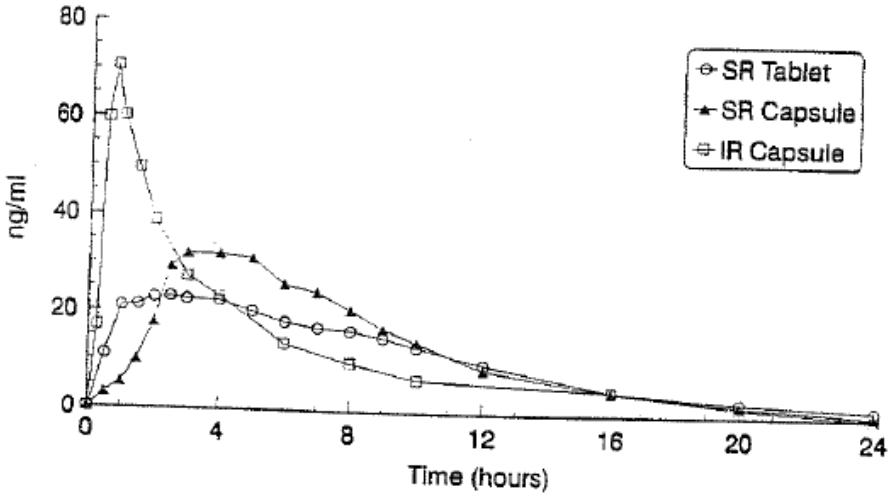


Table 20: Mean non-transformed pharmacokinetic parameters

Parameter	PD12907 SR Tablet	4n806 SR Capsule	PS644 IR capsule
AUC (infinity)	285.88 ± 37.81 P=0.43 vs IR capsule *(95.02 - 113.66)* P=0.35 vs SR capsule *(86.79 - 103.82)*	299.97 ± 53.59 P=0.09 vs IR capsule *(100.16 - 118.80)*	274.00 ± 59.16
Cmax	28.51 ± 4.27 P=0.0001 vs IR cap *(22.01 - 46.28)* P=0.13 vs SR capsule *(48.72 - 102.41)*	37.73 ± 9.85 P=0.0001 vs IR cap *(33.05 - 57.32)*	83.50 ± 23.46
Tmax	2.92 ± 2.38 p=0.0008 vs IR cap	3.63 ± 0.91 P=0.0001 vs IR cap	0.79 ± 0.28

2.5.5 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

The impact of concomitant food intake on the rate and extent of absorption of fampridine-SR tablet was assessed in Studies 0494-006, 0195-001US, FeFa25F-SR112003 (fampridine-SR 25 mg tablet) and FeFa10F-SR-2008. Data from Study FeFa10F-SR-2008 (fampridine-SR 10 mg tablet) was not reviewed since food effect was evaluated on several higher strength fampridine-SR formulations.

Data from these three studies showed that food had relatively small impact on Cmax and AUC (a slight increase of approximately 17% and approximately 5% respectively) under fed conditions (Table 21). Tmax values were delayed by approximately 2 hours. Since fampridine is indicated for chronic dosing, the delay in Tmax is not considered clinically significant. The results justify administration of fampridine-SR tablets with or without regard to food.

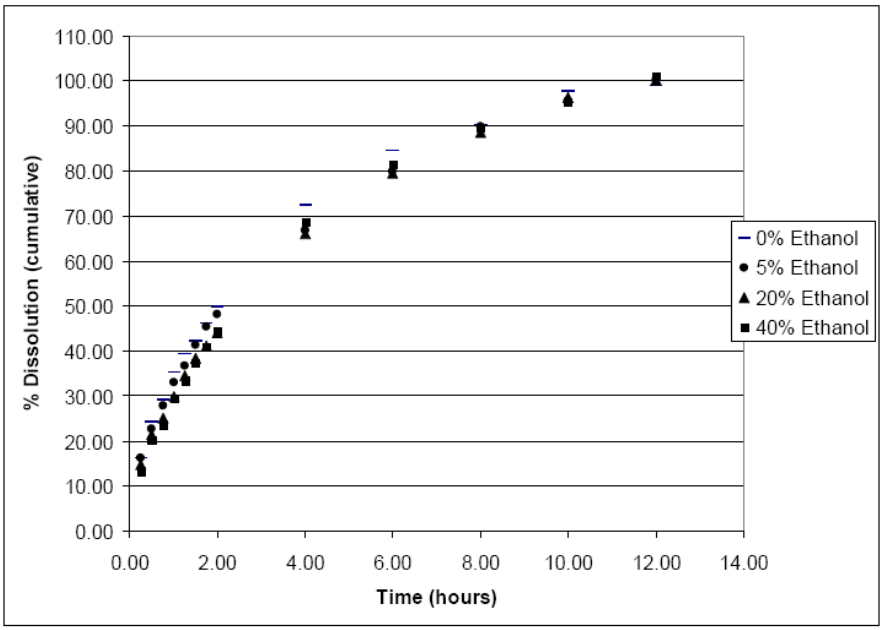
Table 21: Food-Effect Analysis of Fampridine SR 25 mg Tablet Formulation (FeFa25F-SR112003)

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine SR 25 mg Fed/ Fampridine SR 25 mg Fasted	C_{max}	115.4	95.8	139
	$AUC_{0-\infty}$	96.9	81.1	96.9

2.5.6 What is the effect of alcohol intake on the bioavailability (BA) of the drug from the dosage form?

To evaluate potential for ‘dose dumping’ of fampridine-SR tablet formulation with alcohol, *in vitro* dissolution studies were conducted to assess the performance of the formulation at concentrations of 0%, 5%, 20%, or 40%. There is no evidence of dose dumping from *in vitro* dissolution studies in the presence of alcohol as shown in the Figure below.

Figure 14 Dissolution Profile of 10 mg Fampridine-SR in Ethanol



2.6 ANALYTICAL

2.6.1 Have the analytical methods been sufficiently validated?

Yes (both pre-study validation and within study performance).

Method: Human plasma samples were analyzed for fampridine by an LC/MS/MS procedure using (b) (4) as an internal standard. Chromatography was performed on a silica column (b) (4) using a mobile phase composed of 90% CH_3CN, 0.1% TFA, and 5 mM ammonium acetate. The mass spectrometer source was equipped with a (b) (4) was employed. Mass selection was performed by MRM (multiple reaction monitoring) analysis. Sample preparation included the extraction of 0.200-mL of plasma with 1.00-mL of ethyl acetate, prior to introduction into the LC/MS/MS system.

Analyte	Fampridine
Internal standard (IS)	(b) (4)
Method description	HPLC-Reverse phase liquid chromatography with MS/MS detection
Inter-Day Precision And Accuracy	QC samples %CV 5.81% to 9.37%
Intra-Day Precision And Accuracy	QC samples %CV 2.49% to 8.28%
Limit of quantitation	2 ng/mL

Average recovery of drug (%)	70.9%
Standard curve concentration range (ng/mL)	2-200 ng/mL
Potentially Interfering Drugs:	No effect on analyte quantitation
Freeze and Thaw Stability Precision at -80ºC:	5 Cycles
Long-Term Stability of Analyte in Matrix at -4ºC:	17 days
Long-Term Stability of Analyte in Solution at -20ºC:	60 days

Pre-Study Bioanalytical Method Validation

Validation Summary of Metabolite Assays

Reference standards for the two major metabolites of fampridine, 3-hydroxy-4-aminopyridine and its sulfate conjugate, were synthesized and the analytical method (LC/MS/MS) was updated to include these analytes. Human plasma samples (0.200 mL) were analyzed for 3-hydroxy-4- aminopyridine and 3-hydroxy-4-aminopyridine sulfate by LC/MS/MS performed on a (b) (4) system equipped with an (b) (4) . The mobile phases consisted of acetonitrile: water: formic acid (90:10:0.25) (*v/v*) with 5 mM ammonium acetate. Mass selection was by MRM (Multiple Reaction Monitoring) analysis. Sample preparation consisted of precipitation of 0.200 mL of plasma with 0.400 mL of acetonitrile containing the internal standards (b) (4)] prior to injection into the LC/MS/MS.

Analyte	**3-OH-4Aminopyiridine**
Internal standard (IS)	(b) (4)
Method description	HPLC-Reverse phase liquid chromatography with MS/MS detection
Inter-Day Precision And Accuracy	QC samples Precision: %CV 5.74% to 10.8% Accuracy: +0.67% to +4.2%
Intra-Day Precision And Accuracy	QC samples Precision: %CV 5.50% to 6.01%

	Accuracy: -0.5% to +12%
Dilution Integrity	QC samples Precision: %CV 14.2% Accuracy: +8.3%
Limit of quantitation	2 ng/mL
Average recovery of drug (%)	108%
Standard curve concentration range (ng/mL)	2-32 ng/mL
Potentially Interfering Drugs:	No effect on analyte quantitation
Freeze and Thaw Stability Precision at -20ºC:	5 Cycles
Processed Sample Stability at 4 ºC	2 weeks
Stock Solution Stability at Room Temperature	6 hours
Matrix Effect	No interference
Long-Term Stability of Analyte in Solution at -20ºC:	3 months

Analyte	**3-OH-4Aminopyiridine Sulfate**
Internal standard (IS)	(b) (4)
Method description	HPLC-Reverse phase liquid chromatography with MS/MS detection
Inter-Day Precision And Accuracy	QC samples Precision: %CV 6.94% to 9.17% Accuracy: -4.0% to +1.0%
Intra-Day Precision And Accuracy	QC samples Precision: %CV 3.95% to 6.26% Accuracy: -2.2% to -0.75%
Dilution Integrity	QC samples Precision: %CV 2.75% Accuracy: -8.5%
Limit of quantitation	1 ng/mL
Average recovery of metabolite (%)	73.7%
Average recovery of Internal Standard (%)	72.9%
Standard curve concentration range (ng/mL)	2-50 ng/mL

Potentially Interfering Drugs:	No effect on analyte quantitation
Freeze and Thaw Stability Precision at -20ºC:	5 Cycles
Processed Sample Stability at 4 ºC	2 weeks
Stock Solution Stability at Room Temperature	6 hours
Bechtop Stability at Room Temperature	6 hours
Matrix Effect	No interference
Long-Term Stability of Analyte in Solution at -20ºC:	3 months

3.0 LABELING RECOMMENDATIONS

The Office of Clinical Pharmacology (OCP/DCP-1) has reviewed the package insert labeling for AMAYA™ and finds it acceptable pending the following revision:

(~~Strikethrough text~~ is recommended to be deleted and **underlined text** is recommended to be added.)

23 Page(s) of Draft Labeling have been Withheld in Full following this page as B4 (CCI/TS)

4.0 APPENDIX

4.1 INDIVIDUAL STUDY REVIEWS

4.1.1 PHARMACOKINETICS STUDIES

AN751-101: An Open-Label Dose Proportionality Study to Assess the Pharmacokinetics of Esca1ating Doses of Fampridine (4-Aminopyridine) in Subjects with Multiple Sclerosis

Objectives:

To assess dose proportionality of the sustained release fampridine tablet. The t1/2, systemic clearance, and Vd/F were also to be measured to characterize fampridine kinetics in multiple sclerosis patients.

To assess the safety and tolerability of fampridine as determined by adverse events in addition to any effects on laboratory tests, vital signs, and the pre- and post-treatment physical examinations.

To assess the effects of fampridine on the 12 lead electrocardiogram (ECG), and/or any observed effects on fampridine pharmacokinetics attributable to concomitant drug therapy

Study Design	This study was an open-label, multi-center, dose proportionality study of orally administered fampridine in patients with multiple sclerosis.
Study Population	Male and female subjects with multiple sclerosis Age: 18-65 years 24 subjects were enrolled, and 23 completed the study
Treatment Groups	Treatment A = 1 x 5 mg 4-aminopyridine Treatment B = 1 x 10 mg 4-aminopyridine Treatment C = 1 x 15 mg 4-aminopyridine Treatment D = 2 x 10 mg 4-aminopyridine Treatments were separated by atleast 4 days period
Dosage and Administration	Single doses of fampridine tablets were to be given in escalating doses (5 mg, 10 mg, 15 mg, and 20 mg) with at least a four-day interval between administrations of each dose of drug. Patients were to be monitored for 24 hours following each dose of fampridine, and were to be advanced to the next dose of fampridine only after consideration of the safety and pharmacokinetic parameters of the previous dose.
Sampling: Blood	Blood samples were collected during each study period at hour 0 (pre-dose), hours 1-8, and hours 10, 12, l4, 18, and 24.

<table>
<tr><td>Analysis</td><td>Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>5, 40, and 150 ng/mL</td><td>2, 4, 8, 15, 30, 50, 100 and 200 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>9.8to 10.6</td><td>2.5 to 9.3</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.999</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">2 to 200 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">2 ng/mL</td></tr>
</table></td></tr>
<tr><td>Urine</td><td>None</td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>PK Assessments</td><td>The primary pharmacokinetic parameters determined in this study (Cmax, AUC, and t1/2) were derived from the plasma levels of fampridine.</td></tr>
<tr><td>PD Assessments</td><td>None</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetics: Geometric means and geometric coefficients of variation were provided for AUC(0-t), AUC(0-inf), and Cmax.

Analysis of variance (ANOVA) was performed on the dose-normalized In-transformed AUC(0-inf), AUC(0-t), and Cmax.</td></tr>
</table>

Demographic Characteristics

Gender	
Male	10 (42%)
Female	14 (58%)
Race	
Caucasian	24 (100%)
Age (years)	
Mean ± SEM	45.4 ± 1.5
Minimum	29
Maximum	56
Age (years) at MS onset	
Mean ± SEM	29.6 ± 1.7
Minimum	19
Maximum	50
Weight (pounds)	
Mean ± SEM	158.5 ± 5.1
Minimum	105.4
Maximum	192
Height (inches)	
Mean ± SEM	67.2 ± 0.66
Minimum	59
Maximum	72

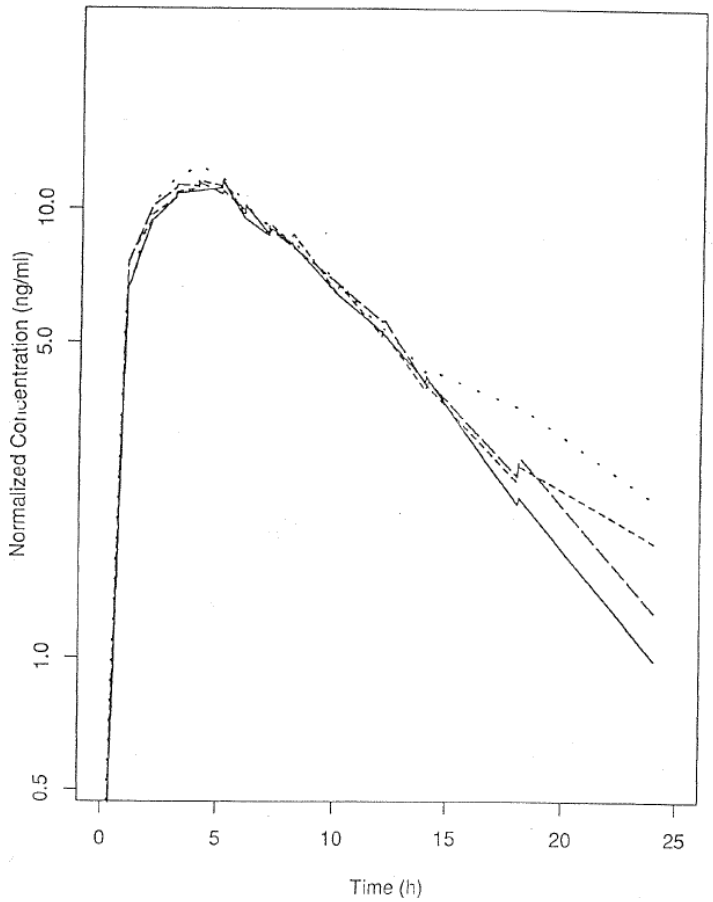
SEM = standard error of mean
Data extracted from Section 14, Table 2

RESULTS:

Twenty-three subjects received all 4 treatments, and one subject received only 3 treatments; data from all treatments were analyzed. Dose-dependent parameters (e.g., peak plasma concentration and areas-under-the-curve) were norma1ized to a 5 mg dose for among-dose comparisons.

Following figure represents plasma concentration time profile for different treatments normalized to 5 mg dose.

Fampridine Dose Normalized Concentration vs. Time



Normalized to 5 mg dose
Dotted 5 mg; short dashed 10 mg; long dashed 15 mg; solid 20 mg

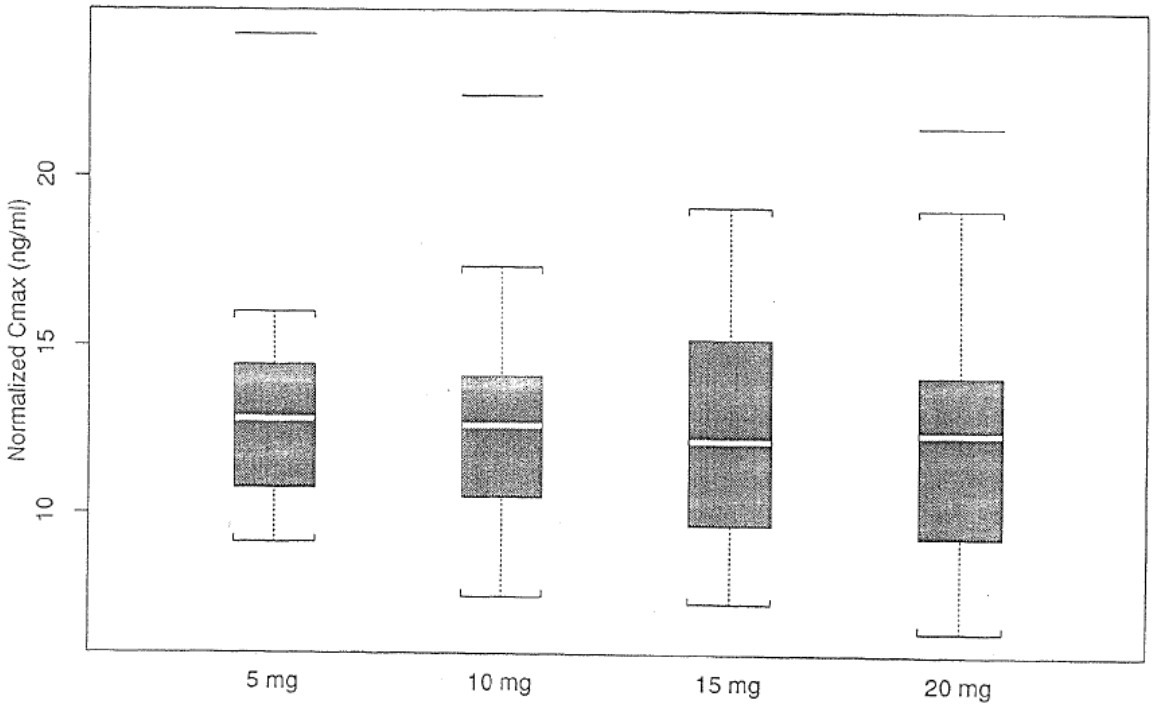
Following table lists PK summary for different treatments normalized to 5 mg dose.

Fampridine pharmacokinetic summary

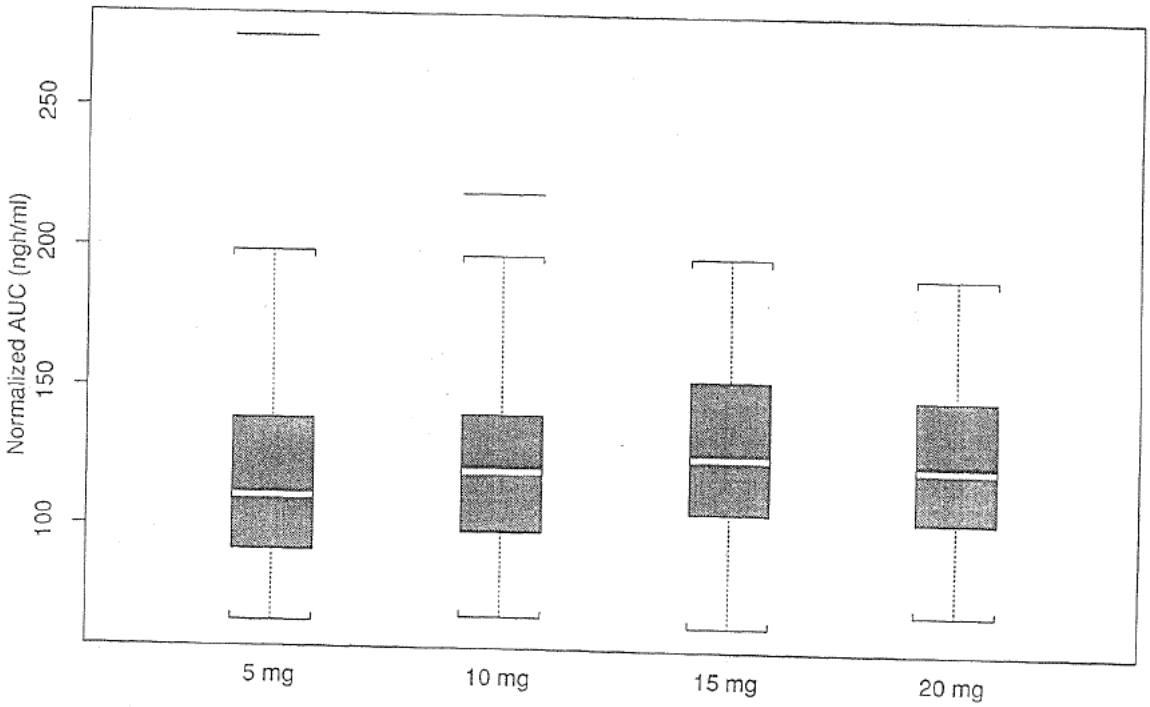
	t_{max} (h)	NC_{max}* (ng/ml)	NAUC* (ng·h/ml)	NAUCX* (ng·h/ml)	k (h^{-1})	$t_{1/2}$ (h)	CL/F (ml/min)
5 *mg*							
mean	3.88	13.13	122.1	146.4	0.143	5.76	619.8
s.e.	0.20	0.64	9.4	10.0	0.012	0.53	36.2
10 *mg*							
mean	3.92	12.61	126.1	141.6	0.143	5.55	641.4
s.e.	0.25	0.69	7.8	6.9	0.011	0.44	39.1
15 *mg*							
mean	3.63	12.34	131.5	143.0	0.138	5.50	632.4
s.e.	0.25	0.66	7.4	8.5	0.008	0.38	39
20 *mg*							
mean	3.57	12.31	127.8	136.7	0.148	5.05	653.9
s.e.	0.25	0.79	6.9	7.9	0.008	0.32	37.1
overall							
mean	3.75	12.60	126.9	142.0	0.143	5.47	636.7
s.e.	0.12	0.35	3.9	4.4	0.005	0.21	18.7

Following box plots represent Cmax and AUC vs dose for different treatments normalized to 5 mg dose.

Fampridine Normalized Cmax vs. Dose



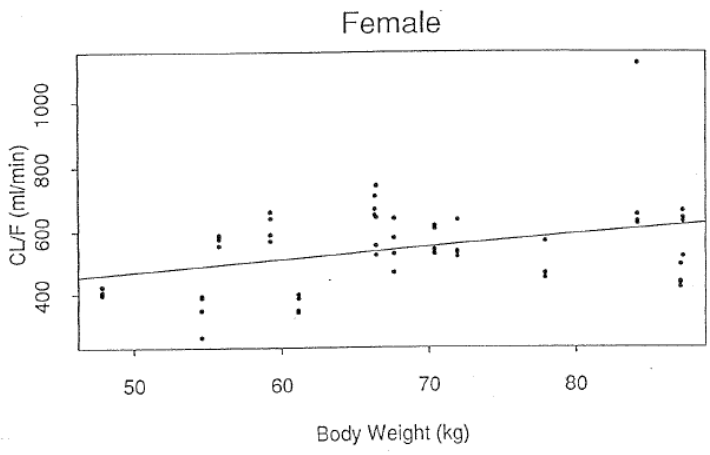
Fampridine Normalized AUC vs. Dose

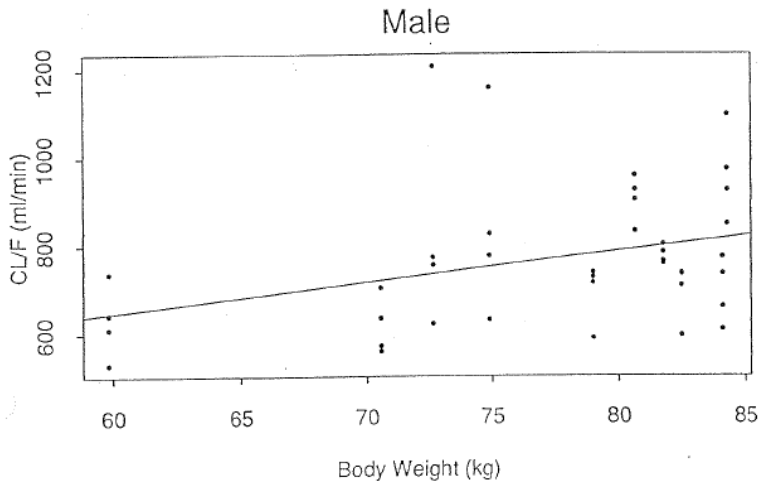


Overall observed time of the peak plasma concentration (mean and its 95% confidence interval) was 3.75 (3.52, 3.98) h, observed peak plasma fampridine concentration (normalized to a 5 mg dose) was 12.6 (11.9, 13.3) ng/ml, area-under-the-concentration-time-curve (normalized to a 5 mg dose) was estimated to be 127 (119, 135) ng.h/ml, extrapolated area-under-the- concentration-time curve (normalized to a 5 mg dose) was 142 (133, 151) ng.h/mL terminal rate constant equaled 0. l4 (0.13, 0.15) h^{-1}, terminal half-life was 5.47 (5.05, 5.89) h and clearance divided by bioavailability (CL/F) was equal to 637 (600, 674) ml/min.

Following scattered plot represent CL/F vs body weight for different treatments.

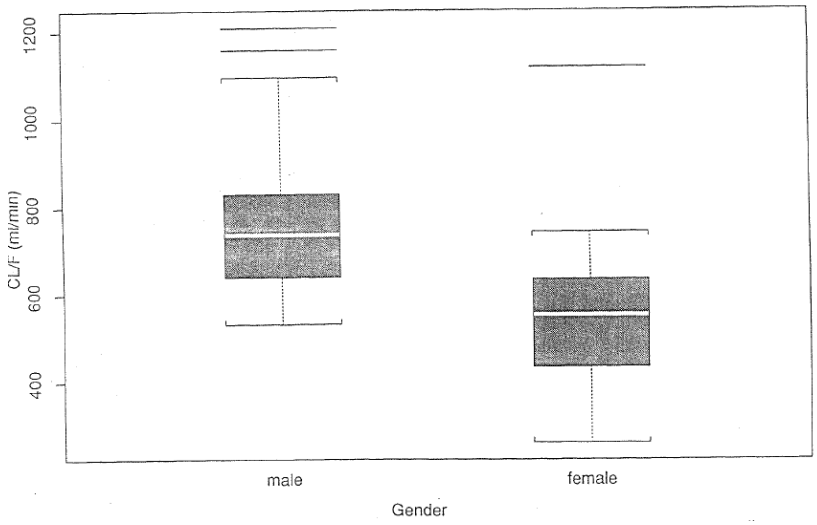
CL/F vs. Body weight in Male and Female





Following box plots represent CL/F vs Gender.

Fampridine CL/F vs. Gender



Conclusions

- Pharmacokinetics of fampridine appeared to be linear with single oral fampridine doses ranging from 5 to 20 mg/day.

- CL/F was influenced significantly by body weight (higher with larger weights), age (lower in older individuals) and gender (lower in women).

0195-001US: Pharmacokinetic and Tolerability Evaluation of Single Dose Fampridine SR Tablets Under Fed and Fasted Conditions, Single Dose Fampridine SR Capsules Under Fasted Conditions, and Multiple Dose Fampridine SR Tablets in Patients with Multiple Sclerosis

<u>Objectives:</u>

The primary objectives of this study were to compare the in-vivo performance under fasting conditions of a sustained release fampridine tablet and capsule formulation in MS patients in order to determine the substitutability of the tablet for the capsule in MS patients. The tablet formulation was further characterized under different conditions i) single dose with food; ii) multiple-dose.

Study Design	This study was a four period, crossover, unrandomized study of fampridine in patients with multiple sclerosis.
Study Population	Male and female subjects with multiple sclerosis Age: 18-65 years 12 subjects (6 male and 6 female) were enrolled, and 12 completed the study
Treatment Groups	**Single Dose** Treatment A: 12.5 mg fampridine SR capsule under fasting conditions Treatment B: 12.5 mg fampridine SR tablet under fasting conditions Treatment C: 12.5 mg fampridine SR tablet under fed conditions **Multiple Dose** Treatment D: 12.5 mg fampridine SR tablet (b.i.d) for 4 days
Dosage and Administration	The study consisted of four treatment periods in which the treatments were administered sequentially as outlined below: Period 1: Subjects received a 12.5mg SR capsule following an overnight fast and fasted for a further 4 hours after dosing. Period 2: Subjects received a 12.5mg SR tablet 30 minutes after receiving a standard breakfast (0.51 full fat milk, cereal, toast or roll with 60g ham and 60g full fat cheese). Period 3: Subjects received a 12.5mg SR tablet following an overnight fast and fasted for a further 4 hours after dosing. Period 4: Subjects received a 12.5mg sustained release tablet twelve-hourly for four days. The evening dose was administered 2.5 hours after dinner and the morning dose was administered 0.5 hours after breakfast on days 1-3. On Day 4, the pharmacokinetic sampling day. parients fasted overnight before dosing and 4 hours after dosing.
Sampling: Blood	Venous blood samples (no more than 5 ml) were withdrawn at the following times Single dose administration: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 24, and 32 hours. Steady state administration: 0 hours (pre-dose) sample on days 1-4 and the following sampling schedule on day 4 at the following times after the second dose. 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 24 and 32 hours.

<table>
<tr><td>Analysis</td><td>Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (µg/mL)</td><td>5, 40 and 150 ng/mL</td><td>2, 4, 8, 15, 30, 50, 100 and 200 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>9.84 to 10.6</td><td>2.54 to 9.31</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.999</td></tr>
<tr><td>Linear Range (µg/mL)</td><td colspan="2">2 to 200 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, µg/mL)</td><td colspan="2">2 ng/mL</td></tr>
</table>

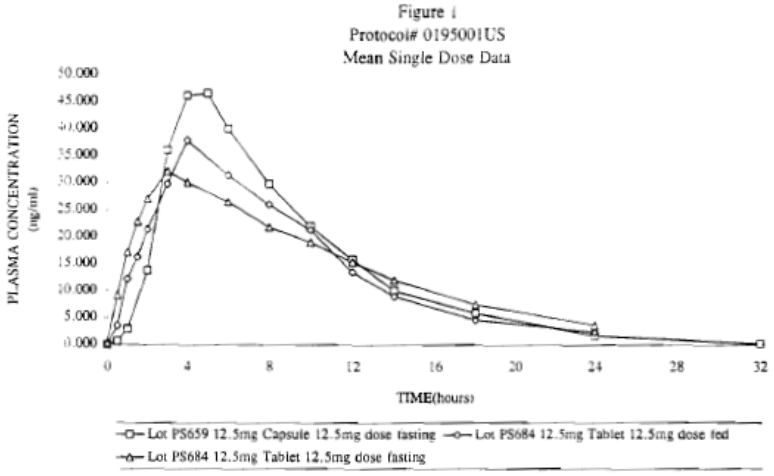
Reviewer's Comment: Atleast 50% of the QCs should pass the acceptance criteria (%CV ±15) at each concentration level for the run to be accepted. However, several runs were accepted eventhough more than 50% QCs failed at mid QC concentration in the following runs listed in the table.
<table>
<tr><th>Run#</th><th>Subject ID</th><th>Number of mid QCs Failed/Total Mid QCs Used</th></tr>
<tr><td>4</td><td>2005</td><td>5/8</td></tr>
<tr><td>7</td><td>2008</td><td>5/8</td></tr>
<tr><td>10</td><td>2011</td><td>7/8</td></tr>
<tr><td>18</td><td>1022</td><td>5/8</td></tr>
</table></td></tr>
<tr><td>Urine</td><td>None</td></tr>
<tr><td>PK Assessments</td><td>AUC(0-24h), AUC 0-12h, AUC(0-inf), Cmax, tmax, kel and t1/2.</td></tr>
<tr><td>PD Assessments</td><td>None</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetics: Geometric means and geometric coefficients of variation were provided for AUC(0-24h), AUC(0-inf) and Cmax.

Analysis of variance (ANOVA) was performed on the pharmacokinetic parameters.</td></tr>
</table>

RESULTS:

The tablet formulation had a lower Cmax value than the capsule formulation in MS patients. The tablet and capsule formulations following single-dose administration had similar AUC values.

Plasma Concentration Time Profile for Different Treatments



Food had little effect on the tablet formulation, with bioequivalence terms of AUC for the fasted and fed administration of this formulation and a slight increase in Cmax with food. The ratio of fed to fasted Cmax was 1.15.

The Cmax values for the tablet formulation administered with food were still lower than the peak concentrations achieved by the capsule formulation. The geometric means as well as the 90% confidence intervals are presented below:

Geometric Means

Parameter	Fampridine 12.5mg capsule fasted	Fampridine 12.5mg tablet fed	Fampridine 12.5mg tablet fasted
Cmax	49.54 (1.40)	37.47 (1.41)	32.57 (1.33)
AUC(inf)	400.35 (1.43)	359.87 (1.44)	392.05 (1.38)
AUC(0-24)	383.33 (1.42)	340.64 (1.43)	357.51 (1.34)

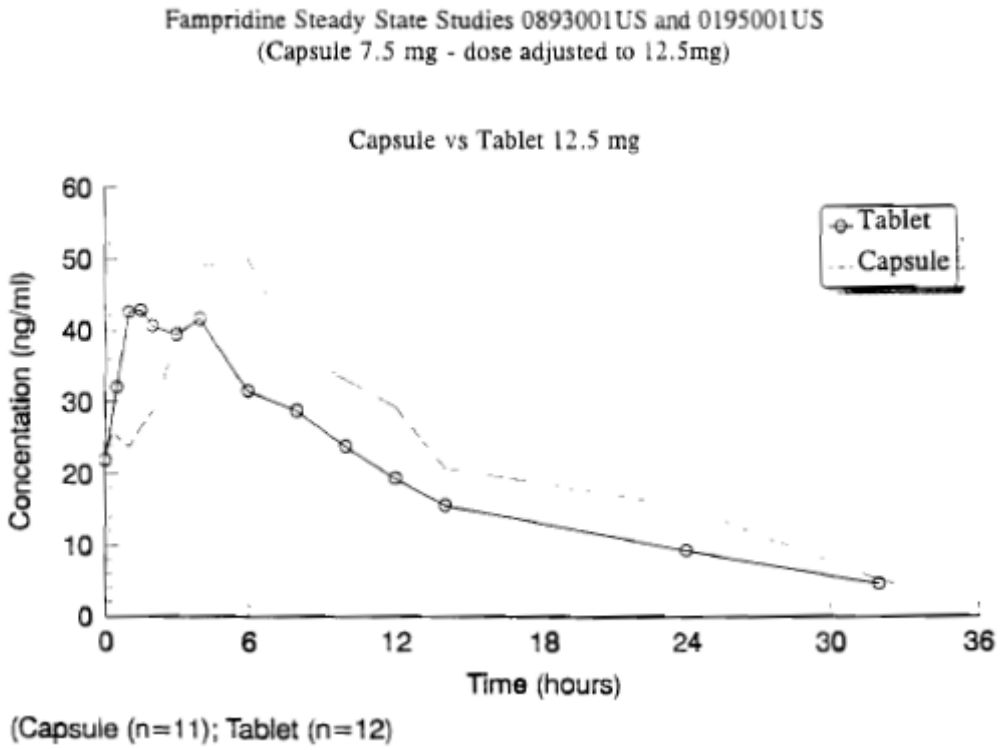
90% Confidence Intervals

Parameter	Comparisons	90% Confidence intervals
Cmax	Tablet fed vs Capsule	68 - 84
	Tablet fasted vs Capsule	59 - 73
	Tablet fed vs Tablet fast	104 - 127
	Ratio tablet fed/fasted	1.15
AUC(inf)	Tablet fed vs Capsule	80 - 100
	Tablet fasted vs Capsule	88 - 109
	Tablet fed vs Tablet fast	82 - 103
	Ratio tablet fed/fasted	0.92
AUC(0-24)	Tablet fed vs Capsule	81 - 99
	Tablet fasted vs Capsule	85 - 103
	Tablet fed vs Tablet fast	87 - 105
	Ratio tablet fed/fasted	0.95

Multiple Dose Pharmacokinetics:

Steady State Cmax for fampridine was higher than at single dose indicating accumulation after multiple dosing. The AUC was comparable between the single dose and steady state regimens.

Figure below represents fampridine plasma concentration time data on day 4 of multiple dosing. Dashed line indicates dose normalized data for 7.5 mg capsule from the previous study.



Sponsor compared data from previous study (Bio 0893001) with a group of subjects receiving a 7.5 mg fampridine capsule twice daily for four days. The dose adjusted data from the previous study showed that the mean Cmax values were similar following steady state administration of the tablet and the capsule. There was no significant difference in AUC between the capsule and tablet following steady state dosing according to the sponsor analysis.

Table below shows the single dose state data from day 1 of this study.

Mean non-transformed pharmacokinetic parameters, Mean ± SD, n=12

Parameter	Capsule	Tablet-fed	Tablet-fasted
Cmax	52.06 ± 16.60*	39.61 ± 14.55**	33.76 ± 9.34
AUC(0-24)	405.50 ± 141.74	361.09 ± 131.81	371.68 ± 106.29
AUC(inf)	424.46 ± 152.92	382.23 ± 138.82	410.41 ± 128.29
tmax	4.50 ± 0.67	3.96 ± 1.71**	3.42 ± 1.08
thalf	4.43 ± 1.32	4.54 ± 1.31**	5.90 ± 1.50***
kel	0.17 ± 0.05	0.17 ± 0.06**	0.12 ± 0.03***

Table 2 Mean log-transformed pharmacokinetic data, single dose data

Means (gSD)

Parameter	Capsule	Tablet fed	Tablet fasted
Cmax	49.54 (1.40)*	37.47 (1.41)**	32.57 (1.33)***
AUC(0-24)	383.33 (1.42)*	340.64 (1.43)	357.51 (1.34)
AUC(0-inf)	400.35 (1.43)	359.87 (1.44)	392.05 (1.38)

* Statistically significant ($p<0.05$) comparing Tablet fed vs Capsule

** Statistically significant ($p<0.05$) comparing Tablet fasted vs Capsule

*** Statistically significant ($p<0.05$) comparing Tablet fed vs Tablet fast

Table below shows the steady state data from this study and a previous study 0893001US for comparison.

Table 3 90% confidence intervals for log-transformed single dose data

Parameter	Comparisons	90% Confidence intervals
Cmax	Tablet fed vs Capsule	68 - 84
	Tablet fasted vs Capsule	59 - 73
	Tablet fed vs Tablet fast	104 - 127
	Ratio tablet fed/fasted	1.15
AUC(inf)	Tablet fed vs Capsule	80 - 100
	Tablet fasted vs Capsule	88 - 109
	Tablet fed vs Tablet fast	82 - 103
	Ratio tablet fed/fasted	0.92
AUC(0-24)	Tablet fed vs Capsule	81 - 98
	Tablet fasted vs Capsule	85 - 103
	Tablet fed vs Tablet fast	87 - 105
	Ratio tablet fed/fasted	0.95

Table 4 Steady State pharmacokinetic parameters

Parameter	Capsule Fed (Bio 0893001)	Tablet fast (day 5)
Cmax (ng/ml)	52.23 ± 12.67	50.19 ± 11.74
$AUC_{0\text{-}tau}$(ng.ml.h)	447.64 ± 125.53	384.58 ± 118.22
Tmax (hours)	4.91 ± 1.04	2.00 ± 1.28

Discussion: Data from the analytical runs from atleast 40% of the subjects (5/12) has to be excluded as they did not meet run acceptance criteria. Integrity of the study data cannot be assured as noted in the analytical section of the review.

Note: Following conclusions are based on the whole data sets. Reanalysis was not conducted as significant numbers of subjects have to be excluded.

CONCLUSIONS:

- The total systemic exposure of fampridine from the tablet at steady state was comparable to single dose.

- Maximum plasma concentration (Cmax) at steady state was slightly higher when compared to Cmax after single dose indicating accumulation after multiple dosing.

- Food had little effect on the extent of availability of fampridine following administration of an SR tablet formulation. In fed conditions the Cmax for fampridine was slightly higher for the tablet compared to the fasted treatment.

AN751-102: An Open-Label Protocol to Assess the Steady State Pharmacokinetics of Orally Administered Fampridine (4-aminopyridine) in Subjects with Multiple Sclerosis

Objectives:

To assess the steady state pharmacokinetics over a two week period of sustained release fampridine tablets as determined by the following pharmacokinetic parameters: AUC, Cmax, t1/2, systemic clearance, and Vd/F.

To assess the safety and tolerability of fampridine at steady state as determined by adverse events, and any effects on the measured laboratory tests, vital signs, 12-lead ECG, and the pre- and post-treatment physical examinations.

To assess, as secondary safety measures, any effects of fampridine on the eight meter walking time, and pharmacokinetics which could be attributed to age, gender, accumulation in p1asma, concomitant drug therapy, or other factors, In addition, any elucidation of any dosage regimen alterations necessary for achievement of optimal steady state plasma 4-aminopyridine concentrations were to be evaluated.

<table>
<tr><td>Study Design</td><td>This study was an open-label, multi-center, single treatment period, multiple dose study of orally administered fampridine in patients with multiple sclerosis.</td></tr>
<tr><td>Study Population</td><td>Male and female subjects with multiple sclerosis
Age: 18-65 years
21 subjects were enrolled (10 Male and 11 female), and 20 completed the study</td></tr>
<tr><td>Treatment Groups</td><td>Treatment A: 2 x 2.5 mg 4-aminopyridine b.i.d for 14 days
Treatment B: 2 x 5 mg 4-aminopyridine b.i.d for 14 days
Treatment C: 2 x 7.5 mg 4-aminopyridine b.i.d for 14 days
Treatment D: 2 x 10 mg 4-aminopyridine b.i.d for 14 days</td></tr>
<tr><td>Dosage and Administration</td><td>
<table>
<tr><th>Maximum Tolerated Dose</th><th>Titration Phase Days 1-2</th><th>Chronic Administration Phase Days 3-14</th></tr>
<tr><td>5.0 mg</td><td>5.0 mg morning
2.5 mg evening</td><td>5.0 mg morning
5.0 mg evening</td></tr>
<tr><td>10.0 mg</td><td>10.0 mg morning
5.0 mg evening</td><td>10.0 mg morning
10.0 mg evening</td></tr>
<tr><td>15.0 mg</td><td>15.0 mg morning
7.5 mg evening</td><td>15.0 mg morning
15.0 mg evening</td></tr>
<tr><td>20.0 mg</td><td>20.0 mg morning
10 mg evening</td><td>20.0 mg morning
20.0 mg evening</td></tr>
</table>
All subjects enrolled in this multiple dose study had previously completed the single dose study with fampridine (AN751-101). Furthermore, all the subjects enrolled in the present study had previously achieved a maximum tolerated dose of 20 mg in 1he single dose study. Consequently, subjects in the current study initially received twice daily doses of 20 mg of fampridine, for a total daily dose of 40 mg (20 mg in the morning, 20 mg in the evening).</td></tr>
<tr><td>Sampling: Blood</td><td>Blood samples for determining drug plasma levels were collected immediately prior to drug administration (baseline) and at the following hours after drug administration: hours 1-8, hour l0, hour l2, and hour 24. Additional blood samples were to be drawn at hours 14, 18, and 20 on Day 14 and on Day l5 additional samples were to be drawn at hours 30 and 36.</td></tr>
<tr><td>Analysis</td><td>Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (µg/mL)</td><td>5, 40 and 150 ng/mL</td><td>2, 4, 8, 15, 30, 50, 100 and 200 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>9.87 to 16.7</td><td>2.92 to 10.6</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.999</td></tr>
<tr><td>Linear Range (µg/mL)</td><td colspan="2">2 to 200 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, µg/mL)</td><td colspan="2">2 ng/mL</td></tr>
</table></td></tr>
</table>

<table>
<tr><td></td><td>

Reviewer's Comment: Atleast 50% of the QCs should pass the acceptance criteria (%CV ±15) at each concentration level for the run to be accepted. However, several runs were accepted though more than 50% QCs failed at mid QC concentration in the following runs listed in the table.

<table>
<tr><th>Run</th><th>Subject ID</th><th>Number of mid QCs Failed/Total Mid QCs Used</th></tr>
<tr><td>5</td><td>1005</td><td>4/6</td></tr>
<tr><td>9</td><td>2004</td><td>4/6</td></tr>
<tr><td>11</td><td>2007</td><td>4/6</td></tr>
<tr><td>12</td><td>2012</td><td>4/6</td></tr>
<tr><td>15</td><td>1006</td><td>4/6</td></tr>
<tr><td>17</td><td>2009-2011</td><td>7/8</td></tr>
</table>

</td></tr>
<tr><td>Urine</td><td>None</td></tr>
<tr><td>PK Assessments</td><td>Pharmacokinetics: Pharmacokinetic variables, peak plasma concentration (Cmax), time of the peak plasma concentration (t), trough concentration (Cτ), area under the curve (AUC), and half-life t1/2 were to be determined from measurements of plasma levels of fampridine. Detai1ed concentration time measurements were to be performed for the 24 hour period following initiation of dosing on Day 1, Day 7, and Day 14 with termination on Day 15.</td></tr>
<tr><td>PD Assessments</td><td>None</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetics: Geometric means and geometric coefficients of variation were provided for AUC(0-t), AUC(0-inf), and Cmax.

Analysis of variance (ANOVA) was performed on the dose-normalized In-transformed AUC(0-inf), AUC(0-t), and Cmax.</td></tr>
</table>

Gender	
Male	10 (48%)
Female	11 (52%)
Race	
Caucasian	21 (100%)
Age (years)	
Mean ± SEM	45.1 ± 1.6
Minimum	29
Maximum	57
Age (years) at MS onset	
Mean ± SEM	29.8 ± 1.8
Minimum	19
Maximum	50
Weight (pounds)	
Mean ± SEM	162.1 ± 4.8
Minimum	120
Maximum	192
Height (inches)	
Mean ± SEM	67.6 ± 0.63
Minimum	62
Maximum	72

SEM = standard error of mean
Data extracted from Section 14, Table 2

RESULTS:

Pharmacokinetic results:

Mean (95% CI) Fampridine Pharmacokinetic Parameters

Parameter	Occasion				Statistical Comparison†
	Single Dose Treatment	Day 1	Day 8	Day 15	
t_{max} h	3.76 (3.46, 4.06)	3.78 (3.24, 4.32)	3.33 (2.79, 3.87)	3.25 (2.64, 3.86)	C B S A
C_{max} ng/ml	50.5§ (45.2, 55.7)	48.6 (42.0, 55.3)	66.7 (57.5, 76.0)	62.6 (55.7, 69.4)	A S C B
C_τ ng/ml	21.4§ (18.8, 24.1)	21.4 (18.2, 24.6)	23.9 (19.6, 28.3)	23.0 (19.5, 26.5)	A S C B
C_{max}/C_τ	2.43 (2.23, 2.63)	2.44 (2.00, 2.88)	2.90 (2.69, 3.11)	2.88 (2.51, 3.25)	S A C B
AUC ng·h/ml	569§ (501, 637)	NE	531 (452, 610)	499 (446, 552)	C B S
CL/F ml/min	635 (567, 703)	NE	700 (557, 844)	703 (621, 786)	S B C
λ h^{-1}	0.14 (0.12, 0.16)	NE	NE	0.13 (0.11, 0.15)	C S
$t_{1/2}$	5.13 (4.53, 5.73)	NE	NE	5.75 (4.95, 6.55)	S C

* Study AN751-102 for Days 1, 8 and 15; Study AN751-101 for single dose treatment.
† Occasions under same bar do not differ significantly;
S: single dose treatment, A: Day 1, B: Day 8 and C: Day 15.
§ Normalized to a dose of 20 mg.
NE = not evaluable

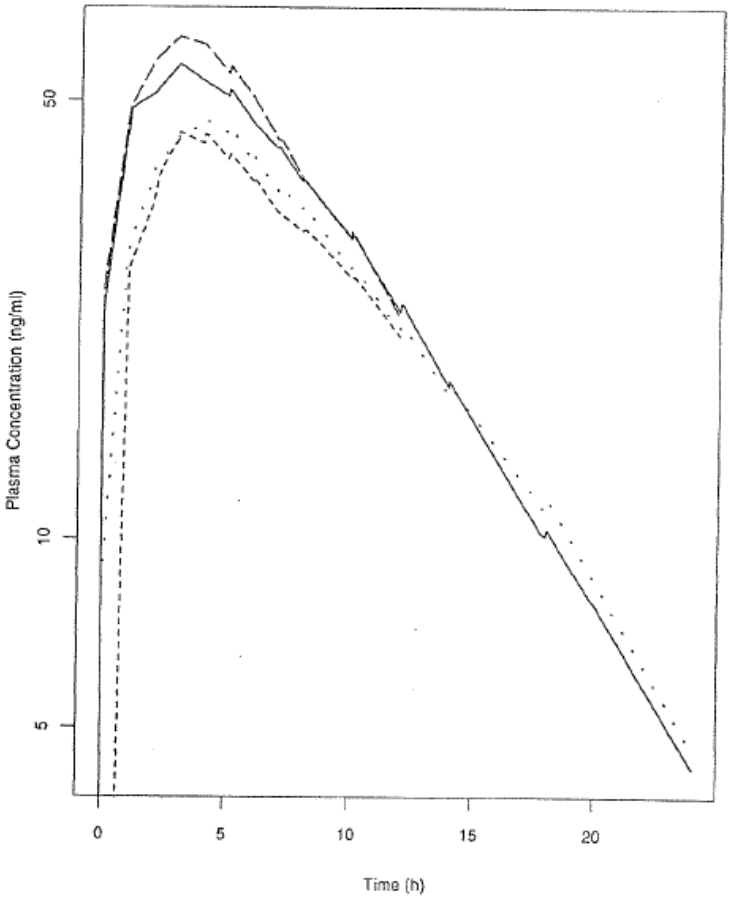
SD treatment refers to study AN75-101.

Fampridine pharmacokinetic parameter estimates of the first dose in this study (Day 1) are similar with those obtained with single dose study (Study AN751-101).

Cmax, and Cmax/Cτ on Days 8 (Cmax = 66.7 ng/mL) and 15 (Cmax= 62.6 ng/mL) were significantly greater than those of the single dose treatment and of Day 1 (Cmax= 48.6 ng/ml).

No significant difference in tmax, was detected among the four means (Single dose = 3.76 h; Day 1 = 3.78 h; Day 8 = 3.33 h; Day 15 = 3.25 h),

There was no significant difference among the four occasions with regard to either tmax, or Cτ and no difference in Cmax, Cmax/Cτ, CL/F or AUC between Days 8 and 15. Further, AUC0-t, on Days 8 and 15 did not differ significantly from total AUC with single dose treatment. Likewise, the estimates of CL/F on Days 8 and l5 and of λ and t1/2 on Day 15 did not differ significantly from those with single dose.



dotted: single dose (20 mg normalized); short dashed: Day 1
long dashed: Day 8; solid: Day 15

Discussion: Data from the analytical runs from atleast 40% of the subjects (8/20) has to be excluded as they did not meet run acceptance criteria. Integrity of the study data cannot be assured as noted in the analytical section of the review.

Note: Following conclusions are based on the whole data sets. Reanalysis was not conducted as significant numbers of subjects have to be excluded.

CONCLUSIONS:

- Fampridine accumulation occurs with multiple dosing as there was significant increase in Cmax and Cmax/Cτ on Days 8 and 15 relative to that on Day 1 and with single dose treatment.

- Steady state appears to have reached as there were no significant differences in Cmax as well as in Cτ, AUC and CL/F between Days 5 and 15.

4.1.2 INTRINSIC FACTORS

RD10F-SR012004: A Single-Dose, Open-label, Two-stage Study of Fampridine-SR in Healthy Volunteers and Renally Impaired Adults

Objective:

The objective of this study was to assess the pharmacokinetics of fampridine (including the measurement of fampridine metabolites in plasma and urine) in adults with mild, moderate, and severe renal impairment as compared to subjects with normal renal function.

Study Design	This was a 2-stage, multicenter, non-randomized, parallel-group, open-label, single-dose study.
Methodology	In the first stage, subjects with moderate renal impairment were enrolled and dosed with a single Fampridine-SR 10 mg tablet, healthy subjects and subjects with mild and severe renal impairment mere enrolled and dosed in the second stage. The same dose was administered in the second stage (10 mg) after reviewing the safety and pharmacokinetic profile in subjects with moderate renal impairment from the first stage.
Study Population	Renally impaired subjects (Men and Women) Age: 18-80 years BMI: ≤ 35 kg/m^2 20 subjects were enrolled, and 20 completed the study Group 1 (Normal renal function): creatinine clearance of > 80 mL/min Group 2 (Mild renal impairment): creatinine clearance of >50 - 80 mL/min Group 3 (Moderate renal impairment): creatinine clearance of 30 - 50 mL/minute Group 4 (Severe renal impairment): creatinine clearance of < 30 mL/minute
Test Product, Dose, Duration, Mode of Administration	All subjects received a single oral 10 mg dose (1 x 10 mg tablet) of Fampridine-SR commercial formulation administered with 240 mL water, in a standard fasting condition.
Sampling: Blood and Urine	Blood samples were obtained at the following times: predose (within 30 minutes prior to drug administration) and 0.5 (30 minutes), 1, 1.5 (90 minutes), 2, 3, 4, 5, 6, 8, 12, 16, 24, 30, and 36 hours postdose. Sampling continued for subjects with renal impairment, with blood

samples obtained 48, 72, 96, and 320 hours after the dose. For each time point, a minimum of 7 mL, of blood was obtained.

All urine excreted during study participation was collected for 36 hours postdose in healthy subjects and for 120 hours in subjects with renal impairment. Subjects voided prior to dosing. Thereafter, urine collected during the following intervals was pooled: 0-2, >2-4, >4-6, >6-8, >8-12, >12-24, >24-36, >36-48, >48-72, >72-96, and >96-320 hours postdose.

Analysis

Fampridine concentration was determined in plasma and urine samples using a validated method for high performance liquid chromatography-with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.

Summary of analytical performance of fampridine assay in plasma

Parameter	**Quality Control Samples**	**Standard Curve Samples**
Quality Control or Standard Curve Concentration (ng/mL)	6, 40, and 150 ng/mL	2, 4, 8, 15, 30, 50, 100 and 200 ng/mL
Between Batch Precision (%CV)	7.98 to 11.5	1.28 to 8.37
Linearity	Weighted linear equation ($1/X^2$), mean r= 0.999	
Linear Range (ng/mL)	2 to 200 ng/mL	
Sensitivity (LLOQ, ng/mL)	2 ng/mL	

Summary of analytical performance of fampridine assay in urine

Parameter	**Quality Control Samples**	**Standard Curve Samples**
Quality Control or Standard Curve Concentration (ng/mL)	1500, 5000, and 15000 ng/mL	500, 1000, 1500, 2000, 5000, 10000, 15000 and 20000 ng/mL
Between Batch Precision (%CV)	0.31 to 0.51	0.22 to 0.44
Linearity	Weighted linear equation ($1/X^2$), mean r= 0.998	
Linear Range (ng/mL)	500 to 20000 ng/mL	
Sensitivity (LLOQ, ng/mL)	500 ng/mL	

Assay performance of for metabolites in plasma and urine was reviewed and found to be acceptable.

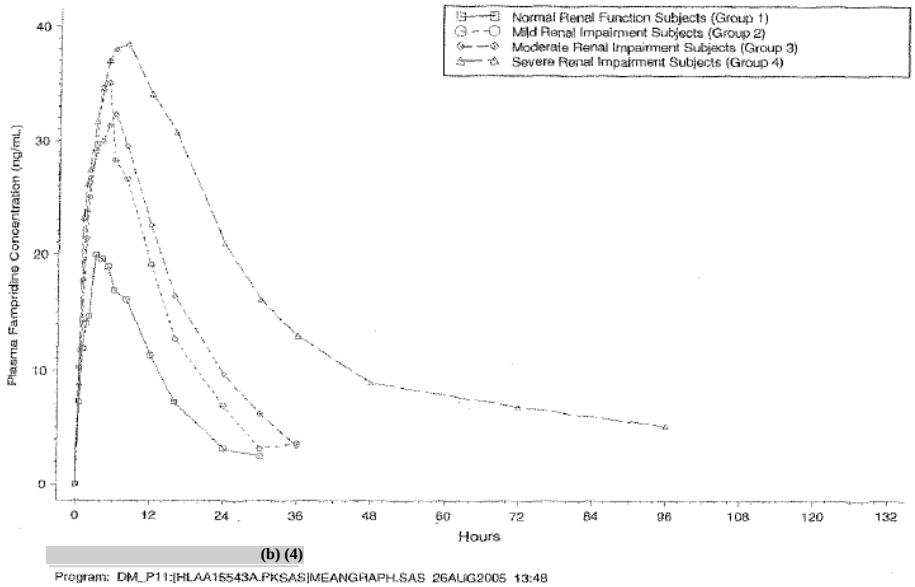
Pharmacokinetic

Pharmacokinetic parameters were calculated from the individual plasma concentrations of fampridine and its metabolites (3-hydroxy-4-aminopyridine and 3-hydroxy-4-aminopyridine-sulfate) using

	noncompartmental methods. Plasma pharmacokinetic parameters calculated were Cmax, Tmax, Tlag, AUC(0-t, AUC(0-inf), T1/2, Kel, MRTpo, AUCR, and CL/F (CL/F for parent drug only). CumAe, % of dose, and CLr parameters were calculated based on urinary drug concentration of fampridine and its metabolites.
Safety	Safety was evaluated by analyzing physical examination findings, vital sign measurements, adverse events (AEs), and laboratory values.
PD Assessments	None
Statistical Methods	Pharmacokinetics: No formal a priori criteria for between group differences were defined. The analysis of variance (ANOVA) was performed on the natural log-transformed AUC(0-t), AUC(0-inf), and Cmax. The ANOVA model included renal function group as a fixed effect. Each ANOVA included calculation of least- squares means (LSM) for each group and the standard errors associated with the differences between each of the renally impaired groups (Groups 2, 3, and 4) and normal renal function group (Group 1). Ratios of LSW were calculated using the exponentiation of the LSM from the analyses on natural log-transformed AUC(0-t), AUC(0-inf), and Cmax. These ratios were expressed as a percentage of the value in subjects with renal impairment (Groups 2, 3, and 4) relative to the values in normal renal function subjects (Group 3), The 90% confidence intervals for the ratios were derived by exponentiation of the confidence intervals obtained for the difference between impairment category LSM resu1ting from the analyses on the natura1 log-transformed AUC(0-t), AUC(0-inf), and Cmax. The confidence interva1s were expressed as percentage relative to the healthy group. The relationship between fampridine and its metabolites exposure and renal function was investigated by linear regression of each individual PK parameter [AUC(0-t), AUC(0-inf), Cmax, C/F, and CLr] and creatinine clearance.

RESULTS:

Mean Fampridine Plasma Concentrations Versus Time



Plasma concentrations were higher in renally impaired subjects compared to normal subjects.

Arithmetic Mean ± SD of Pharmacokinetic Parameters for Fampridine

Pharmacokinetic Parameter	Renal Function			
	Normal	Mild Impairment	Moderate Impairment	Severe Impairment
Plasma				
Cmax (ng/mL)	21.6 ± 3.89	36.3 ± 8.01	34.7 ± 6.63	43.5 ± 8.86
Tmax* (hr)	3.00 (1.00, 5.00)	4.00 (4.00, 5.00)	6.00 (2.00, 8.00)	8.00 (3.00, 16.0)
AUC(0-t) (ng*hr/mL)	254.13 ± 35.024	464.61 ± 69.021	529.53 ± 130.70	1170.2 ± 637.16
AUC(0-inf) (ng*hr/mL)	284.81 ± 31.784	500.90 ± 72.001	591.28 ± 120.92	1254.6 ± 691.30
T1/2 (hr)	6.446 ± 1.312	7.355 ± 1.639	8.135 ± 2.287	14.34 ± 6.172
Kel (1/hr)	0.111 ± 0.0206	0.0977 ± 0.0194	0.0914 ± 0.0280	0.0549 ± 0.0199
MRTpo (hr)	11.59 ± 1.672	12.80 ± 1.935	14.41 ± 3.674	24.02 ± 10.62
CL/F (L/hr)	35.47 ± 4.072	20.31 ± 3.011	17.62 ± 4.374	9.681 ± 4.255
Urine				
CumAe(0-36) (mg)	5.68 ± 1.92	6.00 ± 2.30	6.10 ± 0.84	2.57 ± 1.60
Ae(0-120) (mg)	--	6.00 ± 2.30	6.18 ± 0.93	2.69 ± 1.42
% Dose	56.8 ± 19.2	60.0 ± 23.0	61.8 ± 9.3	26.9 ± 14.2
CLr (L/hr)	22.18 ± 5.93	13.12 ± 5.32	12.03 ± 2.02	2.86 ± 1.78

* For Tmax median (min, max) presented
Source Data: Tables 14.2.1.5, 14.2.1.6, 14.2.1.7, 14.2.1.8, 14.2.4.1, 14.2.4.2, 14.2.4.3, and 14.2.4.4.

The exposure to fampridine and its metabolites increased with decreasing renal function. The mean Cmax of fampridine increased from 21.6 ng/mL in normal subjects to 36.3 ng/mL (mildly impaired subjects), 34.7 ng/mL (moderately impaired subjects), and 43.5 ng/mL (severely impaired subjects).

Similarly, the mean AUC(0-inf) of fampridine increased from 285 ng*hr/mL in norma1 subjects to 501 ng*hr/mL (mildly impaired subjects), 591 ng*hr/mL (moderately impaired subjects), and 1255 ng*hr/mL (severely impaired subjects).

Renal excretion is the major route of elimination for fampridine. CL/F decreased, and mean peak and extent of exposure increased in renally impaired subjects compared to normal subjects. The Tmax was delayed in renally impaired subjects compared to normal subjects. T1/2 was also longer in renally impaired subjects.

The fampridine renal clearance, CLr, decreased with renal impairment. The amount of fampridine excreted in urine over 36 hours postdose was similar in normal (5.68 mg), mildly impaired (6.00 mg), and moderately impaired (6.10 mg) subjects. But, the amount of fampridine excreted in urine was decreased by about 55% in severely impaired subjects (2.57 mg) in severely impaired subjects compared to normal subjects (5.68 mg).

A statistical comparison of fampridine plasma pharmacokinetic parameters between renally impaired subjects and normal subjects is presented in the table below.

Statistical Comparison of Pharmacokinetic Parameters for Fampridine

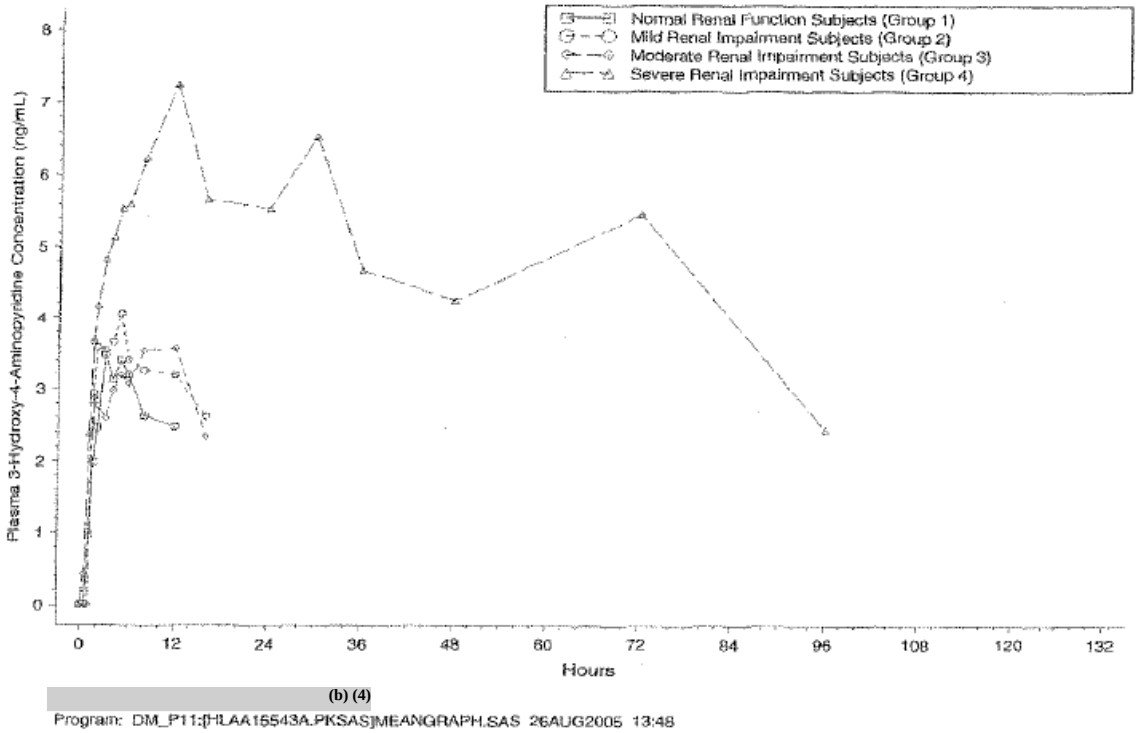
Pharmacokinetic Parameter	Geometric Mean		% Ratio of Geometric Means	90% CI
	Mild Impairment	Normal		
Cmax (ng/mL)	35.5	21.4	166.5	(131.0, 211.5)
AUC(0-t) (ng*hr/mL)	460.4	252.1	182.6	(131.1, 254.3)
AUC(0-inf) (ng*hr/mL)	496.7	283.4	175.3	(127.5, 240.9)
	Moderate Impairment	Normal		
Cmax (ng/mL)	34.1	21.4	159.7	(125.7, 203.0)
AUC(0-t) (ng*hr/mL)	515.1	252.1	204.3	(146.7, 284.5)
AUC(0-inf) (ng*hr/mL)	580.0	283.4	204.7	(148.9, 281.3)
	Severe Impairment	Normal		
Cmax (ng/mL)	42.7	21.4	199.9	(157.3, 253.9)
AUC(0-t) (ng*hr/mL)	1055.4	252.1	418.6	(300.6, 582.9)
AUC(0-inf) (ng*hr/mL)	1129.7	283.4	398.7	(290.1, 547.9)

Source Data: Tables 14.2.1.10, 14.2.1.11, and 14.2.1.12

Based on geometric ratios, when compared to normal subjects, the peak and extent of exposure were 67% and 75% higher in mildly impaired subjects, 60% and 105% higher in moderately impaired subjects, 100% and 299% higher in severely impaired subjects, respectively.

3-Hydroxy-4-Aminopyridine in Plasma and Urine

Mean 3-Hydroxy-4-Aminopyridine Plasma Concentrations versus Time



Arithmetic Mean ± SD of Pharmacokinetic Parameters for 3-Hydroxy-4-Aminopyridine

Pharmacokinetic Parameter	Renal Function			
	Normal	Mild Impairment	Moderate Impairment	Severe Impairment
Plasma				
Cmax (ng/mL)	3.42 ± 1.13	4.39 ± 0.214	4.26 ± 0.872	8.13 ± 2.95
Tmax* (hr)	3.00 (2.00, 5.00)	5.00 (3.00, 5.00)	2.00 (1.47, 12.0)	11.0 (3.00, 12.0)
AUC(0-t) (ng*hr/mL)	26.332 ± 14.012	43.649 ± 7.8394	37.777 ± 16.492	240.50 ± 245.82
AUC(0-inf) (ng*hr/mL)	--	--	--	324.83 ± 327.63
Urine				
CumAe(0-36) (mg)	0.44 ± 0.15	0.50 ± 0.08	0.60 ± 0.08	0.36 ± 0.19
Ae(0-120) (mg)	--	0.50 ± 0.08	0.60 ± 0.08	0.44 ± 0.27
% Dose	3.3 ± 1.1	3.7 ± 0.6	4.5 ± 0.6	3.3 ± 2.0
CLr (L/hr)	23.88 ± 17.49	12.05 ± 4.14	20.44±13.06	2.27 ± 1.00

* For Tmax median (min, max) presented
Source Data: Tables 14.2.2.5, 14.2.2.6, 14.2.2.7, 14.2.2.8, 14.2.5.1, 14.2.5.2, 14.2.5.3, and 14.2.5.4.

Plasma concentrations were not quantifiable after 16 hours postdose in normal subjects, mildly impaired subjects, and moderately impaired subjects. In severely impaired subjects they were quantifiable up to 96 hours postdose.

For 3-hydroxy-4-aminopyridine, mean renal clearance decreased with renal impairment except in moderately impaired subjects. The mean cumulative amount excreted in urine over 36 hours was similar among normal subjects, mildly impaired subjects, moderately impaired subjects, and severely impaired subjects.

Statistical comparison of 3-hydroxy-4-aminopyridine plasma pharmacokinetic parameters between renally impaired subjects and normal subjects are presented in the table below.

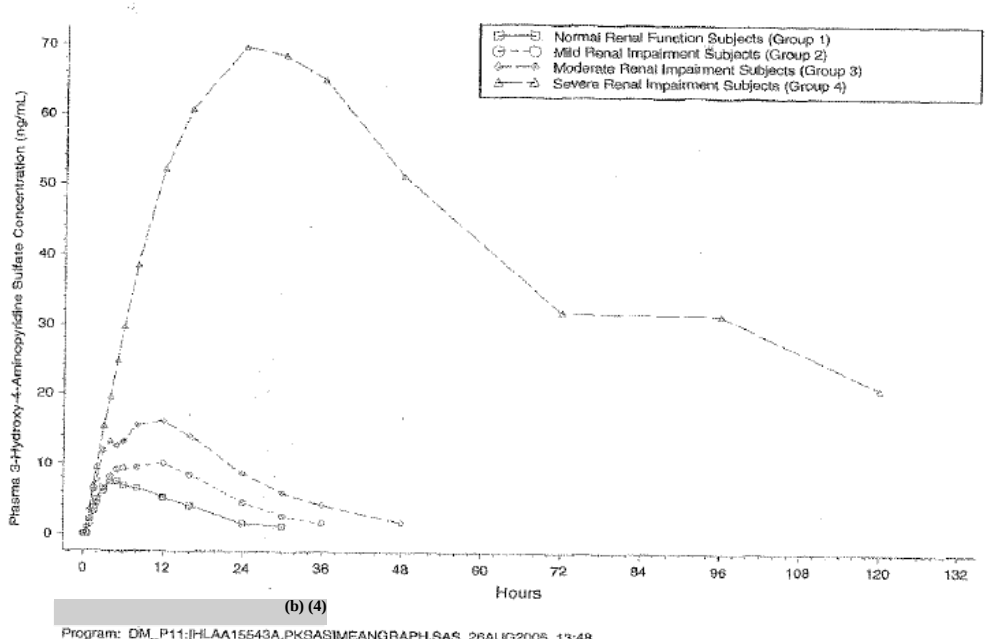
Statistical Comparison of Pharmacokinetic Parameters for 3-Hydroxy-4-Aminopyridine

Pharmacokinetic Parameter	Geometric Mean		% Ratio of Geometric Means	90% CI
	Mild Impairment	Normal		
Cmax (ng/mL)	4.4	3.3	134.5	(101.0, 179.1)
AUC(0-t) (ng*hr/mL)	43.1	21.5	200.3	(92.7, 432.7)
	Moderate Impairment	Normal		
Cmax (ng/mL)	4.2	3.3	128.8	(96.7, 171.4)
AUC(0-t) (ng*hr/mL)	33.6	21.5	156.4	(72.4, 337.9)
	Severe Impairment	Normal		
Cmax (ng/mL)	7.8	3.3	238.8	(179.3, 317.9)
AUC(0-t) (ng*hr/mL)	175.8	21.5	817.2	(378.2, 1765.5)

Source Data: Tables 14.2.2.10, 14.2.2.11, and 14.2.2.12

3-Hydroxy-4-Aminopyridine Sulfate in Plasma and Urine

Mean 3-Hydroxy-4-Aminopyridine Sulfate Plasma Concentrations Versus Time



Arithmetic Mean ± SD of Pharmacokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate

Pharmacokinetic Parameter	Renal Function			
	Normal	Mild Impairment	Moderate Impairment	Severe Impairment
Plasma				
Cmax (ng/mL)	7.69 ± 1.69	10.5 ± 3.23	17.0 ± 3.01	84.8 ± 69.8
Tmax* (hr)	5.00 (4.00, 5.00)	12.0 (5.00, 16.0)	12.0 (4.00, 16.0)	30.0 (12.0, 36.0)
AUC(0-t) (ng*hr/mL)	106.80 ± 23.534	209.09 ± 68.977	382.32 ± 120.95	4668.7 ± 5500.7
AUC(0-inf) (ng*hr/mL)	122.65 ± 19.672	226.91 ± 72.799	400.98 ± 122.59	5452.6 ± 6985.7
T1/2 (hr)	7.606 ± 2.767	8.026 ± 1.120	7.537 ± 0.6448	25.10 ± 13.59
Kel (1/hr)	0.101 ± 0.0355	0.0876 ± 0.0112	0.0925 ± 0.00840	0.0346 ± 0.0171
Urine				
CumAe(0-36) (mg)	0.77 ± 0.17	0.90 ± 0.19	1.33 ± 0.32	1.46 ± 0.51
Ae(0-120) (mg)	--	0.90 ± 0.19	1.37 ± 0.35	2.95 ± 1.12
% Dose	3.2 ± 0.7	3.7 ± 0.8	5.6 ± 1.4	12.1 ± 4.6
CLr (L/hr)	7.61 ± 3.01	4.81 ± 2.00	3.76 ± 0.99	1.05 ± 0.55

* For Tmax median (min, max) presented
Source Data: Tables 14.2.3.5, 14.2.3.6, 14.2.3.7, 14.2.3.8, 14.2.6.1, 14.2.6.2, 14.2.6.3, and 14.2.6.4.

The apparent elimination half-life was approximately similar in normal subjects, mildly impaired subjects, and moderately impaired subjects. A 3.3- fold prolongation in mean T1/2 was observed in severely impaired subjects compared to normal subjects.

For 3-hydroxy-4-aminopyridine sulfate, renal clearance decreased with renal impairment. However, the mean cumulative amount excreted in urine over 120 hours gradually increased with renal impairment.

Statistical comparison of 3-hydroxy-4-aminopyridine Sulfate plasma pharmacokinetic parameters between renally impaired subjects and normal subjects are presented in the table below.

Statistical Comparison of Pharmacokinetic Parameters for 3-Hydroxy-4-Aminopyridine Sulfate

Pharmacokinetic Parameter	Geometric Mean		% Ratio of Geometric Means	90% CI
	Mild Impairment	Normal		
Cmax (ng/mL)	10.1	7.5	134.6	(86.1, 210.4)
AUC(0-t) (ng*hr/mL)	201.0	104.9	191.7	(104.4, 351.9)
AUC(0-inf) (ng*hr/mL)	218.6	121.4	180.1	(95.5, 339.5)
	Moderate Impairment	Normal		
Cmax (ng/mL)	16.8	7.5	223.1	(142.8, 348.7)
AUC(0-t) (ng*hr/mL)	364.2	104.9	347.4	(189.2, 637.7)
AUC(0-inf) (ng*hr/mL)	383.2	121.4	315.6	(167.4, 595.0)
	Severe Impairment	Normal		
Cmax (ng/mL)	68.0	7.5	902.8	(577.7, 1410.8)
AUC(0-t) (ng*hr/mL)	3032.6	104.9	2892.2	(1575.3, 5309.9)
AUC(0-inf) (ng*hr/mL)	3288.1	121.4	2707.6	(1436.0, 5105.4)

Source Data: Tables 14.2.3.10, 14.2.3.11, and 14.2.3.12

Based on geometric ratios, when compared to normal subjects, the peak and extent of exposure were 35% and 80% higher in mildly impaired subjects, 123% and 216% higher

in moderately impaired subjects, and 803% (~8 fold) and 1708% (~26 fold) higher in severely impaired subjects, respectively.

The observed renal clearances were 13.12 L/hr, 12.03 L/hr, and 2.86 L/hr in subjects with mild, moderate, and severe renal impairment, respectively. Assuming bioavailability (i.e., 0.633 as F based on the total recovery of the dose), total clearance of fampridine would be about 12.9 L/hr (0.633*20.35 L/hr), 11.2 L/hr (0.633*17.62 L/hr), and 6.12 L/hr (0.633*9.68l L/hr), in subjects with mild, moderate, and severe renal impairment, respectively.

Discussion

Mean renal clearance of fampridine was 22.58 L/hr (370 mL/min) which is much higher than glomerular filtration rate indicating tubular secretion.

Maximum concentration attained after a single dose of fampridine SR 10 mg tablet in this study for normal subjects was approximately 22 ng/mL. However, in controlled clinical trials in MS patients (MS 203 and MS204) 10 mg fampridine-SR tablet b.i.d resulted in average Cmax of approximately 50 ng/ml. Some of the subjects experienced seizure at plasma fampridine concentrations in the range of 100 – 400 ng/mL.

The plasma concentrations for 3-hydroxy-4-aminopyridine sulfate were very high in severely impaired subjects compared to normal subjects. Also, the amount of 3-hydroxy-4-aminopyridne sulfate excreted in urine was increased by about 200% in severely impaired subjects (1.46 mg) compared to normal subjects (0.77 mg).

The pharmacokinetics of 3-hydroxy-aminopyridine sulfate in severely impaired subjects shows contribution of non renal clearance (i.e., biotransformation to metabolites) to total clearance of fampridine. The peak and extent of exposure to fampridine were 2-fold and 4-fold higher in severely impaired subjects compared to normal subjects. However, peak and extent of exposure to 3-hydroxy-4-aminopyridine sulfate were 8-fold and 26-fold higher in severely impaired subjects compared to normal subjects.

The regression analysis results showed a significant relationship between creatinine clearance and peak and extent of exposure for fampridine and its metabolites, and thus a dosage adjustment may be required based on creatinine clearance values in renally impaired subjects.

A pharmacokinetic compartment model along with covariate effect would be useful to ascertain the dose required in patients with different degrees of renal dysfunction and to maintain an appropriate risk-benefit profile for Fampridine-SR with repeated dosing.

Conclusions:

Following single oral administration of 10 mg Fampridine-SR.

- The mean percent fampridine recovered in urine was 56.8%, 60.0%, 61.8%, and 26.9 %, and mean renal clearance was 22.58 L/hr, 13.12 L/hr, 12.03 L/hr, and 2.86 L/hr

in normal subjects, mildly impaired subjects, moderately impaired subjects, and severely impaired subjects, respectively.

- The mean Cmax and AUC(0-inf) of fampridine increased by 67% and 75% in mildly impaired subjects, by 60% and 105% in moderately impaired subjects, and by 100% and 299% in severely impaired subjects, respectively, when compared to normal subjects.

- The CL/F and CLr of fampridine showed significant relationship ($p < 0.0001$ for C/F and $p = 0.0001$ for CLr) with creatinine clearance.

- The mean percent 3-hydroxy-4- aminopyridine sulfate recovered in urine was 3.2%, 3.7%, 5.6%, and 12.1%, and mean renal clearance was 7.61 L/hr, 4.81 L/hr, 3.76 L/hr, and 1.05 L/hr in normal subjects, mildly impaired subjects, moderately impaired subjects, and severely impaired subjects, respectively.

- The mean Cmax and AUC(0-inf) of 3-hydroxy-4-aminopyridine sulfate increased by 35% and 80% in mildly impaired subjects, by 123% and 216% in moderately impaired subjects, and by 803% and 1708% in severely impaired subjects, respectively, when compared to normal subjects.

Labeling Recommendation: Dosage adjustment is required to maintain an appropriate PK profile following repeated dose administration of Fampridine-SR depending on the degree of renal dysfunction in patients with mild to moderate renal impairment.

Based on the simulated plasma concentration versus time data in patients with mild to moderate renal impairment fampridine SR 10 mg once daily or 5 mg twice daily could not provide comparable effective plasma concentrations. However, 7.5 mg b.i.d dose resulted in comparable PK profile to normal MS patients. The dose has to be reduced to 7.5 mg b.i.d in patients with mild and moderate impairment based on safety and efficacy of fampridine.

Fampridine should not be used in patients with severe renal impairment.

4.1.3 EXTRINSIC FACTORS

0496-002: Excretion balance and pharmacokinetics of ^{14}C-radioactivity after single dose oral intake of ^{14}C-labelled 4-aminopyridine in healthy volunteers.

Objective:

The objective of this study was to determine the pharmacokinetics and excretion balance of ^{14}C-radioactivity after a single oral dose of ^{14}C-labelled 4-aminopyridine and to determine the parent compound and possible metabolites in plasma and urine.

Study Design	This was a single center, open-label, single-dose, pharmacokinetic and excretion study.
Study Population	Healthy male volunteers Age: 18-45 years

	Weight: ±15% deviation from normal range 4 subjects were enrolled, and 4 completed the study
Test Product, Dose, Duration, Mode of Administration	Single dose, oral administration of 15 mg (a 100 µCi ^{14}C-radioactivity) 4-aminopyridine as a solution.
Sampling: Blood, Urine and Faeces	Blood Sampling: 10 ml blood samples were obtained at the following times: 0, 1, 1.5 (90 minutes), 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144 and 168 hours postdose (17 samples per subject; total 68 samples). 15 mL samples for metabolite determination: 2, 4, 6, 8, 12 and 24 h after drug administration (6 samples per subject; total 24 samples) Urine sampling: -12-0, 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h after drug administration (11 samples per subject; total 44 samples) Faeces sampling: blank, and all stools for 168 h after drug administration (8 samples (24-h pooled fractions) per subject; total 32 samples)
Bioanalysis	Total ^{14}C-radioactivity was measured in medication (4 samples in triplicate), in plasma (68 samples in single mode + 15% in duplicate), in urine (44 samples in single mode + 15% in duplicate), in faeces (32 samples in duplicate); quick counts in urine and faeces on days 7 and 8 onwards (8 urine and 8 faeces samples); determination of 4-aminopyridine and its metabolites and metabolic profiling in plasma and urine. The ^{14}C-radioactivity was determined by means of liquid scintillation counting. The shape of all quench curves was good for all matrices. The quench range used was sufficient for all samples. • The ^{14}C-counting efficiency: 96.2% and 97.1%. • The ^{14}C-background : 20.3 and 21.0 dpm. • The CV of the quality control (QC) samples at the three concentrations levels were equal to or below 2.0% in plasma, equal to or below 2.2% in urine and equal to or below 4.9% in faeces. • The bias ranged from -4.1 to +1.2%, -0.6 to +0.4% and -2.2 to +4.1% for plasma, urine and faeces, respectively.
Pharmacokinetic Evaluation and Statistics	Pharmacokinetic parameters: for total ^{14}C-radioactivity: C_{max}, T_{max}, λ_{el}, $t_{1/2}$, $AUC_{0-\infty}$, A^e_{urine}, A^e_{faeces} and A^e_{total}; for ^{14}C-4-aminopyridine and metabolites: cumulative amounts in urine, faeces and total.
PD Assessments	None

RESULTS:

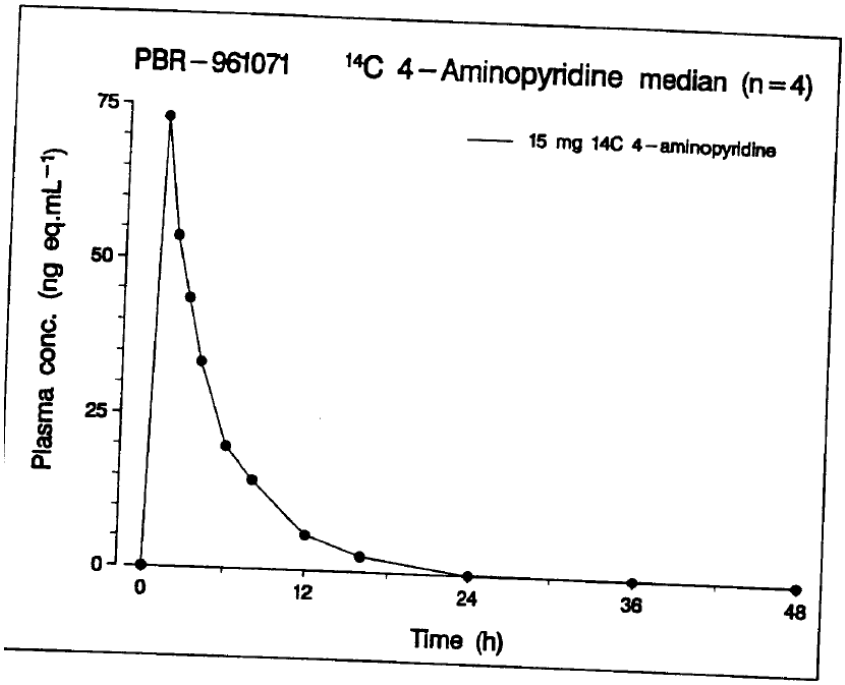
Recovery and Pharmacokinetic parameters: Recovery of ^{14}C-radioactivity and pharmacokinetic parameters derived from plasma ^{14}C-radioactivity are summarized as follows:

Recovery and Pharmacokinetic Parameters for Fampridine

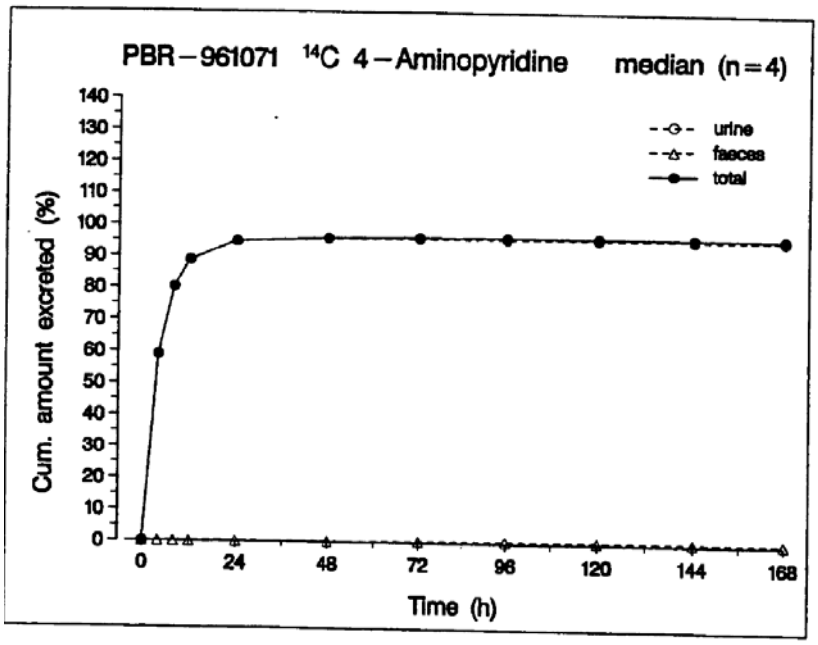
parameter	median	min - max
A^e_{urine} (%)	95.85	93.80 - 97.50
A^e_{feces} (%)	0.51	0.45 - 0.53
A^e_{total} (%)	96.36	94.33 - 97.95
C_{max} (ng eq.mL^{-1})	72.9	63.1 - 86.6
T_{max} (h)	1	1 - 1
λ_{el} (h^{-1})	0.220	0.151 - 0.237
$t_{1/2}$ (h)	3.14	2.92 - 4.59
$AUC_{0-\infty}$ (ng eq.h.mL^{-1})	341.2	258.0 - 416.3

^{14}C-4-aminopyridine was rapidly absorbed and eliminated. The median of Cmax of total radioactivity was 72.9 ng eq.mL^{-1}(range 63.1-86.6 ng eq.mL^{-1}), which was attained 1 hour after dosing. The median elimination rate constant of total ^{14}C-radioactivity was calculated to be 0.220 h^{-1} corresponding with a half-life of 3.14 h. The median area under the curve extrapolated until infinity was calculated to be 341.2 ng eq.h.mL^{-1}.

^{14}C-radioactivity concentrations (median) in plasma versus time after single oral dose administration of 15 mg ^{14}C-4-aminopyridine



Cumulative excretion (median) of ^{14}C-radioactivity (% of dose) after single oral dose administration of 15 mg ^{14}C-4-aminopyridine



CONCLUSIONS:

- Excretion of ^{14}C-radioactivity following oral administration of 15 mg ^{14}C-labelled 4-aminopyridine (100 µCi) was fast and almost complete.
- Cumulative excretion in urine amounted to 95.85% whereas only 0.51% was recovered from faeces resulting in a total excretion of 96.36%.

Study Title	**^{14}C-4-Aminopyridine Human Urine Metabolite Identification Studies**
Study number	ELN 14/972921
Objective	To identify metabolites of [^{14}C]-4-aminopyridine in human urine and plasma samples

METHODS:

Study Design:

A single oral dose of ^{14}C-4-aminopyridine (15 mg; 100 µCi; specific activity 6.667 µCi/mg) was administered to four human volunteer subjects and blood, urine and faeces were collected during the first 168 hours after dose administration.

The major radioactive components detected in urine were isolated and purified by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) and the components were then identified by TLC co-chromatography with available reference compounds and by mass spectrometry. In addition, metabolite profiling was carried out on selected plasma samples (up to 6 hours post-dose) by TLC.

Sample Details

The following samples were obtained from mass balance study 0496-002 for each of Subjects 1 – 4

(a) urine collected at pre-dose (-12 - 0 hours), 0 - 4, 4 - 8, S - 12, 12 - 24, 24 - 48, 48 - 72, 72 - 96, 96 - 120, 120 - 144 and 144 - 168 hours after dose administration, and
(b) plasma obtained at pre-dose, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144 and 168 hours after dose administration.

RESULTS:

Three major metabolites were identified in pooled 0-4 hour urine and these were 3-hydroxy-4- aminopyridine (M1), 4-aminopyridine (M2) and 3-hydroxy-4-aminopyridine sulphate (M3).

Additional evidence that metabolite M3 was a sulphate conjugate of 3-hydroxy-4-aminopyridine was obtained by hydrolysis of urine with aryl-sulphatase which caused an increase in the proportion of 3- hydroxyl-4-aminopyridine and a concomitant decrease in the proportion of M3.

At least four radioactive components (M1 - M4) were detected in methanol extracts of human plasma and both TLC co-chromatography and treatment with aryl-sulphatase indicated that components Ml - M3 were chromatographically equivalent to those detected in urine. Component M4 represented a quantitatively minor radioactive component in human plasma at all times investigated and in each case accounted for about 2% of extract radioactivity, which was close to the limit of accurate quantification. The presence of a fifth radioactive component was also indicated in most of the plasma samples profiled but the levels were below the limit of accurate quantification and the proportion of this component was estimated to be 2% or less in all cases.

There was about 3 times more unchanged 4-aminopyridine (M2) than 3-hydroxy-4-aminopyridine sulphate (M3) and about 8-10 times more 4-aminopyridine than its 3-hydroxy metabolite (M1) in human plasma during the first 6 hours after dose administration. The calculated plasma concentrations of each of the four metabolites were maximal at 1 hour post-dose, the time at which the first sample was collected.

The proportion of 4-aminopyridine in human plasma was less than that detected in 0-4 h urine, whereas the proportion of the sulphate conjugate was much higher in plasma than in urine. The proportion of the 3-hydroxy metabolite was broadly similar in both plasma and urine.

CONCLUSIONS:

- Most of the radioactivity detected in plasma during the first 6 hours post-dose corresponded with unchanged 4-aminopyridine (M2; 61.0% to 69.4% of extract radioactivity).
- 3-Hydroxy-4- aminopyridine sulphate (M3) accounted for 13.7% to 20.3% of extract radioactivity and

- Unconjugated 3-hydroxy-4-aminopyridine accounted for just 6.1% to 8.6% of extract radioactivity over the same period.

0194-002: A study to evaluate the pharmacokinetics of the simultaneous administration of Elan's controlled release fampridine formulation and Lioresal (Baclofen, Ciba-Geigy).

Objective:
The primary objective of this study was to characterize interaction effects, of fampridine and baclofen.

Study Rationale:
Baclofen is used widely in the treatment of spasticity in MS patients, therefore the probability of co-administration of these compounds in a clinical setting is high.

Twelve healthy male subjects were enrolled in the study. The study consisted of three treatment periods, with a minimum of one week between treatment periods. Subjects received either a single 15 mg controlled release fampridine capsule, a single 10mg Lioresal tablet or a combination of these treatments on each treatment period under fasting conditions based on a randomized cross-over design.

Study Design	Single-centre, open, randomized, three-way crossover trial
Study Population	Healthy Male Age: 18-40 years 13 subjects were enrolled, and 12 completed the study
Treatment Groups	Treatment A = 15 mg Fampridine-CR formulation Treatment B = 10 mg Lioresal (Baclofen) Treatment C = 15 mg Fampridine-CR formulation + 10 mg Lioresal (Baclofen) The treatments were administered under fasting conditions, all the treatments were separated by a 1-week washout period
Sampling: Blood	Blood samples (7 -10ml) for determination of plasma drug levels were taken into lithium heparin tubes at the following times: Treatment A (4-AP): 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20 and 24 hours. Treatment B (Lioresal): 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16 and 24 hours Treatment C (4-AP + Lioresal): 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20 and 24 hours

<table>
<tr><td>Analysis</td><td>Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>6, 40, and 150 ng/mL</td><td>2, 4, 8, 15, 30, 50, 100 and 200 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>7.42 to 9.36</td><td>2.72 to 9.52</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.992</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">2 to 200 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">2 ng/mL</td></tr>
</table></td></tr>
<tr><td>Urine</td><td>None</td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>PK Assessments</td><td>The pharmacokinetic parameters Cmax, Tmax, AUC(0-inf), were calculated from the plasma fampridine and baclofen concentration-time data using noncompartmental analysis.</td></tr>
<tr><td>PD Assessments</td><td>None</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetics: Plasma concentration-time data and pharmacokinetic parameters were presented in the electronic data sets.

Analysis of variance (ANOVA) was performed on the ln-transformed AUC(0-inf), AUC(0-t), and Cmax. The ratio of the means was expressed as a percentage of the least-squares means LSM (test/reference). The 90% confidence intervals (CI) for the ratio of the test and reference means were calculated for the ln-transformed parameters to show the effect of baclofen on PK of fampridine and the effect of fampridine on the PK of baclofen.</td></tr>
</table>

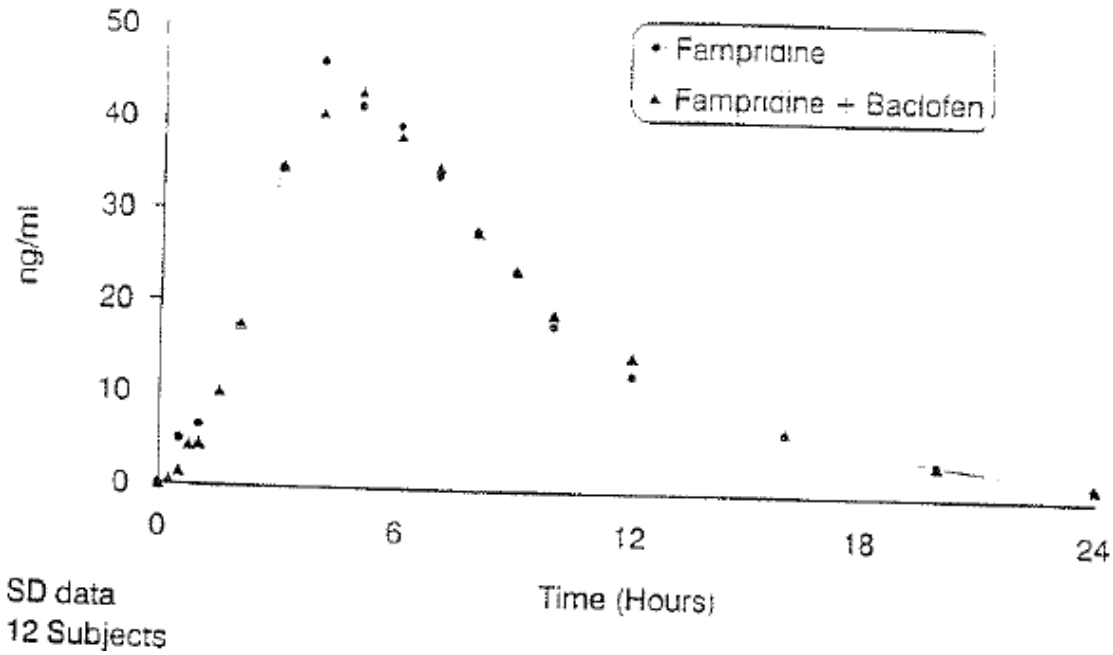
Dose Selection:

Subjects were dosed with 15 mg fampridine CR. Dose selected for Lioresal was 10 mg in this study. The determination of optimal dosage for Lioresal requires individual titration according to manufacturer's label. Started at low dosage and increased gradually until optimum effect is achieved (usually between 40-80 mg daily).

RESULTS:

Figure below shows the mean plasma concentration versus time curves for fampridine administered with and without baclofen co-administration.

Fampridine Plasma Concentrations



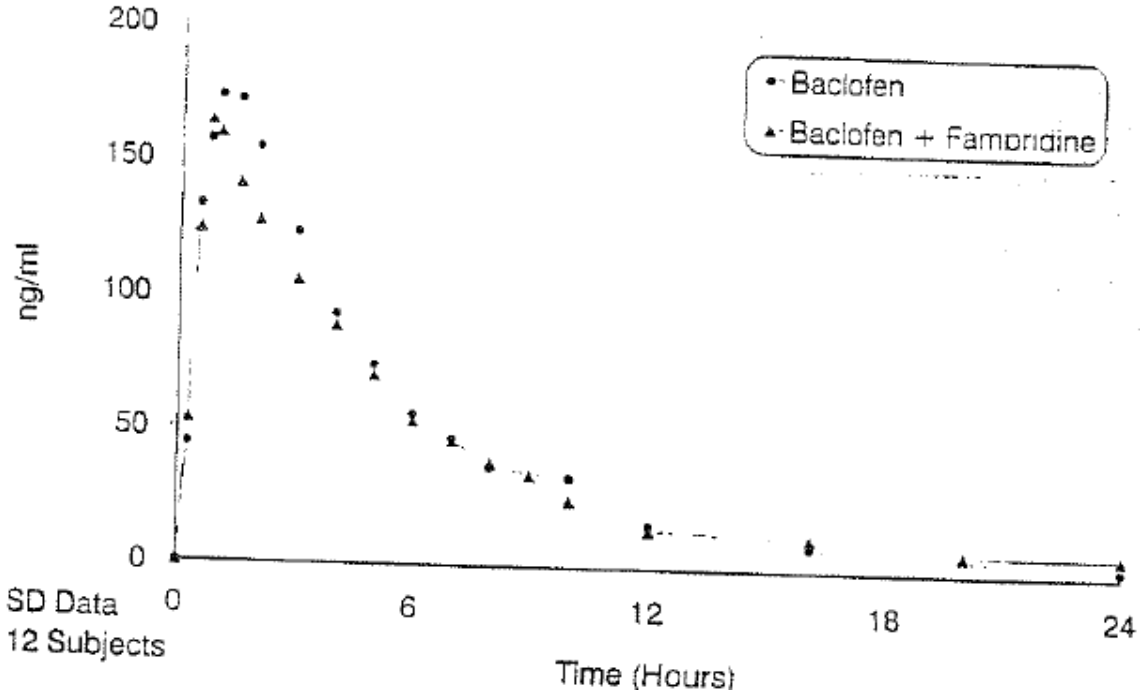
The mean PK data and 90% CIs for these treatments is presented in the Table below.

Parameter	Fampridine	Fampridine + Baclofen	Ratios	Lower 90% CI	Upper 90% CI	Upper 90% CI
$AUC_{0-\infty}$	399.8 ± 78.3	402.4 ± 99.0	97.9	91.9	109.4	109.4
C_{max}	47.2 ± 6.1	46.2 ± 8.1	100.5	90.1	105.5	105.5
T_{max}	4.3 ± 0.6	4.2 ± 0.9				

The 90% CI values were within 80 -125% for AUC and Cmax for the treatments when administered separately or simultaneously.

Figure below shows the mean plasma concentration versus time curves for baclofen administered with and without fampridine co-administration.

Baclofen Plasma Concentrations



The mean PK data and 90% CIs for these treatments is presented in the Table below.

Parameter	Baclofen	Baclofen + Fampridine	Ratio	Lower 90% CI	Upper 90% CI
$AUC_{0-\infty}$	1055 ± 206	1024 ± 245	97.06	87.2	106.9
C_{max} (ng.mL)	199.5 ± 39.3	201.0 ± 41.1	100.7	87.3	114.2
T_{max}	1.02 ± 0.8	0.81 ± 0.3			

The 90% CI values were within 80 -125% for AUC and Cmax for the treatments when administered separately or simultaneously.

Reviewer's Comment:
Study was performed using single dose of 15 mg fampridine CR capsules. The maximum concentration (Cmax) achieved was comparable to Cmax of fampridine-SR 10 mg b.i.d chronic dosing. Fampridine 10 mg sustained release tablet b.i.d was sought for the approval. However, there was no interaction seen between the treatments.

Population PK analysis indicates that concomitant administration of baclofen with fampridine did not effect the PK of fampridine as shown in the figure below.

Pharmacokinetic parameter-covariate relationships for baclofen. Circles are the individual parameter estimates (normalized for males, 50 years, CrCl=105 μmol/L), the red line represents the typical (population) predicted covariate relationship and the blue square is the mean of the observed values.

(b) (4)

CONCLUSIONS

There was no drug-drug interaction between fampridine and baclofen administered simultaneously.

1194-001US: The effect of treatment with subcutaneous beta-interferon on the pharmacokinetics of orally administered fampridine in patients with multiple sclerosis

Objective:
The objective of this study was to compare the pharmacokinetics and safety of single and multiple doses of immediate re1eese (IR) fampridine with and without concurrent administration of Betaseron® in MS patients.

Study Design	Single-centre, open, randomized, three-way crossover trial
Study Population	Multiple Sclerosis Patients Age: 38-55 years 12 subjects were enrolled, and 9 completed the study
Dosage and Administration	Fampridine administration Patients received a single oral dose (7.5 mg) of fampridine IR on day 0, the following day (Day 1) patients began a multiple dose regimen taking fampridine every 8 hours (Q8h). Serial blood samples were collected on Day 0 for the single dose pharmacokinetics and on Day 3 for steady state pharmacokinetics. The last dose of fampridme in this phase of the study was the 8 am dose on Day 3. Fampridine + Beta-interferon On day 6 patients began beta-interferon therapy. On Day 37 these patients received a single oral dose (7.5 mg) of fampridine IR and serial blood samples obtained for pharmacokinetic analysis. On day 39 patients began a multiple dosing regimen taking fampridine every 8 hours with the last dose of fampridine administered on Day 43. Serial blood samples were obtained on Day 43 to evaluate multiple dose pharmacokinetics of fampridine with concomitant administration of beta-interferon. Betaseron Dosing (Day 6-Day 42)

	Patients began every other day (QOD) Betaseron dosing at Day 6 (self- administered subcutaneous injections of 8 million units (0.25 mg) between 9 - 11 p.m.) This regimen continued through Day 42. Accordingly, Betaseron doses were administered on Days 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, and 42.
Sampling: Blood	A 3 ml venous blood samples were obtained for both treatments at the following times: Single dose evaluation of fampridine alone (Day 0) and fampridine co-administered with Betaseron® (Day 37) at 0 hours (pre-dose), 0.25, 0.5, 0.75, 1, l.5, 2, 3, 4, 6, 8, 10, and l2 hours post administration of the dose. Steady state evaluation of fampridine alone (Day 3) and fampridine co-administered with Betaseron® (Day 43) at: 0 hours (pre-dose), 0.25. 0.5, 0.75, l, 1.5, 2, 3, 4, 6, and 8 hours post administration of the dose.
Analysis	Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.

Parameter	**Quality Control Samples**	**Standard Curve Samples**
Quality Control or Standard Curve Concentration (ng/mL)	15, 60, and 150 ng/mL	2, 10, 25, 50, 100 and 200 ng/mL
Between Batch Precision (%CV)	5.37 to 11.2	1.12 to 6.01
Linearity	Weighted linear equation ($1/X^2$), mean r= 0.992	
Linear Range (ng/mL)	2 to 200 ng/mL	
Sensitivity (LLOQ, ng/mL)	2 ng/mL	

Urine	None
Feces	None
PK Assessments	The pharmacokinetic parameters For single dose: AUC (0-24h), AUC(0-inf), Cmax, Tmax, kel, and t1/2 were calculated For multiple dose (Steady State): AUCss (0-8h), Cav ss, Cmax ss, Cmin ss, Tmaxss, Tmin ss from the plasma fampridine concentration-time data using noncompartmental analysis.
PD Assessments	None
Statistical Methods	90% confidence intervals were calculated using least squares mean values for the treatments. The coefficient of variation (% CV) for all the pharmacokinetic parameters reported for single dose and steady state was estimated as a measure of the intersubject variability. Statistically significant differences between the two treatments were detected at a probability level (p) less than 0.05. The power of the

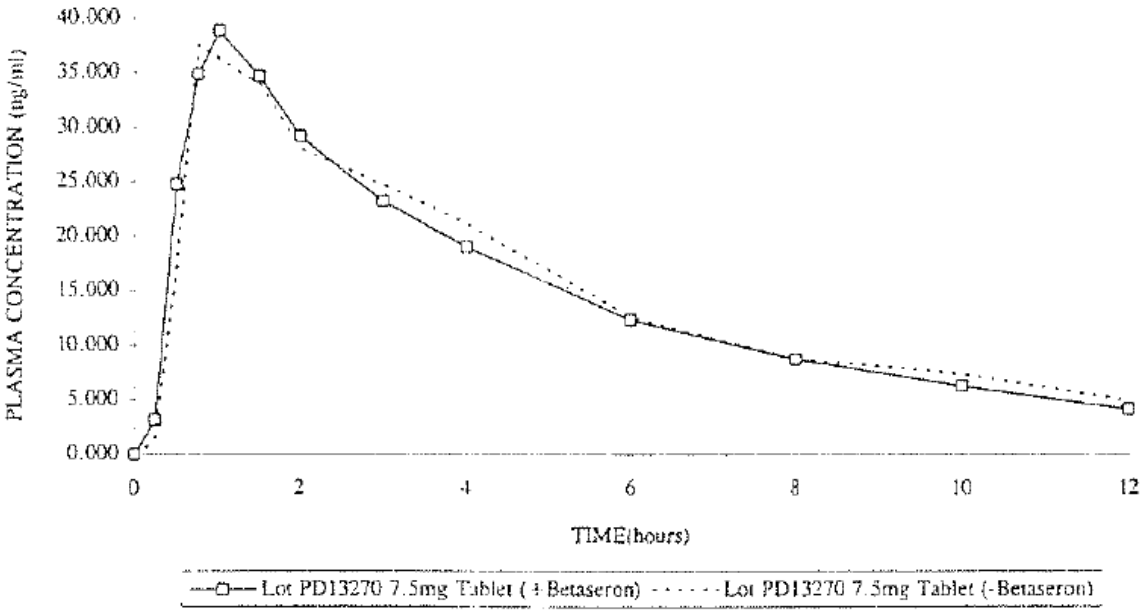
	study to detect a 20% difference between the treatment means and the minimum detectable difference (MDD) at a power of 0.08 were also calculated for the ANOVA analyses. Analysis of variance was also conducted on data transformed to log base 10 of AUC (0-12), AUC (0-∞) and Cmax following single dose administration, and Cmax and AUC(0-8) following steady state dosing.

Dose Selection:
Subjects were dosed with 7.5 mg fampridine IR every 8 hours (Q8h). However, 10 mg fampridine SR b.i.d was sought for approval. Dose selected for betaseron was 8,000 million Units (0.25 mg) as per manufacturers labeling recommendation.

RESULTS:

Figure below shows the mean plasma concentration versus time curves for single dose fampridine administered with and without beta-interferon co-administration.

Fampridine Plasma Concentrations



Mean non- transformed pharmacokinetic parameters, single dose data (mean ± SD, n=9).

Parameter	Fampridine With Betaseron®	Fampridine
Cmax	42.84 ± 14.02	42.94 ± 11.12
AUC(0-12)	176.12 ± 62.44	181.41 ± 56.25
AUC(inf)	200.82 ± 70.73	209.29 ± 66.60
tmax	0.94 ± 0.24	0.94 ± 0.24
thalf	3.68 ± 0.76	3.85 ± 0.79
kel	0.20 ± 0.04	0.19 ± 0.04

Mean log-transformed pharmacokinetic parameters, single dose data Mean (SD) n=9.

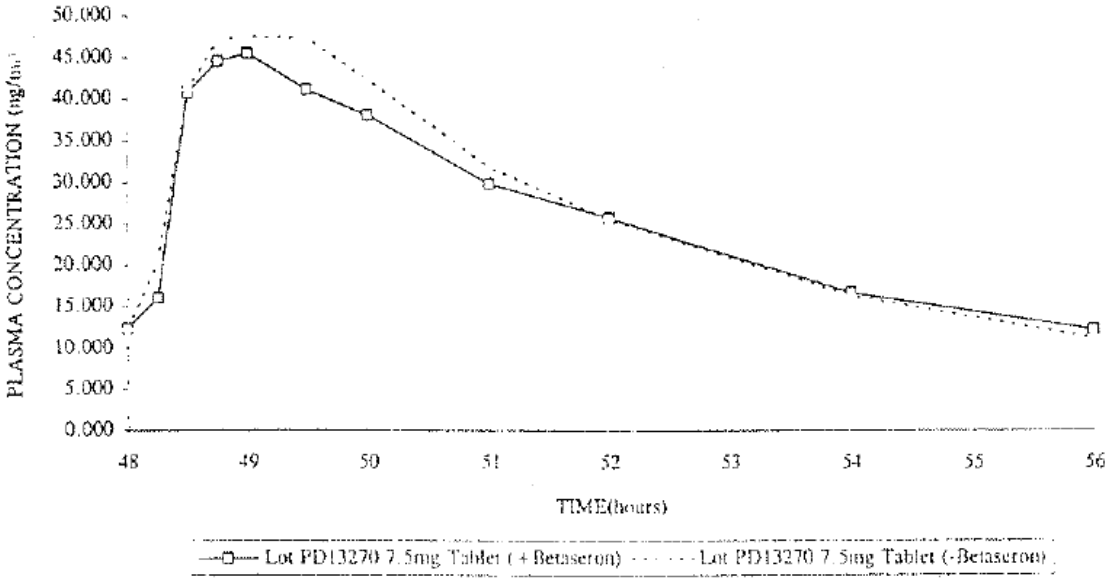
Parameter	Fampridine With Betaseron®	Fampridine	90% CIs
Cmax	40.98 (1.36)	41.73 (1.29)	74.66 - 129.17
AUC(0-12)	167.49 (1.39)	173.11 (1.40)	89.23 - 104.92
AUC(0-inf)	191.05 (1.39)	198.96 (1.42)	86.61 - 106.45

The 90% CI values were within 80 -125% for AUC for the treatments when administered separately or simultaneously with beta-interferon. Lower limits of 90% CI were below 80 (74.66) and upper limit was above 125 (129) for Cmax.

Reviewer's Comment: Dose selected for fampridine IR was 7.5 mg t.i.d may result in approximately similar plasma concentration time profile to fampridine SR 10 mg given b.i.d (dosage sought for approval in this indication). However, effect of coadministration of beta-interferon was also evaluated by population PK analysis of data from pivotal clinical efficacy studies using fampridine SR 10 mg given b.i.d.

Figure below shows the mean steady state plasma concentration versus time curves for fampridine administered with and without Betaseron® co-administration.

Mean steady state fampridine concentrations



Mean non-transformed steady state fampridine pharmacokinetic parameters, (mean ± SD, n=9).

Parameter	Fampridine With Betaseron® (day 5)	Fampridine (day 3)
AUC_{SS} (0-8)	207.22 ± 64.77	216.03 ± 70.60
$Cmax_{SS}$	50.07 ± 9.38	56.73 ± 18.02
$Tmax_{SS}$	0.92 ± 0.38	1.11 ± 0.59
$Cmin_{SS}$	11.28 ± 3.73	9.65 ± 5.20
$Tmin_{SS}$	4.47 ± 4.18	5.39 ± 3.92
Cav	11.28 ± 3.73	9.00 ± 2.94
$Cmax_{SS}$-$Cmin_{SS}$/Cav	4.85 ± 1.89	5.42 ± 1.57
$Cmax_{SS}$/C8	4.64 ± 1.87	6.70 ± 3.91
$Cmax_{SS}$-$Cmin_{SS}$	38.78 ± 9.18	47.08 ± 15.74
$Cmax_{SS}$/$Cmin_{SS}$	4.82 ± 1.70	7.05 ± 3.60
$Cmax_{SS}$-$Cmin_{SS}$/$Cmin_{SS}$	3.82 ± 1.70	6.05 ± 3.60

Mean log-transformed steady state fampridine pharmacokinetic parameters, mean (SD), n=9.

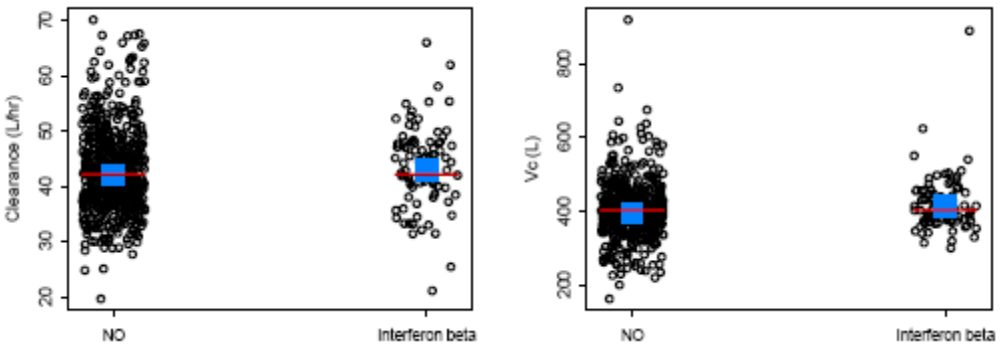
Parameter	**Fampridine + Betaseron®**	**Fampridine (day 3)**	**Lower 90% CI**	**Upper 90%**

	(day 43)			CI
AUCss (0-8)	199.0 ± 1.3	206.1 ± 1.4	88.3	105.6
$C_{max,ss}$	49.33 ± 1.2	54.4 ± 1.3	74.0	111.1

Reviewer's Comment:

Population PK analysis also indicates that concomitant administration of beta-interferon with fampridine did not effect the PK of fampridine as shown in the figure below.

Pharmacokinetic parameter-covariate relationships for Interferon beta. Circles are the individual parameter estimates (normalized for males, 50 years, CrCl=105 μmol/L), the red line represents the typical (population) predicted covariate relationship and the blue square is the mean of the observed values.



Conclusion

There was no interaction between fampridine and beta-interferon administered simultaneously.

4.1.4 BIOPHARMACEUTIC STUDIES

0494-006: Comparative pharmacokinetics of slow-release fampridine tablet formulation administered with and without food and an immediate release fampridine capsule preparation administered fasting in healthy male volunteers

Objective:

The purpose of the study was to compare the bioavailability of an immediate release and slow release formulation of 4 aminopyridine and the effect of food on the bioavailability of the slow release formulation.

Study Design	This was a single centre, pen-label, randomized 3 way cross-over bioavailability study
Study Population	Healthy male Age: 18-35 years 12 subjects

Treatment Groups	Treatment A = Fampridine l2.5 mg immediate release capsule in the fasted state. Treatment B = Fampridine l2.5 mg slow release tablet in the fasted state, and Treatment C= Fampridine l2.5 mg slow release tablet in the fed state Treatments were separated by a 1-week washout period
Dosage and Administration	**Reference:** The reference product was Fampridine-IR 12.5 mg capsule **Test:** The test product was Fampridine-SR 12.5 mg tablet
Sampling: Blood	Blood samples (5 mL) were obtained during each study period at the following times on Days 1 and 2: predose, 15, 30, 45, minutes, 1, 1.5 (90 minutes), 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, and 20 hours postdose.
Analysis	Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.

Parameter	**Quality Control Samples**	**Standard Curve Samples**
Quality Control or Standard Curve Concentration (µg/mL)	15, 45, and 60 ng/mL	2, 10, 25, 50, 100 and 200 ng/mL
Between Batch Precision (%CV)	6.46 to 12.1	1.84 to 8.02
Linearity	Weighted linear equation ($1/X^2$), mean r= 0.993	
Linear Range (µg/mL)	2 to 200 ng/mL	
Sensitivity (LLOQ, µg/mL)	2 ng/mL	

Urine	None
Feces	None
PK Assessments	The pharmacokinetic parameters Cmax, Tmax, and AUC(0-inf), were calculated from the plasma fampridine concentration-time data using noncompartmental analysis.
PD Assessments	None
Statistical Methods	Pharmacokinetics: Plasma concentration-time data and pharmacokinetic parameters were presented by treatment and summarized using descriptive statistics: (number of observations [N], number missing [N missing], mean, standard deviation [SD], coefficient of variation [%CV], standard error of the mean [SEM], geometric mean, geometric coefficient of variation, median, minimum [min], and maximum [max]). Analysis of variance (ANOVA) was performed on the In-transformed AUC(0-inf), AUC(0-t), and Cmax. The ratio of the means was expressed as a percentage of the least-squares means LSM (test/reference). The 90% confidence intervals (CI) for the ratio of the test and reference means were calculated for the In-transformed parameters. Bioequivalence was concluded if the 90% CI of the ratio of the product means for Cmax, AUC(0-t), and AUC(0-inf) fell within the range of 80 - 125%.

RESULTS:

Figure below shows the mean plasma concentration versus time curves for the treatments in this study.

Fampridine concentration vs time for different treatments

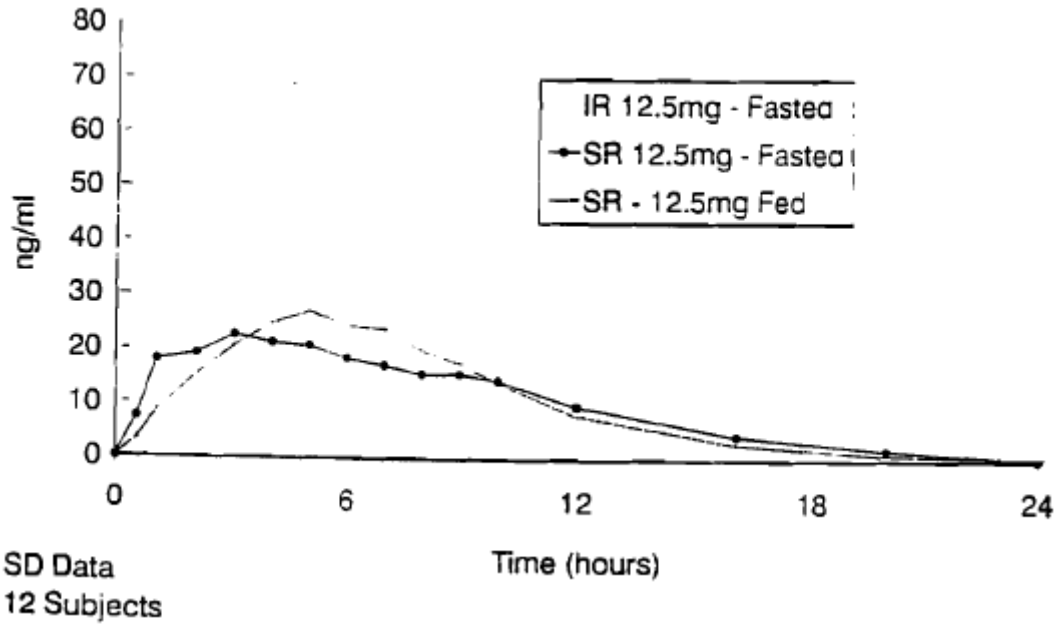


Table below represents the mean PK data for different treatments.

Pharmacokinetic Parameters

Mean (+/-SD) 12 Subjects

Parameter	IR 12.5mg Fasted	SR 12.5mg Fasted	SR 12.5mg Fed	90% CI's
AUC (0-inf)	294.19 +/- 55.60	269.86 +/- 44.39	256.95 +/- 62.73	SR (Fasted)/IR : 83.31 - 100.15 SR (Fed)/SR (Fasted) : 86.03 - 104.40
Cmax	79.31 +/- 16.30	25.58 +/- 3.83	28.73 +/- 4.33	SR (Fasted)/IR : 23.22 - 41.27 SR (Fed)/SR (Fasted) : 84.33 - 140.31
tmax	0.90 +/- 0.39	2.83 +/- 1.34	5.25 +/- 0.75	

The AUC calculated for slow-release tablet formulation was similar to the IR capsule preparation, but had the lower and later peak concentrations.

Food had minor effects on the bioavailability of tablet formulation, with equivalence in AUC and a slight increase in Cmax (Fasted: 25.58 +/- 3.83 ng/ml; Fed: 28.73 +/- 4.33 ng/ml) apparent following administration with food.

CONCLUSIONS:

- Extent of Fampridine exposure (AUC) was comparable for Fampridine IR 12.5 mg capsules and Fampridine SR 12.5 mg tablets.
- Peak plasma concentration of Fampridine SR 12.5 mg tablets was 23%-41% of Fampridine IR 12.5 mg capsules and
- Tmax was prolonged from approximately 1 hr for IR capsules to 3 hrs for SR tablets.
- Food delayed time to maximum concentration by approximately 2 hrs.

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(b) (4)

BE10.25F-SR10OS122003: A Single-Dose, Randomized, Open-Label, Three-way Crossover, Bioequivalence Study of Two Strengths of Fampridine-SR and an Oral Solution of Fampridine in Healthy Adult Volunteers

Objective:

The objective of this study was to assess the relative bioavailability of two strengths of the proposed commercial Fampridine-SR tablets, 10 mg and 25 mg tablets, as compared to a 10 mg buffered solution of fampridine and to assess the relative bioequivalence of the two tablet strengths following a single 10 mg and 25 mg oral dose.

Study Design	This study had a single-dose, open-label, randomized, three-way crossover design. Subjects randomized to all treatments were in a fasted state following a 10-hour overnight fast. There was a 7-day washout interval between the 3 dose administrations.	
Study Population	Healthy male and female Age: 19-45 years 30 subjects were enrolled, and 26 completed the study	
Treatment Groups	Treatment A = Fampridine-SR 10 mg tablets Treatment B = Fampridine-SR 25 mg tablets Treatment C = 4-Aminopyridine 10 mg oral solution The treatments were administered under fasting conditions, all the treatments were separated by a 1-week washout period	
Dosage and Administration	**Reference:** The reference product was 4-Aminopyridine 10 mg oral solution	**Test:** The test product was Fampridine-SR 10 mg tablets and Fampridine-SR 25 mg tablets
Sampling: Blood	Blood samples (7 mL) were collected during each study period at Hour 0 (predose), and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 30, and 36 hours postdose. A total of 15 blood samples were to be drawn during the study for drug analysis.	

<table>
<tr><td>Analysis</td><td>Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (ng/mL)</td><td>6, 40, and 150 ng/mL</td><td>2, 4, 8, 15 30, 50, 100 and 200 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>7.39 to 10.0</td><td>1.84 to 8.02</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.999</td></tr>
<tr><td>Linear Range (ng/mL)</td><td colspan="2">1 to 200 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, ng/mL)</td><td colspan="2">2 ng/mL</td></tr>
</table></td></tr>
<tr><td>Urine</td><td>None</td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>PK Assessments</td><td>The pharmacokinetic parameters Tlag, Cmax, Tmax, AUC(0-t), AUC(0-inf), AUCR, Kel, T1/2, MRTpo, and CL/F were calculated from the plasma fampridine concentration-time data using noncompartmental analysis.</td></tr>
<tr><td>PD Assessments</td><td>None</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetics: Plasma concentrations for fampridine were presented by treatment and summarized using descriptive statistics (mean, standard deviation [SD], coefficient of variation [CV%], standard error of the mean [SEM], number of observations [N], N missing, minimum [min], maximum [max], geometric mean, geometric CV%, and median). All pharmacokinetic parameters were tabulated by treatment and summarized using descriptive statistics (mean, SD, CV%, SEM, N, N missing, min, max, and median). Geometric means and geometric coefficients of variation were provided for AUC(0-t), AUC(0-inf), and Cmax.

Analysis of variance (ANOVA) was performed on the dose-normalized In-transformed AUC(0-inf), AUC(0-t), and Cmax. Tmax was analyzed using nonparametric analysis (Walsh Averages and appropriate quantile of the Wilcoxon Signed Rank Test statistic).

The ratio of the means was expressed as a percentage of the least-squares means (LSM) (for Fampridine-SR 10 mg tablet [Treatment A] versus Fampridine 10 mg oral solution [Treatment C], Fampridine-SR 25 mg tablet [Treatment B] versus Fampridine 10 mg oral solution, and Fampridine-SR 10 mg tablet versus Fampridine-SR 25 mg tablet). The 90% confidence intervals (CI) for the ratio of the test and reference means (Fampridine-SR 10 mg tablet versus fampridine 10 mg oral solution, Fampridine-SR 25 mg tablet versus fampridine 10 mg oral solution, and Fampridine-SR 10 mg tablet versus Fampridine-SR 25 mg tablet) were calculated for the dose-normalized log-transformed parameters. Comparable bioavailability</td></tr>
</table>

	of the test treatments (Fampridine-SR 10 mg tablet and Fampridine-SR 25 mg tablet) to the reference treatment (fampridine 10 mg oral solution), and bioequivalence of Fampridine-SR 10 mg tablet to Fampridine-SR 25 mg tablet, were concluded if the 90% confidence intervals of the ratio of the product means for Cmax, AUC(0-t), and AUC(0-inf) fell within the range of 80% to 125%.

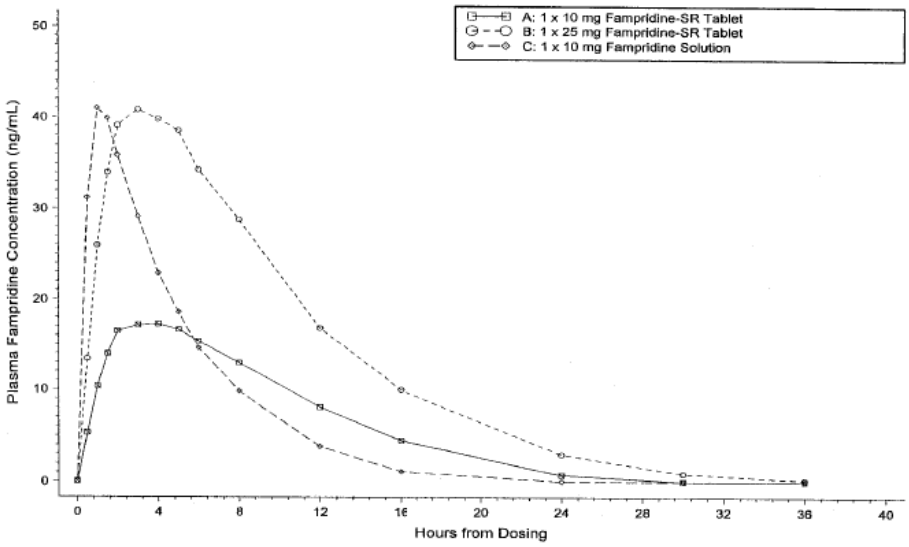
RESULTS:

Relative Bioavailability of Fampridine-SR Tablets

The bioavailability of the two test products, the 10 mg (Fampridine-SR 10 mg tablet) and 25 mg (Fampridine-SR 25 mg tablet) Fampridine-SR tablets, was determined relative to the 10 mg reference oral solution (fampridine 10 mg oral solution).

Following figure represents plasma concentration time profile for different treatments.

Mean Plasma Fampridine Concentration vs Time Following Different Treatments



The individual and mean PK parameter differences, ratios, and log-ratios for fampridine-SR 10 mg tablet versus fampridine 10 mg oral solution are presented in the Table below.

Arithmetic Mean (SD) Pharmacokinetic Parameter Values for Fampridine Following Fampridine-SR 10 mg Tablet and a 10 mg Oral Solution

	Plasma Fampridine							
	Fampridine-SR 10 mg Tablet			Fampridine 10 mg Solution				
Pharmacokinetic Parameters	N	Arithmetic Mean	SD	N	Arithmetic Mean	SD	90% CI	Ratio of Geometric Means**
Cmax(ng/mL)	27	18.69	3.682	29	42.70	8.772	. - .	.
Tmax(hr)	27	3.67	1.32	29	1.14	0.352	. - .	.
Tlag(hr)	27	0.00	0.00	29	0.00	0.00	. - .	.
AUC(0-t)(ng*hr/mL)	27	187.67	38.558	29	214.95	43.567	. - .	.
AUC(0-inf)(ng*hr/mL)	26	218.28	39.126	29	229.48	41.725	. - .	.
T1/2(hr)	26	5.479	1.032	29	3.249	0.9185	. - .	.
Kel(1/hr)	26	0.131	0.0272	29	0.228	0.0587	. - .	.
MRTpo(hr)	26	9.845	1.384	29	5.151	0.9610	. - .	.
AUCR	26	0.864	0.0589	29	0.933	0.0325	. - .	.
CL/F(L/hr)	26	47.33	9.018	29	45.02	8.467	. - .	.
ln(Cmax)	27	2.91	0.198	29	3.73	0.212	. - .	.
ln[AUC(0-t)]	27	5.21	0.204	29	5.35	0.207	. - .	.
ln[AUC(0-inf)]	26	5.37	0.184	29	5.42	0.184	. - .	.
ln(Cmax/Dose)							41.07 - 46.35	43.6
ln[AUC(0-t)/Dose]							80.60 - 93.26	86.7
ln[AUC(0-inf)/Dose]							88.23 - 101.55	94.7

** Geometric Mean Ratio = 100*exp(test-reference)

Reviewer's re-analysis of the data showed similar 90% confidence interval (CI) levels for C_{max} and $AUC_{0\text{-}inf}$ as shown in the table below.

Table 1: Relative Bioavailability Fampridine Formulations [Treatment A (Fampridine SR 10 mg tablet) and Treatment C (Fampridine Oral Solution]

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine SR 10 mg Tablet (A)/Fampridine 10 mg Oral Solution (C)	C_{max}	43.5	41.0	46.2
	$AUC_{0\text{-}\infty}$	94.2	87.7	101

<u>Fampridine-SR 10 mg tablet versus fampridine 10 mg oral solution</u>: The mean ratios for fampridine Cmax, AUC(0-t), and AUC(0-inf), derived from the dose-normalized In-transformed data, were 43.6%, 86.7%, and 94.7%, respectively.

Tmax was approximately 4 hours following Fampridine-SR 10 mg tablet and 1 hour following fampridine 10 mg oral solution and Cmax was 41.07% – 46.35% from sustained release formulation. The extent of absorption from fampridine tablet was similar to oral solution. The 90% CI for AUC(0-t) at 80.60% - 93.26% and AUC(0-inf) at 88.23% – 101.55%.

<u>Fampridine-SR 25 mg tablet versus fampridine 10 mg oral solution</u>: The mean ratios for fampridine Cmax, AUC(0-t), and AUC(0-inf), derived from the dose-normalized In-transformed data, were 41.8%, 84.9%, and 85.3%, respectively.

Arithmetic Mean (SD) Pharmacokinetic Parameter Values for Fampridine Following Fampridine-SR 25 mg Tablet and a 10 mg Oral Solution

	Plasma Fampridine							
	Fampridine-SR 25 mg Tablet			Fampridine 10 mg Solution				
Pharmacokinetic Parameters	N	Arithmetic Mean	SD	N	Arithmetic Mean	SD	90% CI	Ratio of Geometric Means**
Cmax(ng/mL)	28	44.34	9.648	29	42.70	8.772	. - .	.
Tmax(hr)	28	3.48	1.34	29	1.14	0.352	. - .	.
Tlag(hr)	28	0.00	0.00	29	0.00	0.00	. - .	.
AUC(0-t)(ng*hr/mL)	28	454.72	110.38	29	214.95	43.567	. - .	.
AUC(0-inf)(ng*hr/mL)	27	489.96	105.77	29	229.48	41.725	. - .	.
T1/2(hr)	27	5.440	2.779	29	3.249	0.9185	. - .	.
Kel(1/hr)	27	0.152	0.0582	29	0.228	0.0587	. - .	.
MRTpo(hr)	27	9.491	2.342	29	5.151	0.9610	. - .	.
AUCR	27	0.940	0.0420	29	0.933	0.0325	. - .	.
CL/F(L/hr)	27	53.42	11.86	29	45.02	8.467	. - .	.
ln(Cmax)	28	3.77	0.212	29	3.73	0.212	. - .	.
ln[AUC(0-t)]	28	6.09	0.245	29	5.35	0.207	. - .	.
ln[AUC(0-inf)]	27	6.17	0.219	29	5.42	0.184	. - .	.
ln(Cmax/Dose)							39.42 - 44.41	41.8
ln[AUC(0-t)/Dose]							78.98 - 91.21	84.9
ln[AUC(0-inf)/Dose]							79.61 - 91.45	85.3
** Geometric Mean Ratio = 100*exp(test-reference)								

Reviewer's re-analysis of the data showed similar 90% confidence interval (CI) levels for dose normalized C_{max} and $AUC_{0\text{-}inf}$ as shown in the table below.

Table 2: Relative Bioavailability Fampridine Formulations [Treatment B/Dose (Fampridine SR 25 mg tablet) and Treatment C/Dose (Fampridine Oral Solution]

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine SR 25 mg Tablet (B)/Fampridine 10 mg Oral Solution (C)	C_{max}	41.8	39.4	44.3
	$AUC_{0\text{-}\infty}$	85.3	79.5	91.5

Tmax was approximately 3 hours following Fampridine-SR 10 mg tablet and 1 hour following fampridine 10 mg oral solution and Cmax was 39.42% - 44.41% from sustained release formulation. The extent of absorption from fampridine tablet was similar to oral solution. The 90% CI for AUC(0-t) at 78.98% - 91.21% and AUC(0-inf) at 79.61% – 91.45%.

Bioequivalence of Fampridine-SR 10 mg and 25 mg Tablets

The arithmetic means and standard deviations of the plasma fampridine PK parameter values and the statistical comparisons of (dose normalized) Fampridine-SR 10 mg tablet versus Fampridine-SR 25 mg tablet are summarized in the table below.

Arithmetic Mean (SD) Pharmacokinetic Parameter Values for Fampridine Following Fampridine-SR 10 mg and 25 mg Tablets

	Plasma Fampridine							
	Fampridine-SR 10 mg Tablet			Fampridine-SR 25 mg Tablet				
Pharmacokinetic Parameters	N	Arithmetic Mean	SD	N	Arithmetic Mean	SD	90% CI	Ratio of Geometric Means**
Cmax(ng/mL)	27	18.69	3.682	28	44.34	9.648	. - .	.
Tmax(hr)	27	3.67	1.32	28	3.48	1.34	. - .	.
Tlag(hr)	27	0.00	0.00	28	0.00	0.00	. - .	.
AUC(0-t)(ng*hr/mL)	27	187.67	38.558	28	454.72	110.38	. - .	.
AUC(0-inf)(ng*hr/mL)	26	218.28	39.126	27	489.96	105.77	. - .	.
T1/2(hr)	26	5.479	1.032	27	5.440	2.779	. - .	.
Kel(1/hr)	26	0.131	0.0272	27	0.152	0.0582	. - .	.
MRTpo(hr)	26	9.845	1.384	27	9.491	2.342	. - .	.
AUCR	26	0.864	0.0589	27	0.940	0.0420	. - .	.
CL/F(L/hr)	26	47.33	9.018	27	53.42	11.86	. - .	.
ln(Cmax)	27	2.91	0.198	28	3.77	0.212	. - .	.
ln[AUC(0-t)]	27	5.21	0.204	28	6.09	0.245	. - .	.
ln[AUC(0-inf)]	26	5.37	0.184	27	6.17	0.219	. - .	.
ln(Cmax/Dose)							98.07 - 110.88	104.3
ln[AUC(0-t)/Dose]							94.86 - 109.99	102.1
ln[AUC(0-inf)/Dose]							103.20 - 119.25	110.9
** Geometric Mean Ratio = 100*exp(test-reference)								

The mean ratios for fampridine Cmax, AUC(0-t), and AUC(0-inf), derived from the dose-normalized and ln-transformed data, for the comparison of Fampridine-SR 10 mg tablet versus Fampridine-SR 25 mg tablet were 104.3%, 102.1%, and 110.9%, respectively, while the 90% CI were 98.07% - 110.88% for Cmax, 94.86% - 109.99% for AUC(0-t), and 103.20% - 119.25% for AUC(0-inf).

Reviewer's re-analysis of the data showed similar 90% confidence interval (CI) levels for dose normalized C_{max} and $AUC_{0\text{-}inf}$ as shown in the table below.

Table 3: Relative Bioavailability Fampridine Formulations [Treatment B/Dose (Fampridine SR 25 mg tablet) and Treatment C/Dose (Fampridine Oral Solution]

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine SR 25 mg Tablet (B)/Fampridine SR 10 mg Tablet (A)	C_{max}	96.0	90.3	102
	$AUC_{0\text{-}\infty}$	90.6	84.1	97.5

The differences between median Tmax following Fampridine-SR 10 mg tablet (approximately 4 hours) and following Fampridine-SR 25 mg tablet (approximately 3 hours) was not statistically significant (p-value = 0.4800).

CONCLUSIONS

- Mean peak plasma concentrations of 42.7 ng/mL achieved approximately 1 hour after 10 mg oral solution of fampridine administration.
- The peak concentrations following administration as a sustained release tablet are approximately 40% of those achieved with the oral solution and occur approximately 3.5 hours postdose.
- The plasma elimination half-life was prolonged, from 3.2 hours for the oral solution versus 5.4 to 5.5 hours on average for the sustained release tablets.

- The two sustained release tablets were bioequivalent.

BE10F-SR022004: A Single-Dose, Randomized, Open-label, Two-Way Crossover, Bioequivalence Study of 2 Commercial 10-mg Fampridine-SR Tablets (E10 and P10) in Healthy Adult Volunteers.

Objective:

The objective of this study was to assess the bioequivalence of the 10 mg strength of Fampridine-SR tablets manufactured by Elan Corporation (E10) and (b) (4) (P10).

Study Design	Single-centre, open, single-dose, randomized, two-way crossover trial	
Study Population	Healthy male and female Age: 19-45 years 18 subjects were enrolled, and 16 completed the study	
Treatment Groups	Treatment A = Fampridine-SR 10 mg tablets (P10) manufactured by (b) (4) Treatment B = Fampridine-SR 10 mg tablets (E10) manufactured by Elan The treatments were administered under fasting conditions, both treatments were separated by a 1-week washout period	
Dosage and Administration	**Reference:** The reference product was Fampridine-SR 10 mg tablets (E10) administered orally with 240 mL of water. Manufactured by Elan Corporation, Lot No. 22747.	**Test:** The test product was Fampridine-SR 10 mg tablets (P10), administered orally with 240 mL of water. Manufactured by (b) (4) Lot No. R0105F001.
Sampling: Blood	Blood samples (7 mL) were obtained during each study period at the following times on Days 1 and 2 and Days 8 and 9: predose (within 30 minutes prior to drug administration), 0.5 (30 minutes), 1, 1.5 (90 minutes), 2, 3, 4, 5, 6, 8, 12, 16, 24, 30, and 36 hours postdose. A total of 30 blood samples (210 mL) were drawn during the study for pharmacokinetic analysis	
Analysis	Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.	

Parameter	Quality Control Samples	Standard Curve Samples
Quality Control or Standard Curve Concentration (ng/mL)	6, 40, and 150 ng/mL	2, 4, 8, 15 30, 50, 100 and 200 ng/mL
Between Batch Precision (%CV)	4.36 to 8.56	1.61 to 7.63
Linearity	Weighted linear equation ($1/X^2$), mean r= 0.999	
Linear Range (ng/mL)	1 to 200 ng/mL	
Sensitivity (LLOQ,	2 ng/mL	

	ng/mL)
Urine	None
Feces	None
PK Assessments	The pharmacokinetic parameters Cmax, Tmax, Tlag, AUC(0-t), AUC(0-inf), T1/2, Kel, MRTpo, AUCR, and CL/F were calculated from the plasma fampridine concentration-time data using noncompartmental analysis.
PD Assessments	None
Statistical Methods	Pharmacokinetics: Plasma concentration-time data and pharmacokinetic parameters were presented by treatment and summarized using descriptive statistics: (number of observations [N], number missing [N missing], mean, standard deviation [SDJ, coefficient of variation [%CV], standard error of the mean [SEM], geometric mean, geometric coefficient of variation, median, minimum [min], and maximum [max]). Analysis of variance (ANOVA) was performed on the In-transformed AUC(0-inf), AUC(0-t), and Cmax. The ratio of the means was expressed as a percentage of the least-squares means LSM (test/reference). The 90% confidence intervals (CI) for the ratio of the test and reference means were calculated for the In-transformed parameters. Bioequivalence was concluded if the 90% CI of the ratio of the product means for Cmax, AUC(0-t), and AUC(0-inf) fell within the range of 80 - 125%. Tmax was compared using Wilcoxon Signed Rank test.

RESULTS:

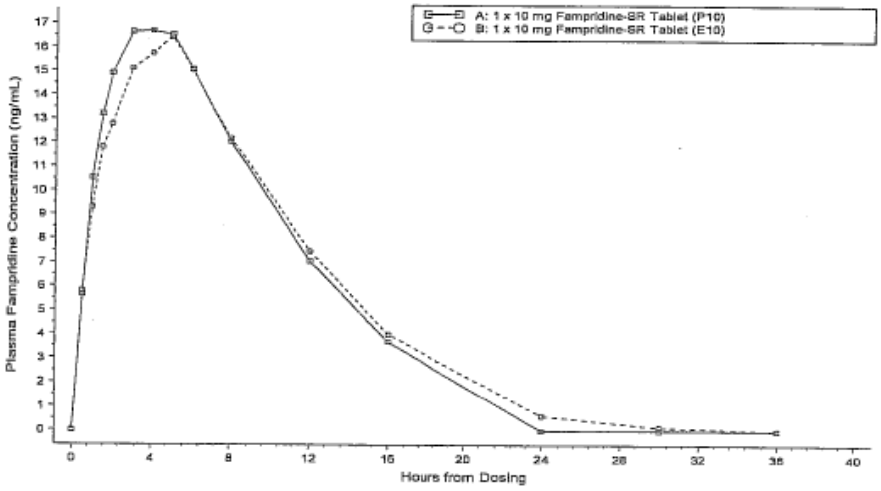
Bioequivalence: The two- formulations were bioequivalent. Following Treatments A (P10) and B (E10), the geometric mean ratios and 90% CI for the geometric mean ratios of ln-transformed Cmax, AUC(0-t) and AUC(0-inf) were 103.8, 97.0, and 96.8 and 97.92 - 109.97%, 90.00 - 104.60%, and 92.68 – 101.01%, respectively.

Fampridine rate and extent of absorption from the P10 test formulation was similar to the rate and extent of absorption from the E10 reference formulation. The 90% CI for the ratio of the test and reference means for the ln-transformed parameters were within the 80% – 125% range.

The median Tmax (4 hours) was identical for both treatments (range 1.50 - 5.00 hours for Treatment A (P10) and 2.00 – 6.00 hours for Treatment B (E10)). Mean T1/2, Kel, MRTpo and CL/F values were all also similar. No lag time (Tlag) was observed following Treatments A (P10) or B (E10).

The geometric mean fampridine plasma concentration (±SD) vs time profiles are presented by treatment formulation in the figure below.

Figure 16: Mean Plasma Fampridine Concentrations (without SD) Versus Time Following Treatments A and B



Following table indicates the PK parameters for different treatments.

Table 4: Mean (± SD) Pharmacokinetic Parameter Values for Fampridine Following Treatment A (P10) and Treaiment B (E10)

Parameter	Arithmetic Mean ± SD		% Ratio of Geometric Means[b]	90% CI[b]
	Treatment A (P10)	Treatment B (E10)		
Cmax (ng/mL)	17.9 ± 3.15 (N=16)	17.3 ± 3.38 (N=16)	103.8	97.92 - 109.97
AUC(0-t) (ng*hr/mL)	168.99 ± 29.537 (N=16)	175.37 ± 35.659 (N=16)	97.0	90.00 - 104.60
AUC(0-inf) (ng*hr/mL)	201.95 ± 33.979 (N=16)	209.36 ± 37.140 (N=15)	96.8	92.68 - 101.01
Tmax [a] (hr)	4.00 (1.50, 5.00) (N=16)	4.00 (2.00, 6.00) (N=16)	-0.0232	-1.00 - 0.00
Tlag (hr)	0.00 ± 0.00 (N=16)	0.00 ± 0.00 (N=16)	-	-
T1/2 (hr)	5.243 ± 1.317 (N=16)	5.789 ± 2.007 (N=15)	-	-
Kel (1/hr)	0.140 ± 0.0343 (N=16)	0.132 ± 0.0410 (N=15)	-	-
MRTpo (hr)	9.281 ± 1.527 (N=16)	10.10 ± 2.231 (N=15)	-	-
CL/F (L/hr)	50.87 ± 8.688 (N=16)	49.28 ± 9.284 (N=15)	-	-
AUCR	0.838 ± 0.0518 (N=16)	0.863 ± 0.0561 (N=15)	-	-

a = For Tmax median (min and max) , median difference and 90% CI of median difference are presented.
b = Percent geometric mean ratios and 90% CI were obtained from LS means of ln-transformed Cmax, AUC(0-t), and AUC(0-inf).

Reviewer's Comment: Re-analysis of the data showed similar 90% confidence interval (CI) levels for C_{max} and $AUC_{0\text{-}inf}$ as shown in the table below.

Table 5: Analysis of Bioequivalence between Fampridine Formulations [Treatment B (P10) and Treaiment A (E10)]

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine P10 (B)/ E10 (A)	C_{max}	103.8	97.9	109.9
	$AUC_{0\text{-}\infty}$	96.7	92.7	101.0

Conclusion

Single dose administration of 10 mg Fampridine-SR test formulation manufactured by (b) (4) (P10) is bioequivalent to reference formulation manufactured by Elan Pharmaceuticals (E10).

FeFa25F-SR112003: A Single Dose, Two-Way Crossover, Bioequivalence Study of Fampridine-SR with and Without Food When Administered to Healthy Adult Volunteers

Objective:

The primary objective of this study was to determine the relative bioequivalence of Fampridine-SR 25 mg tablets, when given in the fasting state and following a high-fat meal.

Study Design	This was a single-dose, two-way crossover, bioequivalence study.
Study Population	Healthy male and female Age: 19-45 years 14 subjects were enrolled, and 14completed the study
Treatment Groups	Treatment A = Fampridine-SR 25 mg tablets under fasting caonditions Treatment B = Fampridine-SR 25 mg tablets following high-fat breakfast The treatments were separated by a 1-week washout period
Dosage and Administration	**Reference:** The reference product was Fampridine-SR 25 mg tablet administered orally after a 10 hour fast. **Test:** The test product was Fampridine-SR 25 mg tablet administered orally following a high-fat breakfast.
Sampling: Blood	Blood samples (7 mL) were collected during each study period at Hour 0 (predose), and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 30, and 36 hours postdose. A total of 15 blood samples were to be drawn during the study for drug analysis.
Analysis	Fampridine concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 2 ng/mL.

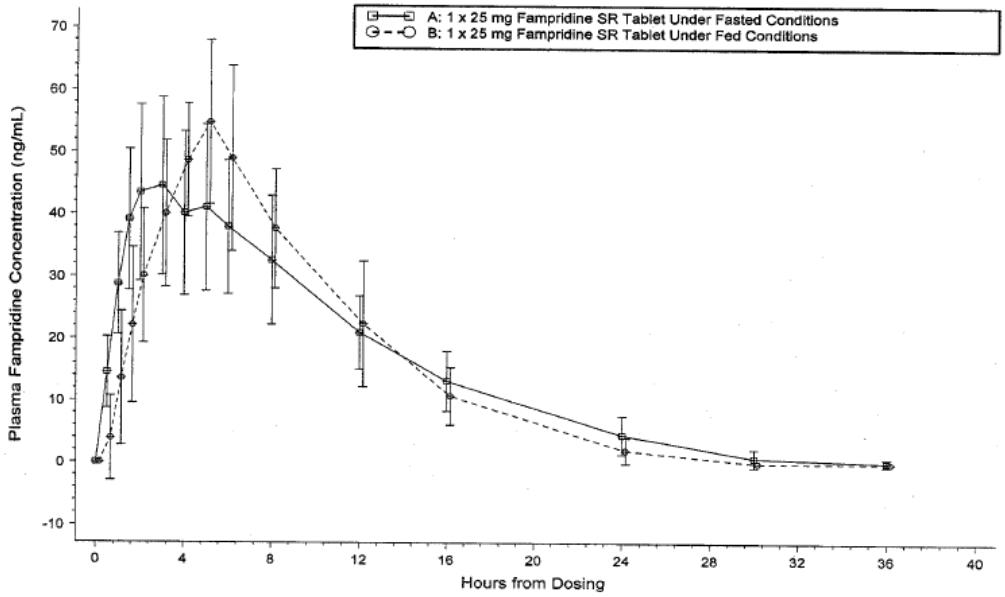
Parameter	**Quality Control Samples**	**Standard Curve Samples**
Quality Control or Standard Curve Concentration (ng/mL)	6, 40, and 150 ng/mL	2, 4, 8, 15 30, 50, 100 and 200 ng/mL
Between Batch Precision (%CV)	7.16 to 8.48	2.14 to 14
Linearity	Weighted linear equation ($1/X^2$), mean r= 0.999	
Linear Range (ng/mL)	1 to 200 ng/mL	
Sensitivity (LLOQ, ng/mL)	2 ng/mL	

Urine	None
Feces	None
PK Assessments	The pharmacokinetic parameters Tlag, Cmax, Tmax, AUC(0-t), AUC(0-inf), AUCR, Kel, T1/2, and MRT were calculated from the plasma fampridine concentration-time data using noncompartmental analysis.
PD Assessments	None
Statistical Methods	Pharmacokinetics: Plasma concentrations were presented by treatment and summarized using descriptive statistics: (mean, standard deviation [SD], coefficient of variation [CV%], standard error of the mean [SEM], and number of observations [N]). All pharmacokinetic parameters were tabulated by treatment and summarized using descriptive statistics. Geometric mean and geometric coefficient of variation were provided

	for AUC(0-t), AUC(0-inf), and Cmax. Analysis of variance (ANOVA) was performed on the In-transformed AUC(0-inf), AUC(0-t), and Cmax. Tmax was analyzed using nonparametric analysis (Walsh Averages and appropriate quantile of the Wilcoxon Signed Rank Test statistic). The ratio of the means was expressed as a percentage of the least-squares means LSM (test/reference). The 90% confidence intervals (CI) for the ratio of the test and reference means were calculated for the log-transformed parameters. No food effect was concluded if the 90% CI of the ratio of the product means for Cmax and AUC(0-inf) fell within the range of 80%-125%.

RESULTS:

Mean Plasma Fampridine Concentrations Versus Time



The arithmetic means and standard deviations of the plasma fampridine pharmacokinetic parameters and the statistical comparisons for Treatment B versus Treatment A are summarized in the following table.

Arithmetic Mean (SD) Pharmacokinetic Parameters for Fampridine

Summary of the Pharmacokinetic Parameters of Plasma Fampridine for Treatments B Versus A

	Plasma Fampridine					
	Treatment B		Treatment A			
Pharmacokinetic Parameters	Arithmetic Mean	SD	Arithmetic Mean	SD	90% CI	Ratio of Geometric Means (B versus A)
Cmax(ng/mL)	55.76	13.23	49.54	15.25		
Tmax(hr)	4.86	0.862	3.26	1.42		
Tlag(hr)	0.394	0.290	0.00	0.00		
AUC(0-t)(ng*hr/mL)	522.64	139.07	541.04	163.64		
AUC(0-inf)(ng*hr/mL)	553.03	136.02	575.54	153.22		
T1/2(hr)	4.027	0.9313	5.300	1.160		
Kel(1/hr)	0.180	0.0395	0.137	0.0333		
MRT	8.778	1.141	9.884	1.511		
AUCR	0.941	0.0358	0.931	0.0508		
ln(Cmax)	4.00	0.220	3.85	0.334	95.78 - 139.09	115.4
ln[AUC(0-t)]	6.23	0.265	6.25	0.335	80.25 - 119.98	98.1
ln[AUC(0-inf)]	6.29	0.247	6.32	0.290	81.06 - 115.89	96.9

Treatment B = 1 x 25 mg Fampridine SR Tablet Under Fed Conditions: test
Treatment A = 1 x 25 mg Fampridine SR Tablet Under Fasted Conditions: reference

The mean ratios for fampridine Cmax, AUC(0-t), and AUC(O-inf), derived from the natural log-transformed data, were 115.4%, 98.1%, and 96.9%, respectively. The 90% CI for AUC(0-t) at 80.25% – 119.98% and AUC(0-inf) at 81.06% – 115.89%. However, the 90% CI for Cmax was 95.78% – 139.09%.

Reviewer's Comment: Re-analysis of the data showed similar 90% confidence interval (CI) levels for C_{max} and $AUC_{0\text{-}inf}$ as shown in the table below.

Table 6: Food-Effect Analysis of Fampridine SR 25 mg Tablet Formulation

Treatment Comparisons	Parameter	Ratio	Lower 90% CI	Upper 90% CI
Fampridine SR 25 mg Fed/ Fampridine SR 25 mg Fasted	C_{max}	115	95.8	139
	$AUC_{0\text{-}\infty}$	96.9	81.1	115

The median Tmax was 3 hours following the fasted treatment (Treatment A) and 5 hours following the fed treatment (Treatment B).

Reviewer's Comment: In this study food effect was evaluated on 25 mg fampridine SR tablet. Fampridine SR 10 mg tablet is the maximum strength indicated in this new drug application. Food effect study was also conducted with 10 mg strength tablet. However, as per preNDA meeting comments food effect study at 10 mg strength was not submitted as per the Agency recommendation.

CONCLUSIONS

- Overall exposure $AUC_{0\text{-}inf}$ did not differ following the fed treatment compared to the fasted treatment.

- Maximum fampridine concentration Cmax differed by 15% and the time to reach maximum plasma concentration, Tmax delayed from approximately 3 hrs to 5 hrs following the fed treatment compared to the fasted treatment.

4.1.5 IN VITRO STUDIES

Study Title	***In Vitro* Protein Binding of14C-4-Aminopyridine in Human, Dog and Rat Plasma Samples**
Study number	HWI-6379-103
Objective	To determine the *in vitro* protein binding of [^{14}C]-4-aminopyridine in human, dog and rat plasma samples

METHODS

The *in vitro* protein binding of 14C-4-Aminopyridine was determined in human, dog, and rat plasma samples by equilibrium dialysis. Plasma samples were fortified to concentrations of 5, 50, and 500 ng/mL with ^{14}C-4-aminopyridine and dialyzed for 3, 4, 5, 6, and 8 hours. The effect of plasma pH on protein binding was determined in human plasma at pH 7.2, 7.4, 7.5, and 7.6.

Plasma Protein Binding Method: Plasma protein binding of ^{14}C-4-Aminopyridine was determined by equilibrium dialysis. Dialysis membranes were 12,000 to 14,000 MW cut-off. The dialysis membranes were placed between two 1.0 ml (b) (4) half-cells, and the ce1l was closed and placed in the cell holder. The cells were filled by placing equal amounts of phosphate buffer and plasma solution containing ^{14}C-Aminopyridine at concentrations of 5, 50 or 500 ng/mL on either of the membrane.

The free fraction amount of 14C-4-aminopyridine was calculated by following equation:

% free 100 – [(Cp-Cb)/Cp] x 100

Where: Cp= Drug concentration in plasma
Cb= Drug concentration in buffer

RESULTS

Following tables provide free drug fractions measured at 6 and 8 hours in human, dog and rat plasma.

Percent Free Drug Fraction of ^{14}C-4- Aminopyridine in Human Plasma at Initial Concentrations of 5, 50, and 500 ng/mL following Equilibrium Dialysis.

Concentration (ng/mL)	Dialysis Time Point (Hours)	% Free	Mean % Free	SD	RSD (%)
5	6	77.11 100.00 89.32	88.81	11.454	12.90
50	6	100.04 93.55 100.36	97.98	3.843	3.92
500	6	94.95 97.46 95.49	95.97	1.321	1.38
5	8	95.81 94.85 89.57	93.41	3.360	3.60
50	8	94.94 96.84 95.30	95.69	1.009	1.05
500	8	95.11 97.56 98.00	96.89	1.557	1.61

Percent Free Drug Fraction of ^{14}C-4- Aminopyridine in Dog Plasma at Initial Concentrations of 5, 50, and 500 ng/mL following Equilibrium Dialysis.

Concentration (ng/mL)	Dialysis Time Point (Hours)	% Free	Mean % Free	SD	RSD (%)
5	6	77.39 79.75 87.84	81.66	5.481	6.71
50	6	95.74 93.73 90.90	93.46	2.432	2.60
500	6	97.50 96.63 98.07	97.40	0.725	0.74
5	8	81.00 86.68 65.05	77.58	11.214	14.46
50	8	66.66 91.24 92.46	83.45	14.556	17.44
500	8	90.48 96.63 97.62	94.91	3.868	4.08

Percent Free Drug Fraction of ^{14}C-4- Aminopyridine in Rat Plasma at Initial Concentrations of 5, 50, and 500 ng/mL following Equilibrium Dialysis.

Concentration (ng/mL)	Dialysis Time Point (Hours)	% Free	Mean % Free	SD	RSD (%)
5	6	78.75 85.98 78.84	81.19	4.149	5.11
50	6	90.66 92.16 92.36	91.73	0.929	1.01
500	6	95.27 96.24 97.59	96.37	1.165	1.21
5	8	64.59 90.70 78.37	77.89	13.062	16.77
50	8	69.49 93.57 88.56	83.87	12.706	15.15
500	8	96.89 93.65 94.18	94.91	1.738	1.83

The Effect of Plasma pH on the Percent Free Drug Fraction of 14C-Aminopyridine in Human Plasma at Initial Concentration of 50 ng/mL Following Dialysis for 4 Hours.

pH Level	% Free	Mean % Free	SD	RSD (%)
7.2	98.05 99.14 96.26 96.83 98.08	97.67	1.137	1.16
7.4	97.26 91.64 98.49 95.98 98.20	96.31	2.790	2.90
7.6	94.38 95.77 95.38 93.16 95.40	94.82	1.061	1.12
7.9	90.20 96.60 94.18 94.81 93.66	93.89	2.342	2.49

CONCLUSIONS

- 14C-4-Aminopyridine was found to be largely unbound with high free drug fractions in human, dog and rat plasma at concentrations 5, 50 and 500 ng/mL.

- Protein binding was independent over the concentration range. The mean free drug fraction was 99% in human, 96% in dog and 92% in rat plasma.

- Plasma protein binding was also found to be independent of pH (range7.2 to 7.9).

Study Title	**Reaction Phenotyping: Identification of CYP enzymes involved in the metabolism of fampridine**
Study number	XT064039
Objective	To identify the cytochrome P450 (CYP) enzyme(s) in human liver microsomes responsible for catalyzing the aromatic hydroxylation of fampridine (4-aminopyridine) to 4-amino-3-hydroxypyridine (3-hydroxyfampridine)

METHODS AND RESULTS

Determination of kinetic constants (K_m and V_{max})

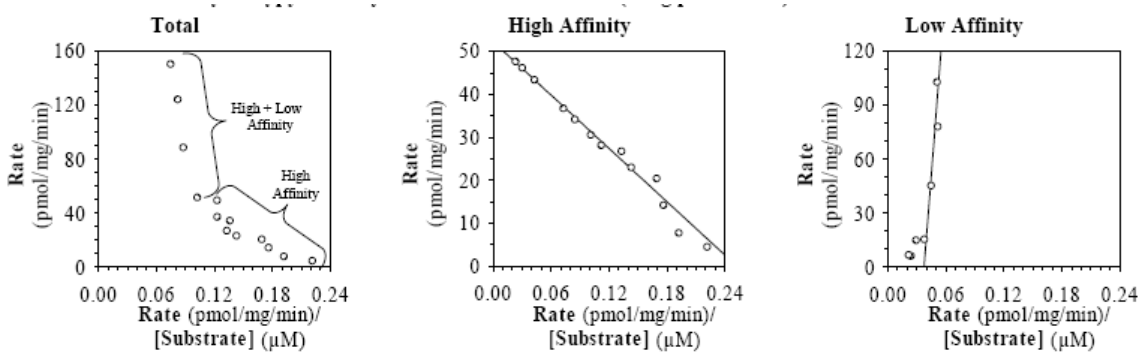
Michaelis-Menten kinetic constants (K_m and V_{max}) for the 3-hydroxylation of fampridine to 4-amino-3-hydroxypyridine by NADPH-fortified human liver microsomes: Fampridine (20, 40, 80, 120, 160, 200, 250, 300, 400, 500, 1000, 1500 and 2000 µM) was incubated in triplicate with human liver microsomes (1 mg protein per mL) at 37 ± 1°C for 60 minutes in 0.2-mL incubation mixtures in a 96-well plate format. The supernatant fractions were diluted 3-fold with stop reagent and analyzed by LC/MS/MS. Zero-time incubations served as controls.

Estimation of the contribution of high affinity and low affinity enzyme(s) to the hydroxylation of fampridine to 4-amino-3-hydroxypyridine by human liver microsomes (1 mg protein/mL)

[Fampridine] (µM)	Total rate of metabolite formation (pmol/mg protein/min)	Estimated high affinity rate (pmol/mg protein/min)	Estimated low affinity rate (pmol/mg protein/min)
20	4.44 ± 0.24	4.44	NA
40	7.70 ± 0.59	7.70	NA
80	14.1 ± 1.2	14.1	NA
120	20.4 ± 1.5	20.4	NA
160	22.9 ± 1.2	22.9	NA
200	26.7 ± 1.3	26.7	NA
250	34.1 ± 0.3	28.1	6.06
300	37.0 ± 0.6	30.4	6.57
400	49.3 ± 1.2	34.0	15.2
500	51.4 ± 1.4	36.6	14.7
1000	88.4 ± 10.9	43.2	45.1
1500	124 ± 3	46.0	77.9
2000	150 ± 7	47.5	103

Rates are the mean of triplicate determinations rounded to three significant figures
Standard deviation rounded to the same degree of accuracy as rate for total rates.
Limit of quantification: 1 ng/mL (equivalent to 1.51 pmol/mg protein/mL)
Data are shown graphically in Figure 5.

Eadie-Holfstee plots: Mean rate of metabolite formation



K_m = 207 μM
V_{max} = 52.2 pmol/mg/min

K_m and V_{max} were negative

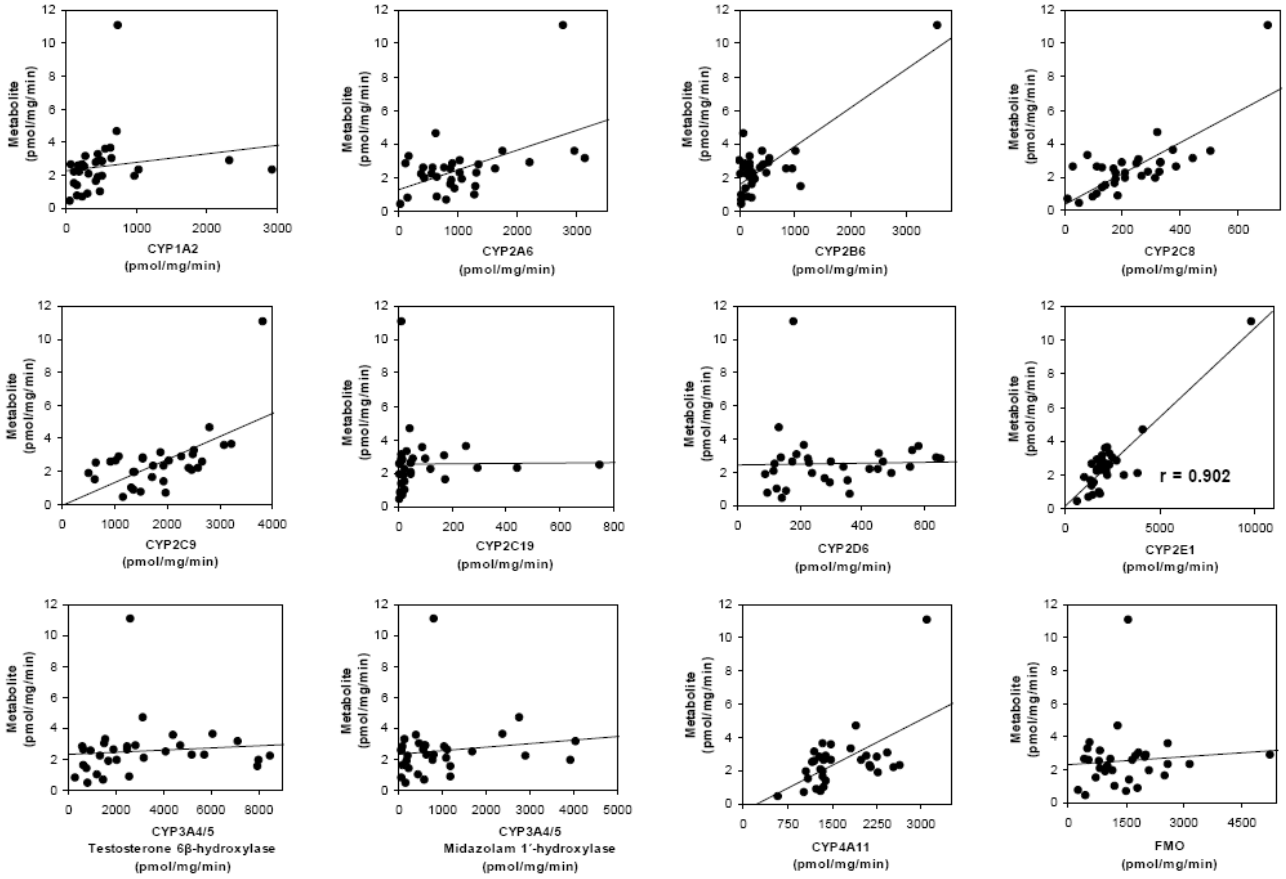
Identification of human CYP enzymes involved in the metabolism of fampridine: Reaction Phenotyping

Fampridine (10 μM) was incubated in duplicate with microsomes from individual samples (1 mg protein per mL) in a solution containing potassium phosphate buffer, $MgCl_2$ and EDTA. Incubations were carried out in duplicate at 37 ± 1°C for 60 minutes in a 96-well plate format.

Differences in the rate of 4-amino-3-hydroxypyridine formation were compared with the sample-to-sample variations in the following activities:

Enzyme	Marker Enzyme Activity
CYP1A2	Phenacetin *O*-dealkylation
CYP2A6	Coumarin 7-hydroxylation
CYP2B6	Bupropion hydroxylation
CYP2C8	Paclitaxel 6α-hydroxylation
CYP2C9	Diclofenac 4′-hydroxylation
CYP2C19	*S*-Mephenytoin 4′-hydroxylation
CYP2D6	Dextromethorphan *O*-demethylation
CYP2E1	Chlorzoxazone 6-hydroxylation
CYP3A4/5	Testosterone 6β-hydroxylation
CYP3A4/5	Midazolam 1′-hydroxylation
CYP4A11	Lauric acid 12-hydroxylation
FMO3	Benzydamine *N*-oxidation

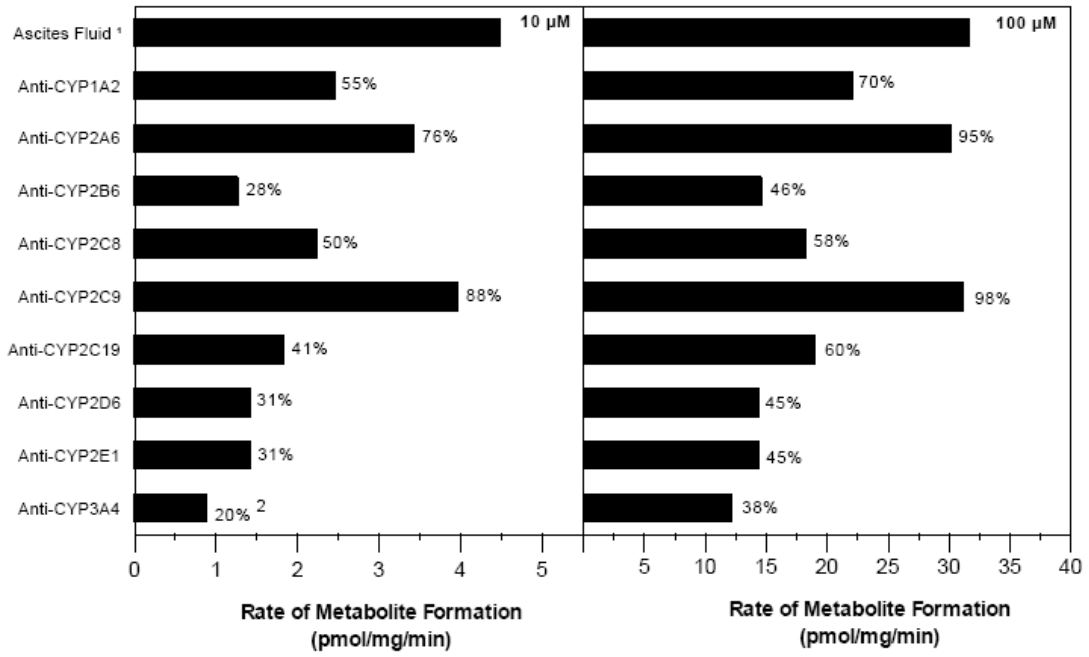
Sample-to-sample variation in CYP/FMO enzyme activity and the conversion of fampridine (10 μM) to 4-amino-3-hydroxypyridine by a bank (n=32) of human liver microsomes (1 mg protein/mL)



Antibody inhibition

Human liver microsomes at 1 mg protein per mL were pre-incubated with ascites fluid containing inhibitory monoclonal antibodies against CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 at room temperature for 15 minutes. Incubations containing control mouse ascites fluid served as negative controls.

Effect of various inhibitory monoclonal antibodies on the conversion of fampridine (10 and 100 µM) to 4-amino-3-hydroxypyridine by human liver microsomes (1 mg protein/mL)



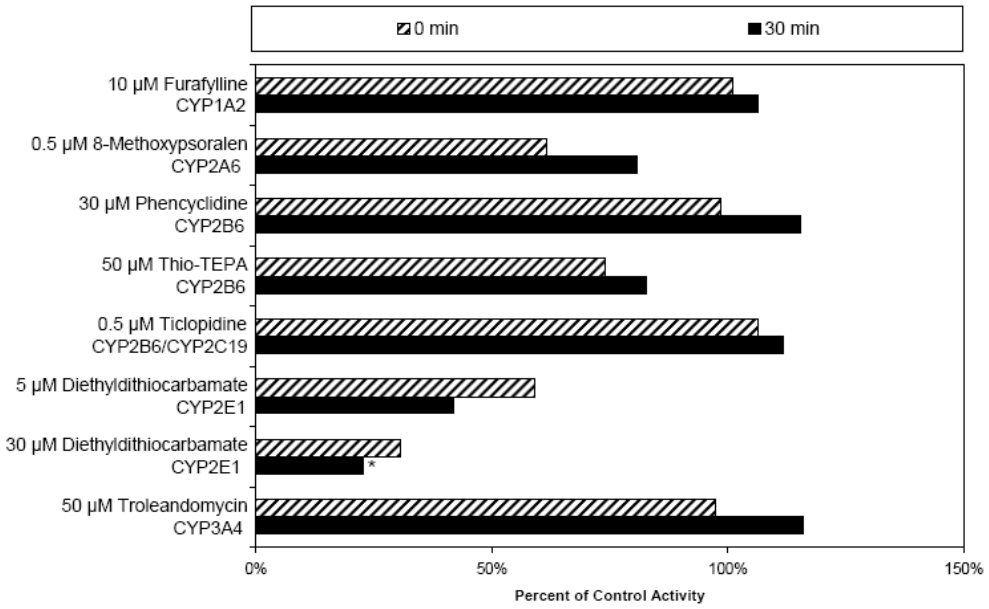
Chemical inhibition

Fampridine (10 µM) was incubated with human liver microsomes (1.0 mg protein per mL) at 37 ±1° C for 60 minutes in the presence of various chemical inhibitors. Solvent control incubations were carried out with the solvents used to add the inhibitors to the incubations (*i.e.*, acetonitrile or water). Metabolism-dependent inhibitors were preincubated for 0 or 30 minutes with human liver microsomes prior to the addition of fampridine to the incubation mixture. All of the remaining inhibitors were used as direct acting inhibitors (*i.e.*, no pre-incubation). Inhibitors used to inhibit specific CYP enzymes are summarized in the following table.

Inhibitor	Inhibited CYP Enzyme	Concentration in incubation (µM)	Solvent
α-Naphthoflavone	CYP1A2	0.1, 1, 10, 50	Acetonitrile (0.5%)
Montelukast	CYP2C8	1, 2.5, 5	Acetonitrile (0.5%)
Sulfaphenazole	CYP2C9	1, 10, 50	Acetonitrile (0.5%)
Quinidine	CYP2D6	0.1, 10, 100	Water
Ketoconazole	CYP3A4	0.01, 0.1, 0.5, 1, 10	Acetonitrile (0.5%)
10-(Imidazolyl)-decanoic acid	CYP4A11	0.1, 0.5, 1	Water
Furafylline*	CYP1A2	10	Water
8-Methoxypsoralen*	CYP2A6	0.5	Water
Phencyclidine*	CYP2B6	30	Water
Thio-TEPA*	CYP2B6	50	Water
Ticlopidine*	CYP2C19/2B6	0.5	Water
Diethyldithiocarbamate*	CYP2E1	5, 30	Water
Troleandomycin*	CYP3A4	50	Acetonitrile (0.5%)

*: Metabolism-dependent inhibitor

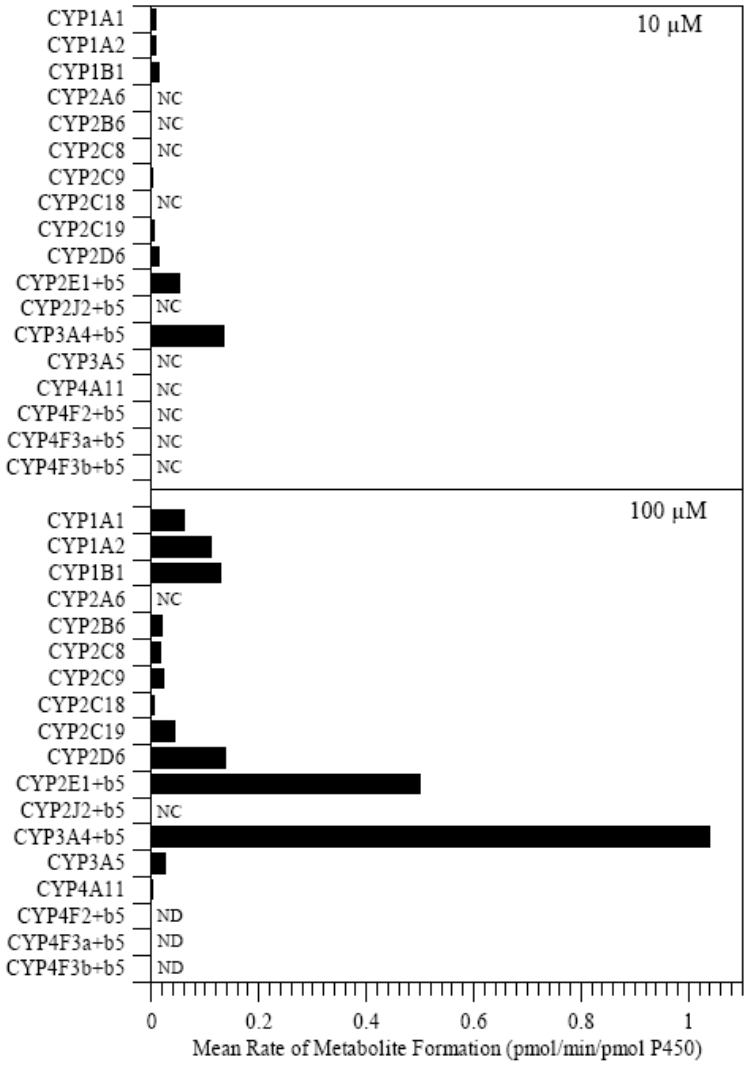
The effect of metabolism-dependent chemical inhibitors of selected CYP enzymes on the conversion of fampridine (10 µM) to 4-amino-3-hydroxypyridine by human liver microsomes (1 mg protein/mL)



Recombinant human CYP enzymes

Fampridine (10 and 100 µM) was incubated with the recombinant human CYP enzymes rCYP1A1, rCYP1A2, rCYP1B1, rCYP2A6, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C18, rCYP2C19, rCYP2D6, rCYP2E1, rCYP2J2, rCYP3A4, rCYP3A5, rCYP4A11, rCYP4F2, rCYP4F3a and rCYP4F3b (25 pmol CYP per incubation) and recombinant FMO enzymes rFMO1, rFMO3 and rFMO5. Incubations of fampridine with Control Bactosomes and microsomes containing only NADPH-cytochrome *c* reductase (Reductase Control) served as negative controls for recombinant CYP enzymes not co-expressed with cytochrome b_5.

Conversion of fampridine (10 and 100 µM) to 4-aminopyridine by a panel of recombinant human CYP enzymes



CONCLUSIONS

- CYP2E1 was the major enzyme responsible for the 3-hydroxylation of fampridine

- The 3-hydroxylation of fampridine to 4-amino-3-hydroxypyridine by human liver microsomes appeared to be catalyzed by two or more kinetically distinct enzymes.

- Several other enzymes (CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP3A5 and CYP4A11) appeared to play a minor role in the 3-hydroxylation of fampridine.

Study Title	**Determination of the Inhibitory Potential of Test Article on CYP450 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in Cryopreserved Human Hepatocytes**
Study number	M-2001-029
Objective	The purpose of this study was to determine the potential for 4-

	aminopyridine to inhibit liver cytochrome P450 (CYP450) isoforms 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in cryopreserved human hepatocytes.

METHODS

Hepatocyte Isolation and Incubation

Hepatocytes prepared and pooled from three male and two female human donors were obtained from the cryopreserved hepatocyte bank maintained at (b) (4). Cryopreserved cells were thawed and counted to determine yield. Viability was measured using Trypan blue exclusion; only cells with ≥ 70% viability were used in this study.

The test article and positive control inhibitors were added to uncoated 24-well plates as appropriate. Aliquots (0.25 mL) of the 2X hepatocyte suspension were then added to each well (0.5 x 10^6 cells/well) and the samples were preincubated for 15 minutes. After this preincubation, 5 µL of 100X probe substrate stocks were added to the groups as appropriate, and the samples were incubated for 60 minutes. The following substrates were evaluated: 15 µM phenacetin (CYP1A2), 8 µM coumarin (CYP2A6), 150 µM tolbutamide (CYP2C9), 20 µM S-mephenytoin (CYP2C19), 8 µM dextromethorphan (CYP2D6), 100 µM chlorzoxazone (CYP2E1), and 50 µM testosterone (CYP3A4). Incubation reactions were terminated with the addition of an equal volume of methanol, except for the incubations with phenacetin, which were terminated with the addition o f 150 µL acetonitrile; and S-mephenytoin, which were terminated with the addition of 50 µL perchloric acid.

RESULTS

CYP1A2

CYP1A2 activity was characterized by the formation of acetaminophen from phenacetin. The percent of inhibition of CYP1A2 activity was 21.0, 16.5, 32.4, 17.6 and 14.2% in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively. As the inhibition was < 50% at all dose levels, the IC50 value was not calculated.

Table 1: CYP1A2 activities in cryopreserved human hepatocytes

Test/Control Article	Conc. (µM)	Acetaminophen Formation (µM)	(pmol/million cells/min)	% VC	% Inhibition
VC	NA	0.598	13.1		
		0.991	21.6		
		0.692	15.1		
		0.746	16.3		
		0.934	20.4		
		0.864	18.9		
	Mean ± SD		17.6 ± 3.3	100	0.00
Furafylline	1	0.125*	2.73		
		0.113*	2.47		
		0.125*	2.73		
		0.118*	2.58		
		0.101*	2.21		
		0.118*	2.58		
	Mean ± SD		2.55 ± 0.19	NA	NA
4-Aminopyridine	0.01	0.687	15.0		
		0.587	12.8		
		0.632	13.8		
	Mean ± SD		13.9 ± 1.1	79.0	21.0
	0.1	0.612	13.4		
		0.630	13.8		
		0.772	16.9		
	Mean ± SD		14.7 ± 1.9	83.5	16.5
	1	0.624	13.6		
		0.588	12.8		
		0.427	9.33		
	Mean ± SD		11.9 ± 2.3	67.6	32.4
	10	0.558	12.2		
		0.625	13.6		
		0.806	17.6		
	Mean ± SD		14.5 ± 2.8	82.4	17.6
	100	0.853	18.6		
		0.692	15.1		
		0.528	11.5		
	Mean ± SD		15.1 ± 3.6	85.8	14.2
CIC	100	0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
	Mean ± SD		0.00	NA	NA

* Observed analyzed value was below quantifiable limit (0.39 µM).
Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CYP2A6

CYP2A6 activity was characterized by the formation of 7-hydroxycoumarin, 7-hydroxycoumarin glucuronide, and 7-hydroxycoumarin sulfate from coumarin. The percent of inhibition of CYP2A6 activity was 0.897, 3.59, 11.2, 15.2, and 11.2% in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively (Table below). As the inhibition was < 50% at all dose levels, the IC50 value was not calculated.

Table 2: CYP2A6 activities in cryopreserved human hepatocytes

Test/Control Article	Conc. (µM)	Total Coumarin Metabolite Formation (µM)	(pmol/million cells/min)	% VC	% Inhibition
VC	NA	0.728	24.5		
		0.692	23.3		
		0.634	21.3		
		0.599	20.2		
		0.608	20.5		
		0.704	23.7		
	Mean ± SD		22.3 ± 1.8	100	0.00
Tranylcypromine	1	0.712	24.0		
		0.638	21.5		
		0.611	20.6		
		0.554	18.7		
		0.580	19.5		
		0.677	22.8		
	Mean ± SD		21.2 ± 2.0	95.1	4.93
4-Aminopyridine	0.01	0.755	25.4		
		0.644	21.7		
		0.570	19.2		
	Mean ± SD		22.1 ± 3.1	99.1	0.897
	0.1	0.560	18.9		
		0.608	20.5		
		0.742	25.0		
	Mean ± SD		21.5 ± 3.2	96.4	3.59
	1	0.690	23.2		
		0.576	19.4		
		0.496	16.7		
	Mean ± SD		19.8 ± 3.3	88.8	11.2
	10	0.510	17.2		
		0.536	18.0		
		0.639	21.5		
	Mean ± SD		18.9 ± 2.3	84.8	15.2
	100	0.682	23.0		
		0.567	19.1		
		0.515	17.3		
	Mean ± SD		19.8 ± 2.9	88.8	11.2
CIC	100	0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
	Mean ± SD		0.00	NA	NA

Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CYP2C9

CYP2C9 activity was characterized by the formation of 4-hydroxytolbutamide from tolbutamide. The percent of inhibition of CYP2C9 activity was -11.3, -15.7, -3.64, -4.00, and -6.82% in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively (Table below). As there was no inhibition of CYP2C9 activity at all dose levels, the IC50 value was not calculated.

Table 3: CYP2C9 activities in cryopreserved human hepatocytes

Test/Control Article	Conc. (µM)	4-Hydroxytolbutamide Formation (µM)	(pmol/million cells/min)			% VC	% Inhibition
VC	NA	0.159	5.37				
		0.236	7.93				
		0.246	8.27				
		0.263	8.87				
		0.332	11.2				
		0.279	9.40				
	Mean ± SD		8.51	±	1.92	100	0.00
Sulfaphenazole	10	0.00	0.00				
		0.00	0.00				
		0.00	0.00				
		0.00	0.00				
		0.00	0.00				
		0.00	0.00				
	Mean ± SD		0.00			0.00	100
4-Aminopyridine	0.01	0.301	10.1				
		0.273	9.20				
		0.270	9.10				
	Mean ± SD		9.47	±	0.55	111	-11.3
	0.1	0.269	9.07				
		0.293	9.87				
		0.315	10.6				
	Mean ± SD		9.85	±	0.77	116	-15.7
	1	0.298	10.0				
		0.248	8.33				
		0.242	8.13				
	Mean ± SD		8.82	±	1.03	104	-3.64
	10	0.249	8.37				
		0.261	8.80				
		0.278	9.37				
	Mean ± SD		8.85	±	0.50	104	-4.00
	100	0.307	10.3				
		0.253	8.53				
		0.250	8.43				
	Mean ± SD		9.09	±	1.05	107	-6.82
CIC	100	0.00	0.00				
		0.00	0.00				
		0.00	0.00				
		0.00	0.00				
		0.00	0.00				
		0.00	0.00				
	Mean ± SD		0.00			NA	NA

Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CYP2C19

CYP2C19 activity was characterized by the formation of 4'hydroxy-S- mephenytoin froom S-mephenytoin. The percent of inhibition of CYP2C19 activity was 15.3, 0.517, 18.1, 16.0, and 22.6% in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively (Table below). As the inhibition was < 50% at all dose levels, the IC50 value was not calculated.

Table 4: CYP2C19 activities in cryopreserved human hepatocytes

Test/Control Article	Conc. (µM)	4-Hydroxymephenytoin Formation (µM)	(pmol/million cells/min)	% VC	% Inhibition
VC	NA	0.292	5.40		
		0.283	5.23		
		0.339	6.27		
		0.298	5.50		
		0.311	5.77		
		0.356	6.60		
	Mean ± SD		5.80 ± 0.54	100	0.00
Omeprazole	25	0.172	3.18		
		0.183	3.40		
		0.185	3.43		
		0.193	3.57		
		0.202	3.73		
		0.237	4.40		
	Mean ± SD		3.62 ± 0.42	62.4	37.6
4-Aminopyridine	0.01	0.283	5.23		
		0.248	4.60		
		0.265	4.90		
	Mean ± SD		4.91 ± 0.32	84.7	15.3
	0.1	0.324	6.00		
		0.281	5.20		
		0.329	6.10		
	Mean ± SD		5.77 ± 0.49	99.5	0.517
	1	0.304	5.63		
		0.245	4.53		
		0.221	4.10		
	Mean ± SD		4.75 ± 0.79	81.9	18.1
	10	0.210	3.90		
		0.274	5.07		
		0.305	5.63		
	Mean ± SD		4.87 ± 0.88	84.0	16.0
	100	0.249	4.60		
		0.259	4.80		
		0.219	4.07		
	Mean ± SD		4.49 ± 0.38	77.4	22.6
CIC	100	0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
	Mean ± SD		0.00	NA	NA

Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CYP2D6

CYP2D6 activity was characterized by the formation of dextrorphan from dextromethorphan. The percent of inhibition of CYP2D6 activity was -23.3, -13.0, -5.83, -4.93, and -6.95% in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively (Table below). The results indicated that there was no inhibition of CYP2D6 activity in cryopreserved human hepatocytes by 4-aminopyridine at all tested concentrations.

Table 5B: CYP2D6 activities in cryopreserved human hepatocytes (re-analysis)

Test/Control Article	Conc. (µM)	Dextrorphan Formation (µM)	(pmol/million cells/min)	% VC	% Inhibition
VC	NA	0.144	4.83		
		0.130	4.37		
		0.129	4.33		
		0.121	4.07		
		0.131	4.40		
		0.141	4.73		
	Mean ± SD		4.46 ± 0.28	100	0.000
Quinidine	2.5	0.022*	0.740		
		0.021*	0.707		
		0.021*	0.707		
		0.020*	0.673		
		0.023*	0.773		
		0.029*	0.977		
	Mean ± SD		0.763 ± 0.110	NA	NA
4-Aminopyridine	0.01	0.191	6.43		
		0.150	5.07		
		0.149	5.00		
	Mean ± SD		5.50 ± 0.81	123	-23.3
	0.1	0.145	4.87		
		0.147	4.93		
		0.158	5.33		
	Mean ± SD		5.04 ± 0.25	113	-13.0
	1	0.159	5.37		
		0.133	4.47		
		0.129	4.33		
	Mean ± SD		4.72 ± 0.56	106	-5.83
	10	0.130	4.37		
		0.134	4.50		
		0.153	5.17		
	Mean ± SD		4.68 ± 0.43	105	-4.93
	100	0.156	5.27		
		0.137	4.60		
		0.132	4.43		
	Mean ± SD		4.77 ± 0.44	107	-6.95
CIC	100	0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
	Mean ± SD		0.00	NA	NA

* Observed analyzed value was below quantifiable limit (0.0955 µM).
Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CYP2E1

CYP2E1 activity was characterized by the formation of 6-hydroxychlorzoxazone from chlorzoxazone. CYP2E1 activity was 21.9, 23.5, 20.7, 22.0, and 16.2 pmol/million cells/minute in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively (Table below). No percent of inhibition was calculated, since the values were below the quantifiable limit of 0.98 µM.

Table 6: CYP2E1 activities in cryopreserved human hepatocytes

Test/Control Article	Conc. (µM)	6-Hydroxychlorzoxazone Formation (µM)	(pmol/million cells/min)	% VC	% Inhibition
VC	NA	0.635*	21.4		
		0.671*	22.6		
		0.665*	22.4		
		0.697*	23.5		
		0.724*	24.4		
		0.811*	27.3		
	Mean ± SD		23.6 ± 2.1	NA	NA
4-Methylpyrazole	250	0.176*	5.93		
		0.175*	5.90		
		0.172*	5.80		
		0.180*	6.07		
		0.187*	6.30		
		0.200*	6.73		
	Mean ± SD		6.12 ± 0.34	NA	NA
4-Aminopyridine	0.01	0.682*	23.0		
		0.637*	21.4		
		0.637*	21.4		
	Mean ± SD		21.9 ± 0.9	NA	NA
	0.1	0.645*	21.7		
		0.704*	23.7		
		0.749*	25.2		
	Mean ± SD		23.5 ± 1.8	NA	NA
	1	0.635*	21.4		
		0.619*	20.8		
		0.594*	20.0		
	Mean ± SD		20.7 ± 0.7	NA	NA
	10	0.591*	19.9		
		0.644*	21.7		
		0.724*	24.4		
	Mean ± SD		22.0 ± 2.3	NA	NA
	100	0.488*	16.4		
		0.475*	16.0		
		0.485*	16.3		
	Mean ± SD		16.2 ± 0.2	NA	NA
CIC	100	0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
	Mean ± SD		0.00	NA	NA

* Observed analyzed value was below quantifiable limit (0.98 µM).
Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CYP3A4

CYP3A4 activity was characterized by the formation of 6β-hydroxytestosterone from testosterone. The percent of inhibition of CYP3A4 activity was 6.09, 0.870, 9.57, 7.83, and 11.3% in cryopreserved human hepatocytes treated with 4-aminopyridine at the tested concentrations of 0.01, 0.1, 1, 10, and 100 µM, respectively (Table below). Since inhibition was < 50% at all dose levels, the IC50 value was not calculated.

Table 7: CYP3A4 activities in cryopreserved human hepatocytes

Test/Control Article	Conc. (µM)	6β-Hydroxytestosterone Formation (µM)	(pmol/million cells/min)	% VC	% Inhibition
VC	NA	3.294	111		
		3.498	118		
		3.434	116		
		3.415	115		
		3.375	114		
		3.447	116		
	Mean ± SD		115 ± 2	100	0.00
Ketoconazole	1	0.286	9.63		
		0.277	9.33		
		0.266	8.97		
		0.269	9.07		
		0.271	9.13		
		0.271	9.13		
	Mean ± SD		9.21 ± 0.24	8.01	92.0
4-Aminopyridine	0.01	3.383	114		
		3.120	105		
		3.160	106		
	Mean ± SD		108 ± 5	93.9	6.09
	0.1	3.145	106		
		3.650	123		
		3.330	112		
	Mean ± SD		114 ± 9	99.1	0.870
	1	3.249	109		
		3.026	102		
		2.966	100		
	Mean ± SD		104 ± 5	90.4	9.57
	10	2.908	98.0		
		3.151	106		
		3.422	115		
	Mean ± SD		106 ± 9	92.2	7.83
	100	3.144	106		
		3.012	101		
		2.914	98.0		
	Mean ± SD		102 ± 4	88.7	11.3
CIC	100	0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
		0.00	0.00		
	Mean ± SD		0.00	NA	NA

Abbreviations: VC, vehicle control (2.0% acetonitrile); CIC, chromatographic interference control; SD, standard deviation; NA, not applicable.

CONCLUSIONS

- No apparent dose-dependent inbibition for CYP1A2, CYP2A6, CYP2C19, CYP2E1, and CYP3A4 activities in cryopreserved human hepatocytes was observed with 4-aminopyridine at concentrations up to 100 µM (9410 ng/mL). The IC50 values could not be calculated.
- No inhibition was observed for CYP2C9 and CYP2D6 activity in cryopreserved human hepatocytes treated with 4-aminopyridine at concentrations up to 100 µM.

Study Title	***In Vitro* Evaluation of 4-aminopyridine as an Inhibitor of Human Cytochrome P450 Enzymes**
Study number	XT075077
Objective	To evaluate, 4-aminopyridine as a direct and time-dependent inhibitor

	of human CYP enzymes

METHODS

Selective inhibition of 4-aminopyridine metabolism:

Pooled human liver microsomes and 4-aminopyridine were incubated with an NADPH generating system, with or without CYP selective inhibitors in potassium phosphate (pH 7.4) at 37°C for 45 min. Comparisons were made to 4-aminopyridine metabolism (depletion of parent) in the absence of inhibitors to determine percent inhibition.

Pooled human liver microsomes (from sixteen individuals) were used as the source of enzymes. Incubations were conducted at approximately 37 °C in 400-µL incubation mixtures (target pH 7.4) containing high purity water, potassium phosphate buffer (50 mM), $MgCl_2$ (3 mM), EDTA (1 mM), an NADPH-generating system (always the mixture of the following: NADP [1 mM], glucose-6-phosphate [5 mM], glucose-6-phosphate dehydrogenase [1 Unit/mL]), and marker substrate at the final concentrations indicated.

CYP enzyme	Positive control	Vehicle	Concentration studied
CYP1A2	α-Naphthoflavone	Methanol	0.5 µM
CYP2A6	Nicotine	Methanol	300 µM
CYP2B6	Orphenadrine	DMSO	750 µM
CYP2C8	Montelukast	Methanol	0.5 µM
CYP2C9	Sulfaphenazole	Methanol	2.0 µM
CYP2C19	Modafinil	DMSO	250 µM
CYP2D6	Quinidine	High purity water	0.5 µM
CYP2E1	4-Methylpyrazole	High purity water	15 µM
CYP3A4/5	Ketoconazole	Methanol	0.15 [a] / 0.075 [b] µM

[a] Testosterone 6β-hydroxylation or Nifedipine oxidation
[b] Midazolam 1´-hydroxylation

For the following time-dependent assays, additional zero-minute and 30-minute preincubations were conducted (in the presence of the following inhibitors) with the normal preincubation time and microsomal protein concentration.

CYP enzyme	Positive control	Vehicle	Concentration studied
CYP1A2	Furafylline	DMSO	1.0 µM
CYP2A6	8-Methoxypsoralen	Methanol	0.05 µM
CYP2B6	Phencyclidine	High purity water	30 µM
CYP2C8	Gemfibrozil glucuronide	Acetonitrile with 0.1% (v/v) formic acid	25 µM
CYP2C9	Tienilic acid	Tris base (0.002 mg/mL)	0.25 µM
CYP2C19	Ticlopidine	High purity water	0.75 µM
CYP2D6	Paroxetine	High purity water	0.3 µM
CYP2E1	3-Amino-1,2,4-triazole	High purity water	10,000 µM
CYP3A4/5	Troleandomycin	Acetonitrile	25 [a] / 7.5 [b] µM

[a] Testosterone 6β-hydroxylation
[b] Midazolam 1´-hydroxylation

Analytical methods
All analyses were performed with validated HPLC/MS/MS methods.

RESULTS

Determination of IC_{50} values

Under the experimental conditions examined, there was no observed direct inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 (as measured by testosterone 6 β -hydroxylation and midazolam 1´-hydroxylation) at 4-aminopyridine concentrations up to 30 µM (Table below). There was evidence of direct inhibition of CYP2E1 by 4-aminopyridine at 30 µM (approximately 12% inhibition); however, the IC_{50} value for CYP2E1 was reported as greater than 30 µM

Table 3: Summary of results: *In vitro* evaluation of 4-aminopyridine as an inhibitor of human CYP enzymes

		Direct inhibition		Time-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation		
Enzyme	CYP Reaction	IC_{50} (µM)	Maximum inhibition at 30 µM (%) [a]	IC_{50} (µM)	Maximum inhibition at 30 µM (%) [a]	Potential for time-dependent inhibition [b]
CYP1A2	Phenacetin *O*-deethylation	>30	NA	>30	NA	Little or no
CYP2A6	Coumarin 7-hydroxylation	>30	NA	>30	NA	Little or no
CYP2B6	Bupropion hydroxylation	>30	NA	>30	NA	Little or no
CYP2C8	Amodiaquine *N*-dealkylation	>30	NA	>30	NA	Little or no
CYP2C9	Diclofenac 4´-hydroxylation	>30	2.0	>30	NA	Little or no
CYP2C19	*S*-Mephenytoin 4´-hydroxylation	>30	NA	>30	NA	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	>30	NA	>30	NA	Little or no
CYP2E1	Chlorzoxazone 6-hydroxylation	>30	12	>30	19	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>30	NA	>30	4.8	Little or no
CYP3A4/5	Midazolam 1´-hydroxylation	>30	NA	>30	3.2	Little or no

Notes: Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values. IC_{50} values were calculated with XLFit.

a Maximum inhibition (%) is calculated with the following formula and data for the highest concentration of test article evaluated (results are rounded to two significant figures): Maximum inhibition (%) = 100% – Percent solvent control.

b Time-dependent inhibition was determined by comparison of IC_{50} values with and without preincubation, by comparison of the maximum inhibition (%) with and without preincubation and by visual inspection of the IC_{50} plot.

NA Not applicable. No value was obtained as the rates at the highest concentration of 4-aminopyridine evaluated (30 µM) were higher than the control rates.

An estimated IC_{50} and K_i for the 4-aminopyridine mediated inhibition of CYP2E1 is 125 µM and 62.5 µM (0.5 * IC_{50}), respectively. The maximum plasma concentration measured in the MS-F203 study was 67 ng/ml (0.7 µM) which gives an I/K_i ratio of 0.01. No additional drug interaction studies were conducted as per the guidance.

Furthermore, under the experimental conditions examined, there was little or no evidence of time-dependent inhibition as inhibition by 4-aminopyridine (up to 30 µM) did not increase substantially after a 30-minute preincubation for any of the enzymes evaluated (*i.e.*, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4/5 [as measured by testosterone 6 β -hydroxylation and midazolam 1´-hydroxylation]).

Reviewer's Comment: In another study (M-2001-029) with cryopreserved human hepatocytes, CYP2E1 inhibition was not seen at 100 µM (higher) 4-aminopyridine concentration. Also, no apparent dose-dependent inbibition for CYP1A2, CYP2A6,

CYP2C19, and CYP3A4 activities or inhibition of CYP2C9 and CYP2D6 activity was observed.

CONCLUSIONS

- 4-aminopyridine at 30 μM resulted in approximately 12% inhibition of CYP2E1. The IC_{50} value for CYP2E1 was reported as greater than 30 μM which is approximately 100 times the average plasma 4-aminopyridine concentration measured for the 10 mg Fampridine-SR tablet.

- There was no observed direct inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 at 4-aminopyridine concentrations up to 30 μM.
- There was no of time-dependent inhibition as inhibition by 4-aminopyridine (up to 30 μM) did not increase substantially after a 30-minute preincubation for any of the enzymes evaluated (*i.e.*, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4/5.

Study Title	***In Vitro* Evaluation of 4-Aminopyridine as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes**
Study number	XT073070
Objective	To investigate the effects of treating primary cultures of human hepatocytes with 4-aminopyridine on the expression of cytochrome P450 (CYP) enzymes.

METHODS

Hepatocytes were isolated and cultured.

Treatment of cultured human hepatocytes
Hepatocytes were obtained from 3 different donors. Hepatocyte cultures were treated daily for three consecutive days with 0.1% DMSO (vehicle, negative control), 0.1% saline (vehicle control for isoniazid), one of four concentrations of 4-aminopyridine (0.025, 0.25, 2.5 or 25 μM) or one of four known human CYP enzyme inducers, including, omeprazole (100 μM), phenobarbital (750 μM), isoniazid (100 μM) and rifampin (10 μM), positive controls.
In this study, it was determined that the average plasma 4-aminopyridine concentration measured for Fampridine-SR tablet treatment group was approximately 30 ng/ml (0.3 μM). The concentration range selected for the *in vitro* induction study, 0.025 to 25 μM represented approximately 0.1 to 100 times the average plasma 4-aminopyridine concentration measured for the 10 mg Fampridine-SR tablet. The maximum proposed marketed strength for Fampridine-SR Tablets is 10 mg.

Microsome preparation from cultured human hepatocytes

Microsomal samples were prepared using acceptable methods. For each hepatocyte preparation, culture dishes within each treatment group were scraped, pooled and frozen

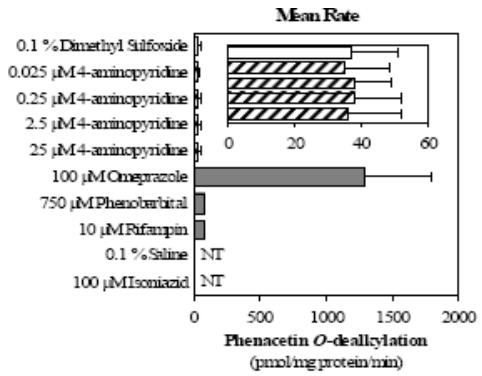
at –80 ± 5 °C. Sonicated lysates were centrifuged at 7,000 to 9,000 × g at 4 °C for 20 minutes, after which the supernatant fraction (S9) was removed and centrifuged at 80,000 to 90,000 × g at 4 °C for 60 minutes. The resulting microsomal pellets were resuspended in 250 mM sucrose and stored at −80 ± 5 °C.

Analytical methods
All the analyses were performed with validated HPLC/MS/MS methods.

RESULTS

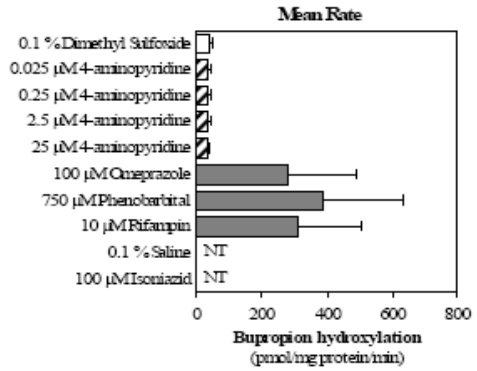
The effect of 4-aminopyridine on human CYP1A2 expression

The effect of treating cultured human hepatocytes with 4-aminopyridine or prototypical inducers on the rate of microsomal phenacetin *O*-dealkylase (CYP1A2) activity



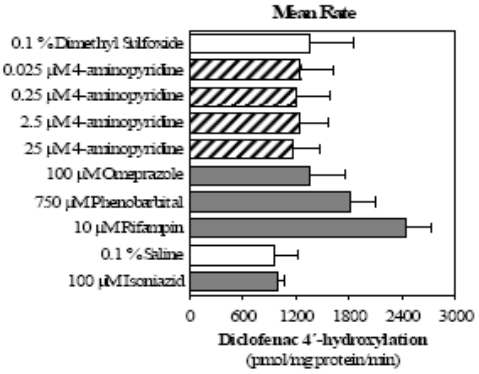
The effect of 4-aminopyridine on human CYP2B6 expression

The effect of treating cultured human hepatocytes with 4-aminopyridine or prototypical inducers on the rate of microsomal bupropion hydroxylase (CYP2B6) activity



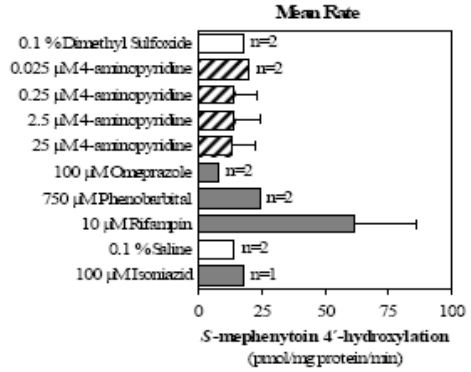
The effect of 4-aminopyridine on human CYP2C9 expression

The effect of treating cultured human hepatocytes with 4-aminopyridine on the rate of microsomal diclofenac 4'-hydroxylase (CYP2C9) activity



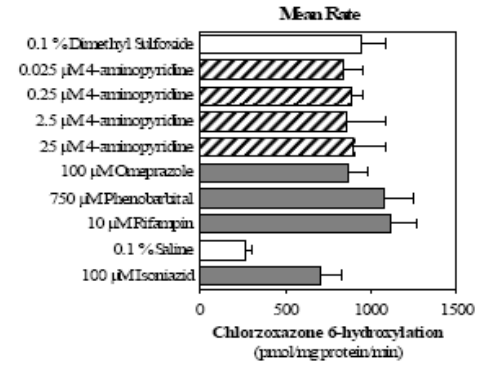
The effect of 4-aminopyridine on human CYP2C19 expression

The effect of treating cultured human hepatocytes with 4-aminopyridine or prototypical inducers on the rate of microsomal *S*-mephenytoin 4´-hydroxylase (CYP2C19) activity



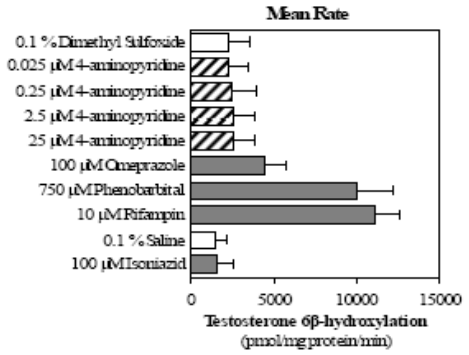
The effect of 4-aminopyridine on human CYP2E1 expression

The effect of treating cultured human hepatocytes with 4-aminopyridine or prototypical inducers on the rate of microsomal chlorzoxazone 6-hydroxylase (CYP2E1) activity



The effect of 4-aminopyridine on human CYP3A4/5 expression

The effect of treating cultured human hepatocytes with 4-aminopyridine or prototypical inducers on the rate of microsomal testosterone 6β-hydroxylase (CYP3A4/5) activity



Reviewer's Comment: Treatment of cultured human hepatocytes with 0.025 µM, 0.25 µM, 2.5 µM and 25 µM 4-aminopyridine had no effect on CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1 or CYP3A4/5 enzyme activities.

CONCLUSIONS

Induction of CYP enzymes in human hepatocytes is unlikely at concentration used in this study which was approximately 100 times the average plasma 4-aminopyridine concentration measured for the Phase 3 Fampridine-SR study (MS-F203).

Study Title	**Assessment of 4-Aminopyridine as Substrate and Inhibitor of P-glycoproteins in Different Cell Systems**
Study number	8ACORP1
Objective	To investigate whether 4-aminopyridine is a substrate and/or inhibitor of P-glycoprotein (P-gp) using bidirectional permeability assay.

METHODS

The ability of 4-aminopyridine to serve as a P-gp substrate was assessed at the dosing concentrations of 0.7, 7, and 70 µM. The net efflux ratio of 4-aminopyridine ranged from 0.8 to 1.0 at these concentrations. Digoxin was used as the positive control in parallel to demonstrate the validity of the experiments. From these results, 4-aminopyridine is not a P-gp substrate.

4-Aminopyridine Bi-Directional Permeability Measurement

The dosing solutions of 4-aminopyridine were prepared in HBSSg buffer from DMSO stock. The cell monolayers used for the study were washed once with HBSSg buffer, and the TEER value for each membrane was measured. The transport buffer in the receiver side was HBSSg buffer.

P-gp Substrate Assessment

The transport of 4-aminopyridine at 0.7, 7, and 70 µM in AP to BL and BL to AP directions was measured in both MDR1-MDCK and MDCK cells. The following procedure was performed: for AP to BL transport, 0.5 mL fresh dosing solutions of 4-

aminopyridine was added to the AP side, and 1.5 mL of buffer was added to the BL side. For BL to AP transport, 1.5 mL 4-aminopyridine dosing solutions were added to the BL side, and 0.5 mL of HBSSg was added to the AP side. Then the cell monolayers were incubated at 37°C (5% CO_2) in a humidified incubator for 120 minutes. Each determination was performed in triplicate. Aliquots (200 µL) were taken from the receiver compartment at 30, 60, and 120 minutes, and the same volume of fresh buffer was replaced back to the receiver side. Aliquots (50 µL) were taken from the donor compartment at 0, 60, and 120 minutes, and no replacement was performed. In parallel, bidirectional permeability of digoxin, a P-gp substrate, was also measured with the same cell batch. For digoxin samples, aliquots (200 µL) were taken from the receiver compartment at 120 minutes. Aliquots (50 µL) were taken from the donor compartment at 0 and 120 minutes.

P-gp Inhibitor Assessment

The bi-directional transport of digoxin, a known P-gp substrate, was measured in the absence and presence of 4-aminopyridine, CsA, and ketoconazole in Caco-2 cells. A 15-minute pre-incubation with 100 µM 4-aminopyridine, 10 µM CsA, or 10 µM ketoconazole solution was performed to inhibit the functions of P-gp. After this pre-incubation, the following procedure was performed: for AP to BL transport, 0.5 mL fresh dosing solutions of digoxin in the absence and presence of 4-aminopyridine and known inhibitors were added to the AP side, and 1.5 mL of buffer in the absence and presence of 4-aminopyridine and known inhibitors was added to the BL side. For BL to AP transport, 1.5 mL dosing solutions of digoxin in the absence and presence of 4-aminopyridine and known inhibitors were added to the BL side, and 0.5 mL of buffer in the absence and presence of 4-aminopyridine and known inhibitors was added to the AP side. Then, the Caco-2 plates were incubated at 37°C (5% CO_2) in a humidified incubator for 120 minutes with shaking. Each determination was performed in triplicate. Samples were taken from the receiver compartment at 60 and 120 minutes and the same volume of fresh buffer was replaced back to the receiver side.

Sample Analysis

4-aminopyridine and digoxin samples were analyzed by LC-MS/MS by validated analytical method.

Permeability and Recovery of 4-Aminopyridine and Digoxin in MDR1-MDCK Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
4-aminopyridine (0.7 µM)	P_{app} ($\times 10^{-6}$cm/s)	3.1	3.7	2.4	3.1 ± 0.6	3.0	3.0	3.0	3.0 ± 0.0	1.0
	% Recovery	78.8	76.7	73.8	76.4 ± 2.5	79.6	79.5	79.6	79.6 ± 0.1	
4-aminopyridine (7 µM)	P_{app} ($\times 10^{-6}$cm/s)	3.7	3.7	3.6	3.7 ± 0.0	3.1	3.2	2.9	3.0 ± 0.1	0.8
	% Recovery	99.6	97.6	97.9	98.4 ± 1.1	103	91.2	101	98.3 ± 6.2	
4-aminopyridine (70 µM)	P_{app} ($\times 10^{-6}$cm/s)	4.0	3.4	3.9	3.8 ± 0.3	3.6	3.8	3.9	3.7 ± 0.1	1.0
	% Recovery	111	105	109	108 ± 2.8	110	109	108	109 ± 1.0	
Digoxin (10 µM)	P_{app} ($\times 10^{-6}$cm/s)	1.9*	0.1	0.1	0.1 ± 0.0	14.1	12.7	16.8	14.5 ± 2.1	123
	% Recovery	89.4*	87.2	89.7	88.4 ± 1.8	92.5	89.4	92.2	91.4 ± 1.7	

*The measured concentration was unusually high and its value was excluded from calculations.

Permeability and Recovery of 4-Aminopyridine and Digoxin in MDCK Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
4-aminopyridine (0.7 μM)	P_{app} ($\times 10^{-6}$cm/s)	0.5*	0.5*	0.5*	0.5	0.5*	0.5*	0.5*	0.5	N.D.
	% Recovery	85.1*	84.0*	84.7*	84.6	84.9*	86.7*	85.0*	85.5	
4-aminopyridine (7 μM)	P_{app} ($\times 10^{-6}$cm/s)	0.5	0.3	0.6	0.5 ± 0.1	0.5	0.5	0.5	0.5 ± 0.0	1.0
	% Recovery	97.8	96.9	99.1	97.9 ± 1.1	105	98.7	104	102 ± 3.2	
4-aminopyridine (70 μM)	P_{app} ($\times 10^{-6}$cm/s)	0.5	0.5	0.5	0.5 ± 0.0	0.6	0.5	0.6	0.5 ± 0.0	1.0
	% Recovery	96.9	96.2	96.8	96.7 ± 0.4	102	96.0	101	100 ± 3.2	
Digoxin (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	0.1	0.1	0.1	0.1 ± 0.0	1.5	1.7	1.3	1.5 ± 0.2	19.5
	% Recovery	67.2	87.7	88.6	81.2 ± 12.1	81.6	77.5	91.1	83.4 ± 7.0	

*The concentrations of the test compound in the receiver side were below the lowest quantification limit (5 nM); the value of 5 nM was used to estimate the permeability and recovery values.
N.D.: Not determined. Because reliable P_{app} values were not obtained, the efflux ratio could not be determined.

Permeability and Recovery of Digoxin in Caco-2 Cells

Treatment	Parameter	A-B Direction				B-A Direction				Efflux Ratio
		R 1	R2	R3	Average ± SD	R1	R2	R3	Average ± SD	
Digoxin (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	0.9	0.9	0.9	0.9 ± 0.0	14.4	13.4	14.9	14.2 ± 0.8	16.0
	% Recovery	101	102	102	102 ± 0.7	103	99.3	114	106 ± 7.7	
Digoxin + 4-aminopyridine (100 μM)	P_{app} ($\times 10^{-6}$cm/s)	1.1	0.9	1.2	1.0 ± 0.2	16.6	19.1	21.9	19.2 ± 2.7	18.3
	% Recovery	88.4	94.5	100	94.3 ± 5.7	105	102	104	104 ± 1.3	
Digoxin + CsA (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	2.8*	3.2*	3.3	3.3	2.7	3.0	3.0	2.9 ± 0.2	0.9
	% Recovery	104*	120*	121	121	110	105	103	106 ± 4.1	
Digoxin + Ketoconazole (10 μM)	P_{app} ($\times 10^{-6}$cm/s)	1.3	2.3	2.3	2.0 ± 0.6	4.6	4.5	5.3	4.8 ± 0.4	2.5
	% Recovery	100	101	109	103 ± 4.7	107	102	100	103 ± 3.1	

*The cell monolayer failed post-experiment LY criteria; thus their values were excluded from average calculation.

CONCLUSIONS

4-aminopyridine is neither a substrate (upto70 μM tested) nor an inhibitor (upto100 μM tested) of P-gp.

New Drug Application Filing and Review Form

Office of Clinical Pharmacology and Biopharmaceutics New Drug Application Filing and Review Form			
General Information About the Submission			
NDA Number	**22-250**	**Brand Name**	**Fampridine**
OCPB Division (I, II, III)	**DCP-1**	**Generic Name**	**4-Amino Pyridine**
Medical Division	**HFD-120**	**Drug Class**	**Potassium Channel Blocker**
OCPB Reviewer	**Jagan Mohan Parepally**	**Indication(s)**	**Treatment of Walking Disability in Multiple Sclerosis**
OCPB Team Leader	**Veneeta Tandon**	**Dosage Form**	**Sustained Release Tablet**
Date of Submission	**01/30/2009**	**Dosage**	**10 mg b.i.d**
Estimated Due Date of OCP Review	**6/11/2009**	**Route of Administration**	**Oral**
PDUFA Due Date	**7/30/2009**	**Sponsor**	**Acorda Therapeutics, Inc.**
Division Due Date	**6/25/2009**	**Priority Classification**	**P**

Clin. Pharm. and Biopharm. Information

Summary: Fampridine is a potassium (K^+) channel blocker proposed as a treatment for walking disability in patients with Multiple Sclerosis (MS).

According to the sponsor the IR capsule formulation showed that fampridine has a narrow therapeutic index. The doses of fampridine required to maintain therapeutic plasma concentration were linked to serious adverse events (most notably a tonic-clonic seizure at a plasma level > 100 ng/mL). Therefore, Sustained release formulation was developed to reduce the peak and trough plasma concentration.

Two commercial drug product manufacturers are proposed, Elan Pharmaceuticals Plc, and (b) (4). Following categories along with the list of studies were included in the NDA submission. Fampridine SR 10 mg tablet is to-be-marketed product.

- Half life of Fampridine SR was 5.2-6.5 hrs,
- Plasma Protein Binding: most of the drug is unbound to plasma proteins (97-99%)
- Metabolites were inactive
- CYP 2E1 is the major enzyme responsible for 3-hydroxylation. Fampridine at 30 µM inhibits CYP 2E1(~12% inhibition) which is approximately 100 times the average plasma concentration measured for 10 mg dose.
- Most (90.3%) of the drug was excreted unchanged in the urine in the 0-4 hr period. Two metabolites were identified: 3-hydroxy-4-aminopyridine (4.3%) and 3-hydroxy-4-aminopyridine sulfate (2.6%).

Mass Balance: Urinary excretion of radiolabel was 95.85% after 24 hours. Recovery in feces was 0.51%. 3-hydroxy-4- aminopyridine, (13.7 to 20.3%) of extract radioactivity; 3-hydroxy-4-aminopyridine sulfate, (6.1 to 8.6%). Two minor unidentified metabolites were also present, one accounting for about 2% of extract radioactivity and the other below the limit of quantitation.

Population PK, pharmacokinetic-pharmacodynamic (PK/PD) and QTc studies (n=2)

- Study FSR_PKPD_2008-00 (PK-PD): Summarizes population PK and pharmacokinetic-pharmacodynamic (PK/PD) analyses conducted to screen for drug interactions (common concomitant drugs) and to identify any key covariate relationships among age, race (Caucasian vs. Non-Caucasian), and gender. The studies included in this analyses were FeFa25F-SR11203; RD10F-SR012004; AN751-101; AN751-102; 0195-001US; MS-F201; MSF202; MS-F203; and MS-F204.
- Study TQTc-F-SR001: QT study (10 and 30 mg bid, SR tablets)

Dose response/efficacy studies (n=5+3) FYI

- Studies MS-F201 (5, 10, 15, 20, 25, 30, 35 and 40 mg SR tablets), MS-F202 (10, 15, and 20mg SR tablets), MS-F203 (10 mg SR tablets) and MS-F204 (10 mg SR tablets)
- Study 0494-001US: Efficacy study to determine effective dose at neurological improvement occurred (SR tablets)
- Long term open-label extension studies MSF202EXT, MS-F203EXT and MS-F204EXT.

Pharmacokinetic Studies:

Dose Escalation in Healthy Subjects (n=1)

- Single Dose Study: ELA/G-9101 (10, 15, 20, 25 mg dose escalation, IR capsule)

Metabolism Studies (n=2)

- Study 0496-002: Radiolabeled mass-balance metabolism
- Study ELN 14-972-921: Urine metabolite identification

***In vivo* drug interaction studies (n=2)**

- Study 0194-002: Drug interaction study with Baclofen (15 mg (b) (4) capsule)
- Study 1194-001US: Drug interaction study with Betaseron (7.5 mg IR capsule)

Note: No link has been established between (b) (4) capsule and SR tablet. No PK interaction was seen on Fampridine plasma concentration.

Special Populations (n=6)

MS Patients

Single Dose Studies

- Study 1091-001 US (IR dose escalation, 2 ranges of target plasma concentrations)
- Study AN751-101: Dose proportionality (5, 10, 15 mg, SR tablets)
- Study 0893-001US: Food effect study (15 mg SR capsule)
- Study 0195-001US: PK, safety and tolerability study, Fampridine-SR tablet and SR capsule formulations, under fasting conditions, Study arms
  - 12.5 mg Fampridine-SR tablet
  - 12.5 mg Fampridine-SR capsule

Multiple Dose Studies

- Study AN751-102(20 mg SR tablets, bid, 13 days)
- Study 0195-001US: PK, safety and tolerability study, Fampridine-SR tablet and SR capsule formulations, under fasting conditions (Part II)

Renally Impaired Patients

Study RD10F-SR012004: Excretion of Fampridine and metabolites in adults with renal impairment (10 mg SR tablets): Decrease in clearance of 42.7% in mild, 50.3% in moderate, and 72.7% in severe. The terminal half-life is prolonged by 3.3-fold in severe renal impairment but not prolonged in mild or moderate impairment.

Bioequivalence studies with Fampridine (n=5)

- Study BE10.25F-SR10OS122003: Relative bioavailability study of Fampridine-SR tablets, under fasting conditions, Study arms
  - 10 mg Fampridine-SR tablets,
  - 25 mg Fampridine-SR tablets
  - 10 mg buffered aqueous oral Fampridine solution (0.83 mg/mL)
- Studies BE10F-SR022004 and BE25F-SR022004: Qualification of alternative drug product manufacturer for Fampridine-SR Tablet, under fasting conditions, Study arms
  - 10 mg Fampridine-SR tablets (manufactured by Elan)
  - 25 mg Fampridine-SR tablets (manufactured by (b) (4))
- Study 0494-006: relative bioavailability study. Food effect assessed in the third arm, Study arms
  - 12.5 mg immediate-release (IR) Fampridine capsule in a fasted state
  - 12.5 mg Fampridine-SR tablet in a fasted state
  - 12.5 mg Fampridine-SR tablet in a fed state
- Study 1194-002: Three-way crossover relative bioavailability study; all treatments were administered in a fasted state. Study arms
  - 12.5 mg Fampridine SR tablet
  - 12.5 mg Fampridine SR capsule
  - 12.5 mg Fampridine-IR capsule
- Study 0497-010: Effect of varying amounts of methocel on bioavailability (15 mg extended release tablet, 4 Elan formulations)

Food effect studies (n=6), 1 study to be reviewed

- Study FeFa25F-SR112003: Food effect study (high-fat breakfast *versus* fasted) of Fampridine-SR tablets, 25 mg conducted in healthy adult volunteers.
- Food effect was also addressed in studies 0494-006 (12.5 mg SR tablet), Study 0195-001US (SR tablet, 12.5 mg in MS patients)
- FeFa10F-SR-2008: Food effect study on 10 mg Fampridine-SR formulation (SR tablet). Study report not submitted as the Agency recommended that this study is not required.
- Study 0791-011: In healthy volunteers, food effect on 5, 7.5 mg IR capsule, standard breakfast and SD PK of two (b) (4) formulations under fasting conditions

- Study 0792-001: 10 and 20 mg (b) (4) capsule, high fat breakfast
- Study 0193-002: Food effect on (b) (4) capsules, 20 mg SD and 30, 40 mg MD

***In vitro* studies (n=5)**

- HWI 6379-103: *In vitro* protein binding study
- Study XT064039 (Metabolism)
- Study M-2001-029 (Inhibition): The extent to which 4-AP inhibits the activity of several CYP isozymes (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) was tested in hepatocytes.
- Study XT075077 (Inhibition): The extent that 4-AP inhibits the activity of predominant CYP isozymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4/5) was tested pooled microsomes.
- Study XT073070 (Induction): The extent that 4-AP induces the activity of predominant CYP isozymes (CYP1A2, 2B6, 2C9, 2C19, 2E1 and 3A4/5) was tested
- Study 8ACORP1(P-gp Study): Assessment of 4-Aminopyridine as substrate and inhibitor of P-glycoproteins in different cell systems.

Early formulation development studies (n=3) FYI

- Study 0492-004: *In vivo* comparison of 20 mg (b) (4) capsule (4) formulations
- Study 0593-005: *In vitro* and *in vivo* comparison of various 15 mg (b) (4) capsule formulations
- Study 0195-006: Effect of phosphate buffer (12.5 mg (b) (4) tablet with and without buffer)

Composition of Fampridine-SR formulations used in most of the clinical trials is presented in appendix 2.

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	3		
I. Clinical Pharmacology				
Mass balance:	X	1	1	Study 0496-002
Isozyme characterization:	X	-		
Blood/plasma ratio:	-	-	-	
Plasma protein binding:	X	1	1	HWI 6379-103

Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	1	-	Studies ELA/G-9101
multiple dose:	X	2	2	Studies 0492-004, 0193-002
Patients-				
single dose:	X	4	-	Studies 1293-001US, 1091-001US, 0893-001US (SD and MD study), 0195-001US
multiple dose:	X	2	-	Studies AN751-102, 0893-001US
Dose proportionality -				
fasting / non-fasting single dose:	X	1	1	Studies AN751-101
fasting / non-fasting multiple dose:	-	-	-	
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	2	1	Studies 0194-002, 1194-001US
In-vivo effects of primary drug:	X	-	-	
In-vitro:	X	5	5	Study XT064039, Study M-2001-029, Study XT075077, Study XT073070 and Study 8ACORP1
Subpopulation studies -				
ethnicity:	X	-	-	As a part of PopPK analysis
gender:	X	-	-	As a part of PopPK analysis
pediatrics:	-	-	-	
geriatrics:	X	-		As a part of PopPK analysis
renal impairment:	X	1	1	Study RD10F-SR012004
hepatic impairment:	-	-	-	
PD:				
Phase 1:	-	-	-	
Phase 3:	X	-	-	
PK/PD:				
Phase 1 and/or 2, proof of concept:	-	-	-	

Phase 3 clinical trial:	X	1+1	-	Studies FSR-PKPD-2008, QTc Study
Population Analyses -				
Data rich:	X	-	-	FSR-PKPD-2008
Data sparse:	-	-	-	
II. Biopharmaceutics				
Absolute bioavailability:	-	-	-	
Relative bioavailability -	X	-		
solution as reference:	X	1	1	Study BE10.25F-SR10OS122003
alternate formulation as reference:	X	1	1	Studies BE10F-SR022004 and BE25F-SR022004
Bioequivalence studies -				
traditional design; single / multi dose:	X	4	4	Study 0195-001US, Study 0494-006, Study 1194-002 and Study 0497-010
replicate design; single / multi dose:				
Food-drug interaction studies:	X	5	1	With Fampridine SR: Studies FeFa25F-SR112003, 0494-006, 0195-001US With Fampridine earlier formulations: 0893-001US, 0791-011, 0792-001, and 0193-002
Dissolution:	X	-	-	
(IVIVC):	X	-		Studies 0593-005, 0497-010
In vitro Alcohol Dose Dumping Analysis	X	1	-	
Bio-waiver request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:	-	-	-	
Chronopharmacokinetics	-	-	-	
Pediatric development plan	-	-	-	
Literature References		-	-	

Total Number of Studies		20 + 5 in vitro+ 1 PopPK + 1 QTc + 3 Assay	12 + 5 in vitro + 3 Assay	
Filability and QBR comments				
	"X" if yes	Comments		
Application filable?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?		
Comments sent to firm?		Attached		
QBR questions (key issues to be considered)	Is the Dose Selection for the Indication Appropriate? What Intrinsic Factors Affect Fampridine Exposure-Response? What Extrinsic Factors Affect Fampridine Exposure Response? What are the General Biopharmaceutic Characteristics of Fampridine Formulations? Are adequate links provided between formulations? What Analytical Methods were used in the Fampridine Development Program?			
Other comments or information not included above				
Primary reviewer Signature and Date				
Secondary reviewer Signature and Date				

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	X			Data sets missing
2	Has the applicant provided metabolism and drug-drug interaction information?	X			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?		X		Analytical reports missing in some studies
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?		X		See filing issues
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?		X		See filing issues
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?		X		No titles for studies in EDR. Analytical reports are not linked
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?		X		See filing issues
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	?			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?			X	
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	X			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	X			
15	Are the pediatric exclusivity studies adequately			X	

	designed to demonstrate effectiveness, if the drug is indeed effective?				
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	X			
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	?			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Clinical Pharmacologist Date

Team Leader/Supervisor Date

CC: NDA 22-250 HFD-850 (Electronic Entry), HFD-120, HFD-860 (Jagan Parepally, Veneeta Tandon, Ramana Uppoor, Mehul Mehta)

Appendix: Tabular listing of clinical studies

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
Clinical Trials in Non-Patients						
Open-Label Clinical Trials						
0593-005 1 Healthy Volunteers	Open-label, randomized, single-dose, 5-period, crossover 08-Nov-93 14-Dec-93	15 mg FAM-IR capsule 15 mg FAM-(b) (4) capsule Single oral dose	10	10 (100%), 0 34.0 yrs. (27, 40) 100% Caucasian	Completed	Module 5.3.1.3
0792-001 1 Healthy Volunteers	Open-label, single-dose, randomized, 4-way crossover 22-Feb-93 04-May-93	10 mg FAM-IR 20 mg FAM-(b) (4) Single oral dose	13	13 (100%), 0 29.7 yrs. (25, 33)	Completed	Module 5.3.1.1
0194-002 1 Healthy Volunteers	Open-label, single-dose, randomized, 3-way crossover 31-Mar-94 31-May-94	15 mg FAM-(b) (4) 10 mg Baclofen 15 mg FAM-(b) (4) and 10 mg Baclofen Single oral dose	12	13 (100%), 0 29.8 yrs. (18, 40)	Completed	Module 5.3.3.4
0195-006 1 Healthy Volunteers	Open-label, 2-way crossover 24-Feb-95 09-Mar-95	12.5 mg FAM-(b) (4) (with or without phosphate buffer) Single oral dose	4	4 (100%), 0 35.3 yrs. (16, 40) 100% Caucasian	Completed	Module 5.3.1.1

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
0496-002 1 Healthy Volunteers	Open-label, single-dose 20-Jun-96 23-Jul-96	15 mg ^{14}C FAM solution Single oral dose	4	4 (100%), 0 20.7 yrs. (18, 22) 100% Caucasian	Completed	Module 5.3.3.1
1194-002 1 Healthy Volunteers	Open-label, randomized, 3-way crossover 31-Mar-94 31-May-94	12.5 mg FAM-SR tablet 12.5 mg FAM-SR capsule 12.5 mg FAM-IR capsule Single oral dose	12	12 (100%), 0 27.0 yrs. (23, 35) 100% Caucasian	Completed	Module 5.3.4.1
BE10-25F-SR10OS122003 1 Healthy Volunteers	Open-label, single-dose, randomized, 3-way crossover 04-Dec-03 20-Dec-03	10 mg FAM-SR tablet 25 mg FAM-SR tablet 10 mg FAM solution Single oral dose	30	17 (57%), 13 (43%) 25.4 yrs. (19, 42) 83.3% Caucasian, 6.6% Black, 3.3% European/ Middle Eastern, 3.3% Hispanic, 3.3% American Indian	Completed	Module 5.3.1.2
FeFa25F-SR112003 1 Healthy Volunteers	Open-label, single-dose, 2-way crossover 06-Nov-03 15-Nov-03	25 mg FAM-SR tablets Single oral dose	14	5 (36%), 9 (64%) 34.0 yrs. (26, 5) 78.6% Caucasian, 14.3% Asian, 7.1% Black	Completed	Module 5.3.1.2
RD10F SR012004 3 15 Patients with Renal Impairment 5 Healthy Volunteers	Open-label, single-dose, non-randomized, 2-stage, parallel group 10-Feb-04 28-Jul-04	10 mg FAM-SR tablets Single oral dose	20	9 (45%), 11 (55%) 60.5 yrs. (19, 80) 75.0% Caucasian, 25% Black	Completed	Module 5.3.3.3

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
BE25F-SRO22004 1 Healthy Volunteers	Open-label, single-dose, randomized, 2-way crossover 05-Mar-04 14-Mar-04	25 mg FAM-SR tablets (from 2 manufacturers, (b) (4) and Elan Corporation) Single oral dose	22	10 (45%), 12 (55%) 24.2 yrs. (19, 38) 95.5% Caucasian, 4.5% Black	Completed	Module 5.3.1.2
BE10F-SR22004 1 Healthy Volunteers	Open-label, single-dose, randomized, 2-way crossover 16-Mar-04 25-Mar-04	10 mg FAM-SR tablets (from 2 manufacturers, (b) (4) and Elan Corporation) Single oral dose	18	10 (56%), 8 (44%) 29.4 yrs. (20, 45) 100% Caucasian	Completed	Module 5.3.1.2
Blinded Clinical Trials						
0492-004 1 Healthy Volunteers	Single-blind, randomized, multiple-dose, 4-period, crossover 17-Jun-92 04-Aug-92	20 mg FAM-(b) (4) Oral b.i.d.	14	14 (100%), 0 28.8 yrs. (21, 47) 100% Caucasian	Completed	Module 5.3.4.1
TQTc-F-SR001 1 Healthy Volunteers	Double-blind, placebo-controlled, randomized, double-dummy, parallel group 17-Oct-07 03-Dec-07	10 mg FAM-SR Oral q12h 30 mg FAM-SR Oral q12h Placebo Oral q12h 400 mg Moxifloxacin/ Placebo Oral q12h	208	113 (54%), 95 (46%) 25.0 yrs. (18, 44) 5% American Indian/Alaskan Native, 1% American Indian/Alaskan Native, White, 2% Asian, 5% Black, 87% Caucasian	Completed	Module 5.3.4.1

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
Clinical Trials in MS Patients						
Open-label Clinical Trials						
0893-001US 1 MS Patients	Open-label, single-dose, 3-way crossover Mar-93 Dec-93	7.5 and 15 mg FAM-SR capsules Single oral dose	12	6 (50%), 6 (50%) 50.4 yrs. (41,64) 100% Caucasian	Completed	Module 5.3.3.2
1194-001US 1 MS Patients	Open-label, single, and multiple dosing 13-Jan-95 25-Mar-95	7.5 mg FAM-IR capsules Oral 8M units Betaseron IM	12	4 (33%), 8 (67%) 43.8 yrs. (38, 55) 8% Native American, 92% Caucasian	Completed	Module 5.3.3.4
0195-001US 1 MS Patients	Open-label, single-dose, 3-way crossover Mar-95 Apr-95	12.5 mg FAM-SR tablets Oral 12.5 mg FAM-SR capsules Single oral dose	13	7 (54%), 6 (46%) 47.5 yrs. (30, 60) 8% Asian, 92% Caucasian	Completed	Module 5.3.3.2
AN751-101 2 MS Patients	Open-label, single-escalating doses 21-Oct-97 21-Jan-98	5 mg FAM-SR 10 mg FAM-SR 15 mg FAM-SR Single oral dose	24	10 (42%), 14 (58%) 45.4 yrs. (29, 56) 100% Caucasian	Completed	Module 5.3.3.2
SCI-101 1 SCI Patients	Open-label, escalating dose 24-Mar-98 19-Jun-98	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR 25 mg FAM-SR Single oral dose	14	9 (64%), 5 (36%) 38 yrs. (17,61) 85.7% Caucasian, 7% Black, 7% Other	Completed	Module 5.3.5.4

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
1293-001US 1 MS Patients	Open-label, dose escalation 07-Feb-94 13-Apr-94	7.5 mg FAM-SR Oral b.i.d., increased by 2.5 mg b.i.d. weekly	12	6 (50%), 6 (50%) 51.3 yrs. (41, 65) 100% Caucasian	Completed	Module 5.3.3.2
0296-003US 1 MS Patients	Open-label study 14-May-96 27-Jul-98	12.5 mg FAM-SR tablet 17.5 mg FAM-SR tablet 22.5 mg FAM-SR tablet Oral b.i.d.	20	10 (50%), 10 (50%) 52.0 yrs. (31, 67)	Completed	Module 5.3.3.2
AN751-102 2 MS Patients	Open-label, multi-center, single treatment period multiple dose study 25-Nov-97 10-Feb-98	20 mg FAM-SR Oral b.i.d. for 13 consecutive days, with a single administration on Day 14	21	10 (48%), 11 (52%) 45.1 yrs. (29, 57) 100% Caucasian	Completed	Module 5.3.3.2
SCI-102 1 SCI Patients	Open-label, escalating dose 24-Mar-98 10-Jul-98	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR 25 mg FAM-SR Oral b.i.d.	16	14 (88%), 2 (12%) 40 yrs. (22, 59) 100% Caucasian	Completed	Module 5.3.5.4

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
SCI-103 1 SCI Patients	Open-label, escalating dose 26-Feb-99 30-Jul-99	5 mg FAM-SR 20 mg FAM-SR 25 mg FAM-SR 30 mg FAM-SR 35 mg FAM-SR 40 mg FAM-SR b.i.d.	16	13 (81%), 3 (19%) 35.7 yrs. (18, 60) 100% Caucasian	Completed	Module 5.3.5.4
Blinded Clinical Trials						
0995-001US 1 MS Patients	Double-blind, placebo-controlled, crossover Sep-95 Nov-95	17.5 mg FAM-SR tablets Placebo Oral doses administered b.i.d. for 1 week/treatment	10	4 (40%), 6 (60%) 48.4 yrs. (33, 57)	Completed	Module 5.3.4.2
MS-F200 1 MS Patients	Double-blind, randomized, placebo-controlled, 4-way, single-ascending dose, crossover 11-Oct-99 11-May-00	5 mg FAM-SR 15 mg FAM-SR 25 mg FAM-SR Placebo Single oral dose	24	8 (33%), 16 (67%) 46.2 yrs. (23, 56) 92% Caucasian, 2% Other	Completed	Module 5.3.1.2
MS-F201 4 MS Patients	Double-blind, randomized, placebo-controlled 08-Nov-00 18-Sep-01	5, 10, 15, 20, 25, 30, 35, and 40 mg FAM-SR Placebo Oral doses administered b.i.d. for 8 weeks	36	17 (47%), 19 (53%) 47 yrs. (30, 61) 97.2% Caucasian 2.7% Black	Completed	Module 5.3.5.1

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
MS-F202 24 MS Patients (Adequate/well-controlled study)	Double-blind, placebo-controlled, 20-week, parallel group 27-Feb-03 18-Dec-03	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR Placebo Oral doses administered b.i.d. for 15 weeks	206	75 (36%), 131 (64%) 49.8 yrs. (28, 69) 92.2% Caucasian 4.9% Black 1.5% Hispanic 1% Other 0.5% Asian/Pacific Islander	Completed	Module 5.3.5.1
SCI-001 1 SCI Patients	Double-blind, randomized, placebo-controlled 05-Nov-96 23-Dec-96	5 mg FAM-IR Placebo Oral doses administered b.i.d. for 7 days	4	4 (100%), 0 44 yrs. (28, 52)	Terminated early by sponsor	Module 5.3.5.4
0293-001US 1 MS Patients (Study terminated prior to planned crossover)	Double-blind, placebo-controlled, 3-period crossover comparison of slow-release fampridine 30 and 40 mg in MS patients 12-May-93 25-Jun-93	30 mg FAM-SR 40 mg FAM-SR Placebo Oral doses administered b.i.d. for 10 days	9	4 (44%), 5 (56%) 50.8 yrs. (41, 58) 100% Caucasian	Terminated early by sponsor	Module 5.3.1.1
0494-001US 9 MS Patients	Double-blind, randomized, placebo-controlled, parallel group 12-Sep-94 16-Dec-94	12.5, 17.5, and 22.5 mg FAM-SR Placebo Oral doses administered b.i.d. for 6 weeks	161	70 (43%), 91 (57%) 48.9 yrs. (29, 74) 3.1% African-American, 95% Caucasian, 1.8% Other	Completed	Module 5.3.4.2

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
MS-F203 33 MS Patients (Adequate/well-controlled study)	Double-blind, randomized, placebo-controlled 07-Jun-05 28-Jun-06	10 mg FAM-SR Placebo Oral doses administered b.i.d. for 14 weeks	300	95 (32%), 205 (68%) 51.4 yrs. (26, 70) 92.9% Caucasian 7.1% Non-Caucasian	Completed	Module 5.3.5.1
MS-F204 35 MS Patients (Adequate/well-controlled study)	Double-blind, randomized, placebo-controlled 22-May-07 27-Feb-08	10 mg FAM-SR Placebo Oral doses administered b.i.d. for 9 weeks	239	77 (32%), 162 (68%) 51.7 yrs. (24, 73) 91.2% Caucasian, 5.0% African-American, 1.7% Hispanic, 1.7% Other, 04% American Indian/Alaskan Native	Completed	Module 5.3.5.1
0295-001US 2 SCI Patients	Double-blind, randomized, placebo-controlled, dose titration, 3-way crossover in chronic SCI patients Sep-95 Jan-96	12.5 mg FAM-SR 17.5 mg FAM-SR Placebo Oral b.i.d. for 1 week	29	28 (97%), 1 (3%) 40.4 yrs. (23, 63) 90% Caucasian, 7% Hispanic, 3% African-American	Completed	Module 5.3.5.4
SCI-200 6 SCI Patients	Double-blind, placebo-controlled, escalating dose, crossover in SCI patients 23-Oct-97 28-Apr-98	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR Oral b.i.d. for 1 week	59	49 (82%), 11 (18%) 38.9 yrs. (20, 64) 82% Caucasian	Completed	Module 5.3.5.4

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
SCI-F201 10 SCI Patients	Double-blind, placebo-controlled, parallel group in chronic incomplete SCI patients 27-Jun-00 06-Mar-01	25 mg FAM-SR 40 mg FAM-SR Placebo Oral b.i.d.	91	72 (79%), 19 (21%) 41.5 yrs. (19, 67) 92% Caucasian	Completed	Module 5.3.5.4
SCI-F203 1 SCI Patients	Double-blind, placebo-controlled, parallel group in chronic complete and incomplete SCI patients 04-Jun-02 29-Oct-03	15 mg FAM-SR 20 mg FAM-SR 25 mg FAM-SR Oral b.i.d.	9	5 (56%), 4 (44%) 46.0 yrs. (30, 73) 100% Caucasian	Completed	Module 5.3.5.4
SCI-F301 51 SCI Patients	Double-blind, placebo-controlled, fixed dose, 2-way crossover 8-Jul-02 16-Feb-04	25 mg FAM-SR Placebo Oral b.i.d.	212	185 (87%), 27 (13%) 40.9 yrs. (17, 70) 82.5% Caucasian	Completed	Module 5.3.5.4
SCI-F302 33 SCI Patients	Double-blind, placebo-controlled, parallel group study in chronic SCI patients 11-Jun-02 14-Nov-03	25 mg FAM-SR Placebo Oral b.i.d.	203	172 (85%), 31 (15%) 40.9 yrs. (18, 73) 88.2% Caucasian	Completed	Module 5.3.5.4

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
Extension Studies						
1293-001US EXT 1 MS Patients	Open-label extension study 14-Mar-94 15-May-96	10-22.5 mg FAM-SR Oral b.i.d., switched from capsule to tablet during extension phase	12	6 (50%), 6 (50%) 51.3 yrs. (41, 65) 100% Caucasian	Completed	Module 5.3.5.2
0195-001US EXT 1 MS Patients	Open-label extension study 15-Jun-95 22-May-96	12.5 mg FAM-SR tablets Oral b.i.d.	12	7 (58%), 5 (42%) 48.9 yrs. (31, 61) 92% Caucasian, 8% Asian	Completed	Module 5.3.5.2
MS-F202 EXT 21 MS Patients	Open-label extension study 15-Mar-04 (ongoing)	10 mg FAM-SR tablet 15 mg FAM-SR tablet 20 mg FAM-SR tablet Oral	177	66 (37%), 111 (63%) 51.9 yrs. (29, 70) 96.6% Caucasian 1.1% Black 2.3% Hispanic	Ongoing	Module 5.3.5.2
MS-F203 EXT 33 MS Patients	Open-label extension study 13-Dec-05 (ongoing)	10 mg FAM-SR tablet Oral b.i.d.	267	87 (33%), 180 (67%) 52.0 yrs. (26, 71) 93.3% Caucasian 4.1% Black 1.1% Hispanic 1.1% Asian/Pacific Islander 0.4% Other	Ongoing	Module 5.3.5.2

Study ID No. of Centers Population	Design Start/End Dates	Planned Doses Route Regimen	No. of Patients Receiving at Least One Dose	Demographics Male, Female Mean Age (min, max) Race[1]	Study Status	Location
MS-F204 EXT 38 MS Patients	Open-label extension study 27-Aug-07 (ongoing)	10 mg FAM-SR tablet Oral b.i.d. Placebo	239	77 (32%), 162 (68%) 51.7 yrs. (24, 73) 91.2% Caucasian 5.0% Black 1.7% Hispanic 0.4% American Indian or Alaskan 1.7% Other	Ongoing	Module 5.3.5.2
SCI-F201 EXT 12 SCI Patients	Open-label, multicenter, extension study 04-Jun-02 07-Jul-04	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR 25 mg FAM-SR 30 mg FAM-SR 35 mg FAM-SR 40 mg FAM-SR Oral b.i.d.	132	111 (84%), 21 (16%) 42 yrs. (19,68) 93.2% Caucasian 3.8% Black 3% Other	Terminated early by sponsor	Module 5.3.5.2
SCI-F300 EXT 40 SCI Patients	Open-label, multicenter, extension study 03-Oct-03 25-Jun-04	10 mg FAM-SR 15 mg FAM-SR 20 mg FAM-SR 25 mg FAM-SR Oral b.i.d.	230	201 (87%), 29 (13%) 42 yrs. (19,74) 82.2% Caucasian 15.2% Black 0.9% American Indian/Alaskan Native 1.7% Other	Terminated early by sponsor	Module 5.3.5.2

1 Race only listed when provided in the demographic data.
b.i.d. = Twice daily; (b) (4) EXT = Extension; FAM = Fampridine; IM=Intramuscular; IR = Immediate Release; Max = Maximum; Min = Minimum; MS = Multiple sclerosis; No. = Number; SCI = Spinal cord injury; SR = Sustained-release; yrs. = Years.
Data from Integrated Summary of Safety Table 1.1.

Appendix 2: Composition of Fampridine SR formulations

Table 3: Composition of Fampridine-SR Tablets Used in MS Clinical Trials

Ingredient	Fampridine-SR Tablet Strengths			
	5 mg	**10 mg**	**12.5 mg**	**25 mg**
Fampridine	5 mg	10.0 mg	12.5 mg	25.0 mg
Hydroxypropyl Methylcellulose, USP	(b) (4)			
Microcrystalline Cellulose, USP				
Colloidal Silicon Dioxide, NF				
Magnesium Stearate, USP				
(b) (4) Film-coat				
Total Weight	410.0 mg	410.0 mg	410.0 mg	410.0 mg

4.2 PHARMACOMETRICS REVIEW

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Is there any significant covariate which affects Fampridine PK?

Yes, age, sex and the level of renal impairment were found to be significant factors for fampridine clearance (CL, L/hr). Especially the changes in fampridine CL were directly proportional to observed changes in creatinine clearance (CrCL). Hence, dose adjustment for the renal impaired patients is recommended.

A one-compartment system with a first order absorption, first order elimination, and lag time was employed to describe the pharmacokinetics of fampridine. The impacts of age, sex, race (Caucasian vs. Non-Caucasian), body mass index (BMI), level of renal impairment, the most commonly used concomitant medications (Bacolfen,Interferon beta, renal transport inhibitor, Tizanidine, Glatiramer Acetate), food intake (fasted vs. fed), study, and subject status (healthy volunteers vs. MS patients) on the population PK model were investigated.

Age and sex were identified as significant covariates for fampridine CL. Fampridine clearance was approximately 14.5% lower for females (36 L/hr) at the same age and CrCL and it decreases with increasing age (49L/hr→39L/hr over 20 years to 80 years). Across a CrCL range of 69 to 149 mL/min (10th to 90th percentile in the patients), the range of model predicted maximal plasma concentration at steady state is from 22.4 to 30.2 ng/mL and the range of mean predicted plasma concentration at steady state varies from 16.5 to 22.4 ng/mL when 10 mg bid dose is administered.

Also, the volume of distribution was found to be significantly lower in females as compared to males and the absorption rate constant was found to be significantly lower when the drug was given with food. However, body mass index, race, subject status, and the most commonly used concomitant medications did not appear to affect the pharmacokinetics of fampridine.

1.1.2 Is there any significant exposure-response relationship? And does the relationship support the proposed dose (10mg bid)?

Yes, there is a significant exposure (AUC (ug·hr/L))-response(the percent change from the baseline at the end of double blind phase in walking speed (ft/sec)) relationship when the placebo group was included in the analysis (reviewer's analysis). However, the relationship is flat without placebo within the observed exposure range.

A phase II study (MS-F202) and two phase III studies (MS-F203, MS-F204) were included in the sponsor's exposure-response analyses for both efficacy and safety.

"Timed Walk Responder" was the primary efficacy endpoint, which was defined as a patient with a faster walking speed on timed 25-foot walk test for at least three of the four visits during the double-blind treatment period, as compared to the maximum walking speed for any of the four pre-treatment visits and the first posttreatment visit. Based on the primary endpoint, fampridine showed superior effectiveness (37.3% 10mg bid.vs. 8.9% placebo for pooled data) in the efficacy analysis and the sponsor's exposure-response analysis also predicted similar results. However, a patient who may have no drug effect at the last visit of double blind treatment phase could be declared as a responder by definition of "timed walk responder". In addition, it is not possible to evaluate how much improvement a patient achieved in actual walking speed by the primary endpoint.

Hence, the reviewer performed the independent analysis using the percent change in walking speed from the baseline at the end of double blind phase as an endpoint.

Figure 8 presents the results from pooled data analysis. First the linear model was applied to the pooled data and linear regression showed the statistically significant relationship between AUC (ug·hr/L) and the endpoint (p-value=0.015). The same model was fitted to the data after excluding placebo data and results are shown in the right panel in the Figure 8. The flat relationship without placebo suggests that the response reached the plateau at 10mg bid and a lower dose may be as efficacious as 10 mg bid. This observation is supported by the similar responder rate at each dose group (Table 2).

The safety of 10mg bid was evaluated using the probability of having at least one CNS-related AE as an endpoint. The sponsor claimed that CNS-related AE incidence was flat up to an AUC of 200 ug·hr/L and 10 mg bid should be safe based on the model prediction.

In order to further evaluate the safety of 10 mg bid compared to placebo, the reviewer focused on the patients who were assigned to 10 mg bid or placebo. Figure 10 shows the observed CNS-related AE incidence at the octile of AUC at the dose of 10 mg bid and placebo. As observed in the sponsor's analysis, the incidence rate appeared to be similar to placebo up to the AUC of 200 ug·hr/L and showed increasing trend for the range of AUC > 200 ug·hr/L with a relatively flat but higher rate within 220 ug·hr/L and 350 ug·hr/L.

Overall, fampridine seems to show the effectiveness based on both primary endpoint (responder analysis) and the reviewer's endpoint (percent change from the baseline in walking speed (ft/sec)). However, the exposure-response relationship is flat within the studied exposure range and for most of the exposure range under 10 mg bid, the incidence rate of CNS-related AE is higher than that for the placebo group. Hence, a lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

1.2 Recommendations

The Division of Pharmacometrics has reviewed the submission (NDA 22250) and has the following recommendation:

- A lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

2 PERTINENT REGULATORY BACKGROUND

The sponsor is seeking the approval for fampridine-SR (Amaya™) for the treatment of patients with multiple sclerosis (MS) for the improvement of walking ability**.** Fampridine has advanced through Phase 3 development, following the results from earlier Phase 2 trials, MS-F202. Based on the analysis performed on MS-F202, a Timed Walk Responders analysis was developed as the primary endpoint in the two subsequent Phase 3 trials, MS-F203 and MS-F204. The response criterion was based on the difference in the percent of responders in walking speed on the Timed 25-Foot Walk (T25FW), a standard MS assessment. Timed Walked Response was the primary efficacy variable in the two prospective protocols (MS-F203 and MS-F204) under Special Protocol Assessments (SPAs) with FDA. The same analysis methodology was applied retrospectively to a previous dose comparison study of similar design (MS-F202).

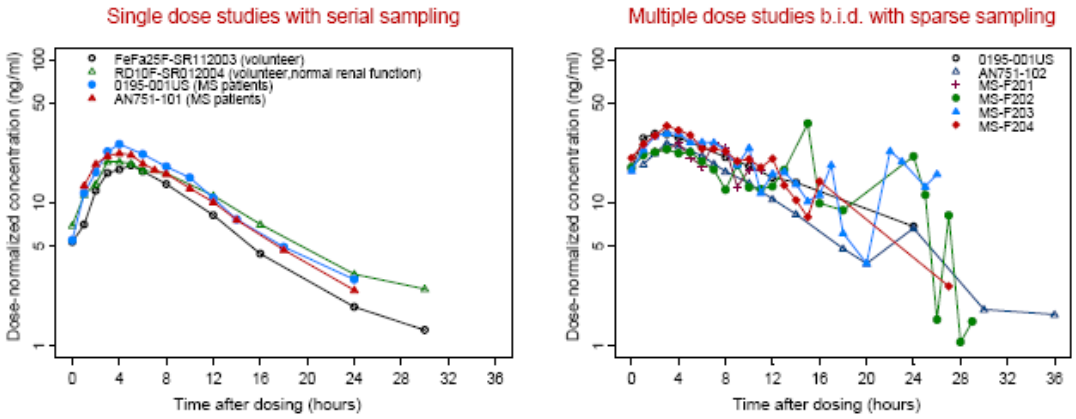
3 RESULTS OF SPONSOR'S ANALYSIS

The sponsor conducted population PK and exposure-response analyses for both efficacy and safety to address the effects of covariates on PK and to evaluate the relationship between exposure and efficacy / safety.

PK analysis

There are five Phase I studies (FeFa25F-SR11203, RD10F-SR012004, AN751-101, AN751-102,0195-001US), two Phase 2 studies (MS-F201, MS-F202), and two Phase 3 studies (MS-F203, MS-F204) included in the population PK analysis.
The pharmacokinetics of fampridine in plasma were evaluated in 19 healthy volunteers from two studies, 15 renal impaired volunteers from one study, and 580 patients with multiple sclerosis from seven studies. A one-compartment system with a first order absorption, first order elimination, and lag time was employed to describe the pharmacokinetics of fampridine. Figure 1 displays the PK profile of fampridine from the single dose studies and multiple dose studies.

Figure 1. The PK profiles of fampridine from single dose studies (left panel) and multiple dose studies (right panel) in healthy volunteers and MS patients normalized to 10 mg dose.



Source: the sponsor's report : A Population Pharmacokinetic and Pharmacokinetic-Pharmacodynamic Analysis of Fampridine-SR Tablets in Healthy Volunteers and Multiple Sclerosis Patients, page15.

The impacts of age, sex, race (Caucasian vs. Non-Caucasian), body mass index (BMI), level of renal impairment, the most commonly used concomitant medications (Bacolfen,Interferon beta, renal transport inhibitor, Tizanidine, Glatiramer Acetate), food intake (fasted vs. fed), study, and subject status (healthy volunteers vs. MS patients) on the population PK model were investigated.

The population median estimated clearance was 42 L/hr for a male patient with an age of 50 years, and creatinine clearance (CrCL) of 105 mL/min. Age and sex were identified as significant covariates for fampridine clearance. Fampridine clearance was approximately 14.5% lower for females (36 L/hr) at the same age and CrCL and it decreases with increasing age (49L/hr→39L/hr over 20 years to 80 years).

Figure 2. The individual predicted CL .vs. significant covariates. The right panel: CrCL, the middle panel: age, the left panel: gender.

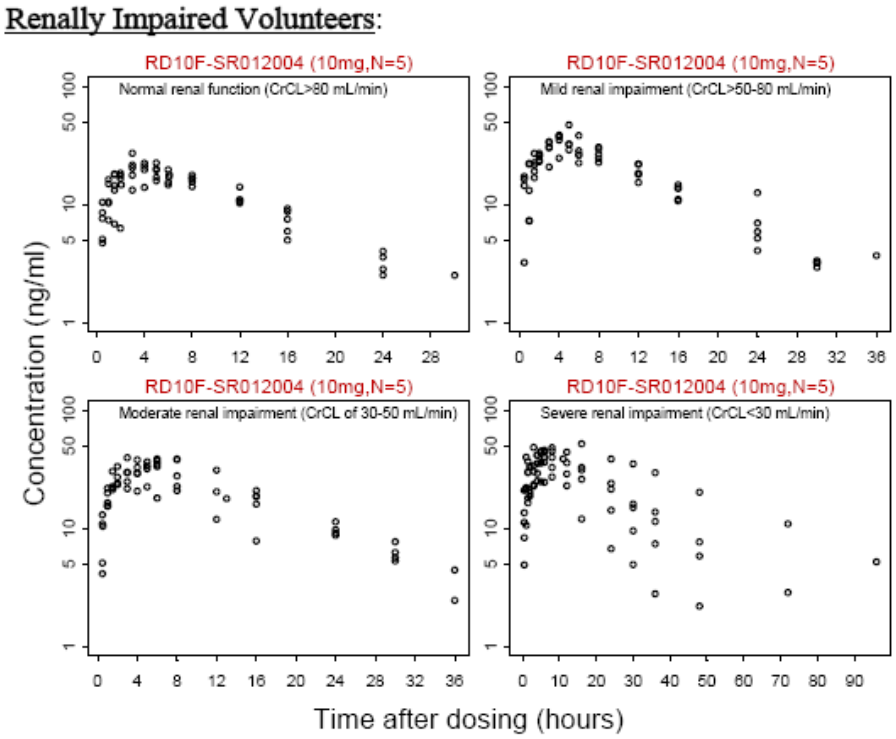
(b) (4)

Source: the sponsor's report : A Population Pharmacokinetic and Pharmacokinetic-

Pharmacodynamic Analysis of Fampridine-SR Tablets in Healthy Volunteers and Multiple Sclerosis Patients, page 44-45.

Also the changes in fampridine clearance were directly proportional to observed changes in creatinine clearance. Across a CrCL range of 69 to 149 mL/min (10th to 90th percentile in the patients), the range of model predicted maximal plasma concentration at steady state is from 22.4 to 30.2 ng/mL and the range of mean predicted plasma concentration at steady state varies from 16.5 to 22.4 ng/mL when 10 mg bid dose is administered.

Figure 3. Fampridine plasma concentration-time profile in study RD10F-SR012004 as a function of renal impairment (creatinine clearance): upper left panel: normal; upper right panel: mild impairment; lower left panel: moderate impairment; lower right panel: severe impairment.



Source: the sponsor's report : A Population Pharmacokinetic and Pharmacokinetic-Pharmacodynamic Analysis of Fampridine-SR Tablets in Healthy Volunteers and Multiple Sclerosis Patients, page 33.

The typical volume of the central compartment in males was estimated to be 403 L (a male adult, 50 years, CrCl=105 mL/min). The corresponding central compartment volume in females was approximately 24.7% lower (304 L).

The absorption rate constant was found to be significantly lower when the drug was given with food.

However, body mass index, race, subject status, and the most commonly used concomitant medications do not appear to affect the pharmacokinetics(see Appendix) .

Reviewer's comments:
Sponsor's population PK analysis is acceptable.

Exposure-response analyses

A phase II study (MS-F202) and two phase III studies (MS-F203, MS-F204) were included in the sponsor's exposure-response analyses for both efficacy and safety.

In the study of MS-F202, the doses of 10mg bid, 15mg bid and 20mg bid with placebo were tested whereas two pivotal studies (MS-F203, MS-F204) included 10mg bid only. Table 1 summarizes the brief overview of three studies. Refer to Appendix 1 for the details.

Table 1. Overview of the pivotal placebo-controlled studies and supportive phase II study in patients with multiple sclerosis.

Study No.	N	Dose	Design
MS-F202	47	placebo	Double-blind, placebo-controlled, 20-week dose comparison study (1 week post-screening; 2-week single blind, placebo lead-in; 2-week dose escalation; 12-week fixed dose treatment period; 1-week down-titration; 2-week off-treatment follow-up visit)
	52	10 mg b.i.d.	
	50	15 mg b.i.d.	
	57	20 mg b.i.d.	
MS-F203	72	placebo	Double-blind, placebo-controlled, multicenter study, 21-week study(1 week post-screening; 2-week single blind, placebo lead-in; 14-week fixed dose treatment period; 4-week off-treatment follow-up visit)
	229	10 mg b.i.d.	
MS-F204	119	placebo	Double-blind, placebo-controlled, multicenter, 15-week study (1 week post-screening; 2-week single blind, placebo lead-in; 9-week fixed dose treatment period; 2-week off-treatment follow-up visit)
	120	10 mg b.i.d.	

Source: the sponsor's report: A clinical-overview, page 14.

A "Timed Walk Responder" was a primary endpoint, which was defined as a patient with a faster walking speed on this test for at least three of the four visits during the double-blind treatment period, as compared to the maximum walking speed for any of the four pre-treatment visits and the first post-treatment visit.

As shown in Table 2, fampridine achieved significant more responders compared to placebo based on the primary endpoint across the studies.

Table 2. Summary of Timed Walk Response for Studies MS-F202, MS-F203 and MS-F204 (ITT Population)

Characteristics	MS-F202	MS-F203	MS-F204
No. of subjects	205	296	237
Responder (N/Y)	143/62	212/84	175/62
Sample size by dose group (0/10/15/20 mg b.i.d.)	47/51/50/57	72/224/n.a./n.a.	118/119/n.a/n.a
Responder by dose group (0/10/15/20 mg b.i.d.)	4/18/18/22	6/78/n.a./n.a.	11/51/n.a/n.a
Response frequency by dose group (0/10/15/20 mg b.i.d.) (%)	8.5/35.3/36.0/38.6	8.3/34.8/n.a./n.a	9.3/42.9/n.a/n.a

Source: the sponsor's report : A Population Pharmacokinetic and Pharmacokinetic-Pharmacodynamic Analysis of Fampridine-SR Tablets in Healthy Volunteers and Multiple Sclerosis Patients, page 20.

The probability of Timed Walk Response was used to build PK/PD model for efficacy and the total adverse events and all CNS AE data were used to build PK/PD models for safety. The area under the plasma concentration versus time curve (AUC) was used as the PK parameter in the exposure-response models for both efficacy and safety.

The probability of a MS patient becoming a Timed Walk Responder was described by a logistic regression model as follows;

$$Logit(P(responder)) = E_0 + Emax\frac{PAR}{(PAR + EC_{50})}$$

where P(responder) represents the probability of timed walk responder, E_0 is the placebo response; PAR is AUC (ug·hr/L), E_{max} is the maximal drug effect, and EC_{50} is the AUC to achieve 50% of E_{max}.

The effect of fampridine plasma concentrations on AEs was best described by a linear model as follows;

Model for all AEs: $Logit(P(AE)) = E_0 + slope \times PAR$

Model for CNS AEs: $Logit(P(AE)) = E_0 + slope \times PAR(Dose >= 10 \ or \ AUC >= 200)$

where

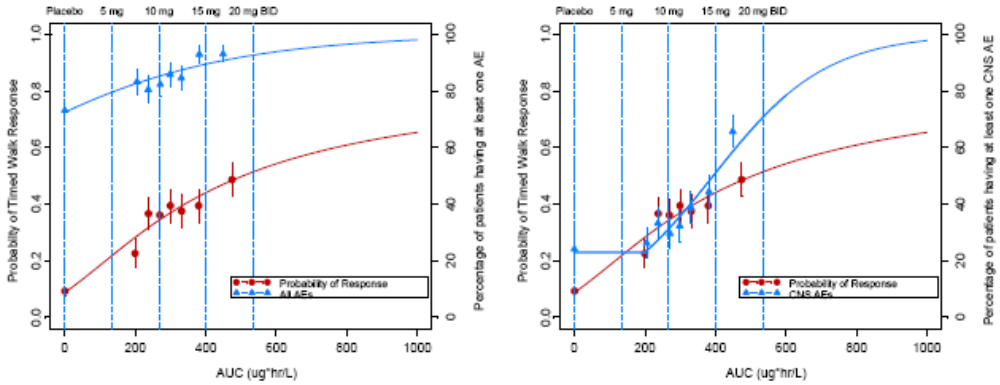
- $P(AE)$ represents the probability of a patient with at least 1 AE
- E_0 is the placebo response
- PAR is dose in mg or the AUC in μg*hr/L
- E_{max} is the maximal drug effect
- E_{50} is dose or AUC to achieve 50% of E_{max}
- k is study

Figure 4 presents the results from the sponsor's exposure-response analyses for both timed walk responder and safety. The sponsor's AUC-timed walk responder model suggests that 10 mg, 15 mg, 20 mg bid will achieve 25.5%, 35.3%, and 42.6% more responders than placebo, respectively (or 34.3%, 44.1%, and 51.4% for fampridine treatment groups, respectively vs. 8.8% for placebo), which slightly under-predicts the observed rate at the dose of 10mg bid and clearly over-predicts at the doses of 15mg and 20mg bid (see Table 2).

The sponsor's safety analysis predicts that the proportion of patients with at least one AE increased from 73% with placebo to approximately 92% in the 20 mg bid dose group and the incidence of CNS-related AEs was similar between placebo and fampridine treatment regimens of 5 mg and 10 mg bid. However, there was a linear dose dependent increase in CNS-related AEs between the 10 mg and 20 mg bid doses.

The sponsor claimed that the exposure-response analysis for efficacy and safety strongly supports selection of the fampridine 10 mg bid dosing regimen in the MS patient population for the balance that it provides between therapeutic benefit and AE profile.

Figure 4. Exposure-response relationship for the probability of Timed Walk Response and all AEs (left) and CNS AEs (right). The symbols are the observations and the error bars are SE[sqrt(P*(1-P)/N)] for quantiles (at 12.5 percentiles) of AUC (plotted at the median value within each quantile). The lines are the model predictions.



Source: the sponsor's report : A Population Pharmacokinetic and Pharmacokinetic-Pharmacodynamic Analysis of Fampridine-SR Tablets in Healthy Volunteers and Multiple Sclerosis Patients, page 63.

Reviewer's comments:

- *The sponsor over-predicts the response rate at the doses of 15mg and 20mg bid (predicted rates: 44% and 51% .vs. observed rates: 36% and 39% at the doses of 15mg and 20mg, respectively).*
    - *The sponsor used the predicted median AUC (ug·hr/L) which was computed based on typical CL to predict the response rates at each dose. However, the predicted median AUC (ug·hr/L) would not be appropriate to represent the dose group when the distribution of individual AUC, computed based on individual CL, does not show the dose-proportionality as seen in Figure 6.*
        - *The non-proportionality could be due to unbalanced CrCL (mL/min) between dose groups (Figure 5).*

4 REVIEWER’S ANALYSIS

4.1 Introduction

The sponsor evaluated the effectiveness of fampridine based on the primary endpoint, timed walk responder. However, a patient who may have no drug effect at the last visit of double blind treatment phase could be declared as a responder by the definition of “timed walk responder”. In addition, it is not possible to see how much improvement a patient achieved in actual walking speed by the current endpoint.

Hence, it would be worthwhile to explore the exposure-response relationship based on the actual continuous scale such as the percent change in walking speed from the baseline at the end of double blind phase.

Also the sponsor claimed that CNS related AE incidence did not show increasing trend between placebo and 10mg bid. However, the reviewer observed meaningful difference in CNS related to AE between placebo and 10mg bid (12% .vs. 30%) in one of pivotal study (MS-F204) so the safety of 10mg bid was further examined by the reviewer.

4.2 Objectives

The reviewer aims to re-evaluate the exposure-response relationship focusing on

- Whether or not there exists exposure-response relationship using a different efficacy endpoint, the percent change from the baseline in walking speed (ft/sec) at the end of double blind phase.
- Whether or not the sponsor’s claim that there is little difference in CNS-related AE incidence between placebo and 10mg bid can be confirmed.
- Whether or not the proposed dose of 10mg bid is appropriate, based on the observation on E-R relationship for both efficacy and safety

4.3 Methods

The percent change from the baseline at the end of double blind phase in walking speed (ft/sec) was used as the primary efficacy endpoint in the reviewer’s exposure-efficacy analysis.

The relationship between CNS-related AE incidence and exposure at the dose of 10mg bid was re-examined based on the subset of the patients who were assigned to the 10mg bid and placebo treatment arms only.

4.3.1 Data Sets

Data sets used are summarized in Table 3.

Table 3. Analysis Data Sets

Study Number	Name	Link to EDR
MS-F202, MS-F203, MS-F204	pddata.csv	

4.3.2 Software

SAS 9.2 was used for the analysis.

4.3.3 Model Results

First, the reviewer summarized the baseline population characteristics (Table 4) to explore whether there exists any difference between studies. Most demographics are comparable across studies while CrCL in study MS-F202 appears to be slightly higher than that in MS-F203 and MS-F204. Also notice that there is slightly unbalanced CrCL (mL/min) distribution shown between dose groups (Figure 5).

Table 4. Baseline population characteristics.

	MS-F202	MS-F203	MS-F204
Age (yrs)	49.9±8.6	51.5±8.7	51.7±9.6
Weight (kg)	74.7±17.2	75.0±18.8	71.4±21.4
CrCL (mL/min)	120.0±32.8	108.5±33.4	101.3±38.4
BMI	26.4±5.9	26.3±5.9	24.4±7.9
Sex (Female/male)	103/55	158/66	86/31
Race (White/black/Asian/other)	145/8/1/4	208/9/3/4	111/2/0/4

Figure 5. The distribution of individual CrCL (mL/min) at each dose for the study of MS-F202.

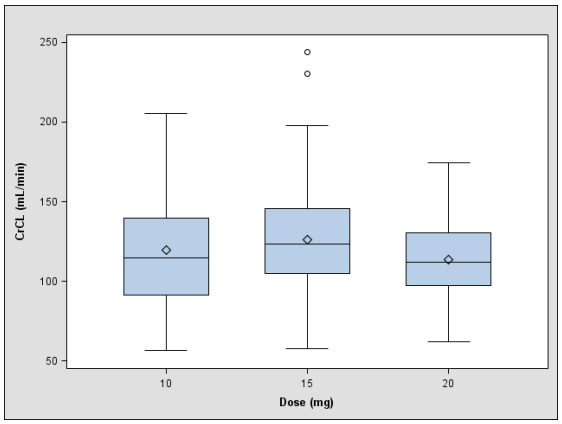


Figure 6 presents the distribution of exposures at each dose group. Cmax was calculated from the observed concentration data within 3-5 hours (Tmax ± 1 hour) post dose from studies MS-F202, MS-F203 and MS-F204. Notice that that there is a large overlap of exposure between doses for both AUC and Cmax.

Figure 6. The distribution of exposure at each dose group. The left panel shows the distribution of AUC (ug·hr/L) and the right panel displays the distribution of Cmax (ug/L).

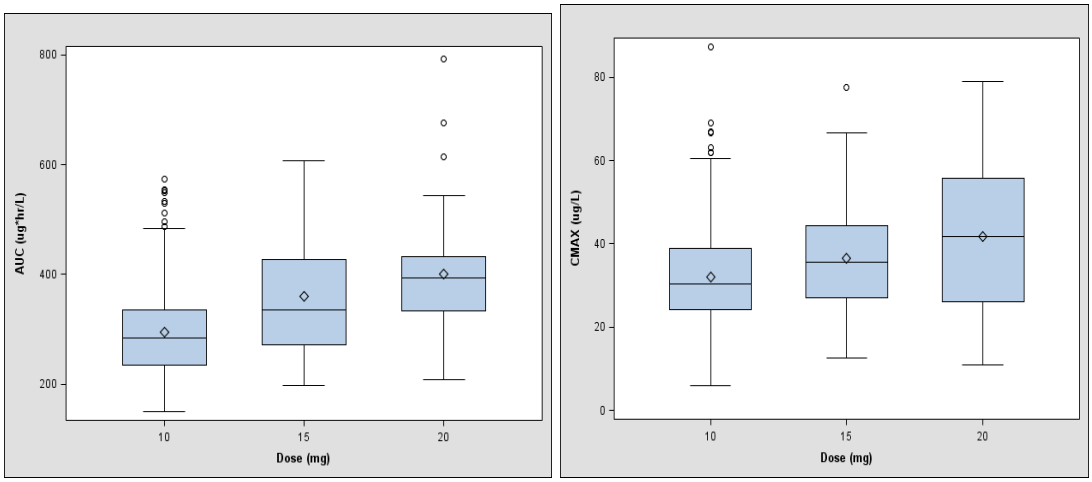
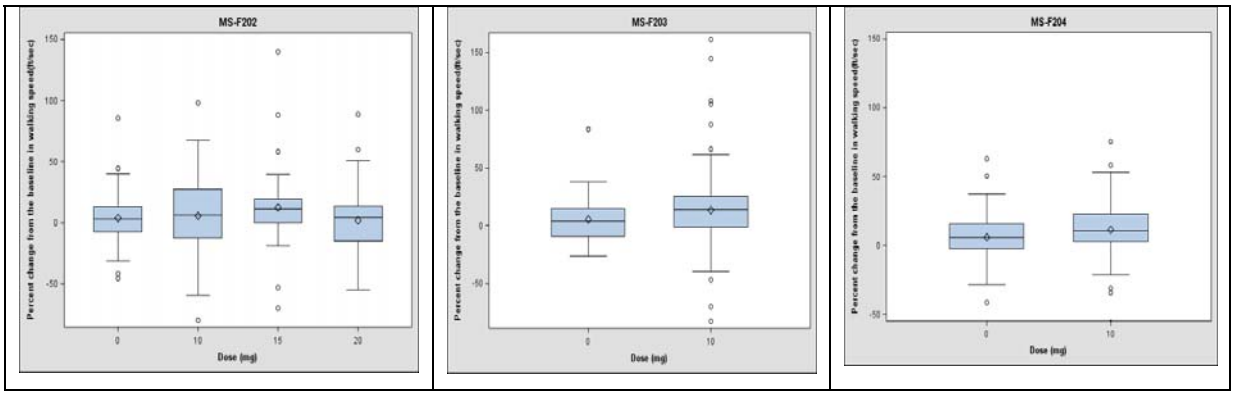


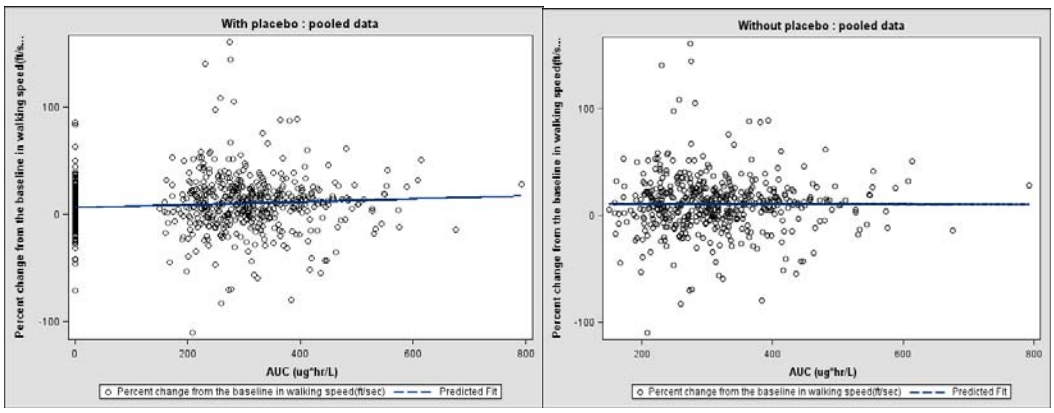
Figure 7 shows the percent change from the baseline in walking speed at each dose group within each study. A dose of 10mg bid showed slightly higher response than placebo across all studies but the doses of 15mg and 20mg bid did not give any additional benefit.

Figure 7. The percent change from the baseline in walking speed by dose group and study.



Linear regression analysis was conducted for the pooled data to explore the relationship between exposure (AUC (ug·hr/L**)**) and the percent change from the baseline in walking speed. Figure 8 presents the results from pooled data analysis. When the patients who were assigned to placebo were included, it showed the statistically significant relationship between AUC (ug·hr/L) and the percent change from the baseline in walking speed (p-value=0.015). The same model was fitted to the data after excluding placebo data and results are shown in the right panel in the Figure 8. The flat relationship without placebo suggests that the response reached the plateau at 10mg bid and a lower dose may be as efficacious as 10 mg bid. This observation is supported by the similar responder rate at each dose group (Table 2).

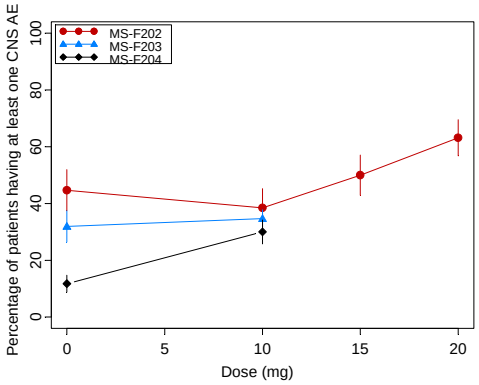
Figure 8. The pooled data analysis : the relationship between AUC (ug·hr/L) and the percent change from the baseline in walking speed (ft/sec) with placebo (left panel, p-value=0.015) and without placebo (right panel, p-value=0.935).



The safety of 10mg bid was evaluated using the probability of having at least one CNS-related AE as an endpoint. The sponsor claimed that CNS-related AE incidence was flat up to an AUC of 200 ug·hr/L and 10mg bid should be safe based on the model prediction.

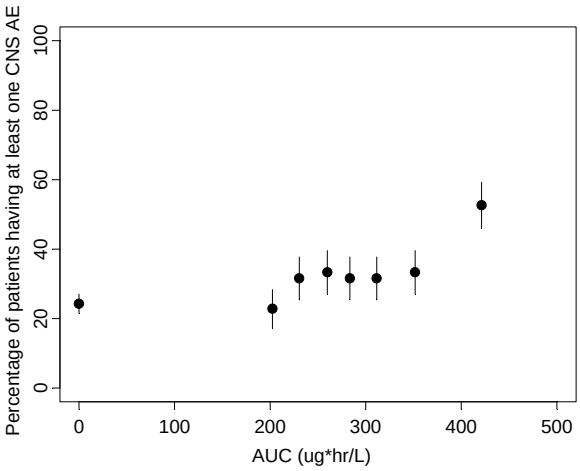
Figure 9 shows CNS-related AE at each dose within each study. It is noticeable that the CNS-related AE incidence for placebo group is highly variable across studies (11%, 32% and 45% for the studies of MS-F204, MS-F203 and MS-F202, respective). It is apparent that there is an increasing trend between placebo and 10mg bid in study MS-F204, although the incidence rate for placebo group is also lowest in this study.

Figure 9. The dose-CNS-related AE relationship for each study.



In order to further evaluate the safety of 10mg bid compared to placebo, the reviewer focused on the patients who were assigned to 10 mg bid or placebo. Figure 10 shows the observed CNS-related AE incidence at the octile of AUC at the dose of 10mg bid and placebo. As observed in the sponsor's analysis, the incidence rate appears to similar to placebo up to the AUC of 200 ug·hr/L and shows increasing trend for the range of AUC > 200 ug·hr/L with a relatively flat but higher rate within 220 ug·hr/L and 350 ug·hr/L.

Figure 10. The probability of having at least one CNS related AE by AUC at the dose of 10mg bid from the observed data. Each dot represents the proportion of having at least one CNS related AE at each octile of AUC and vertical bars indicate one standard error (±SE).



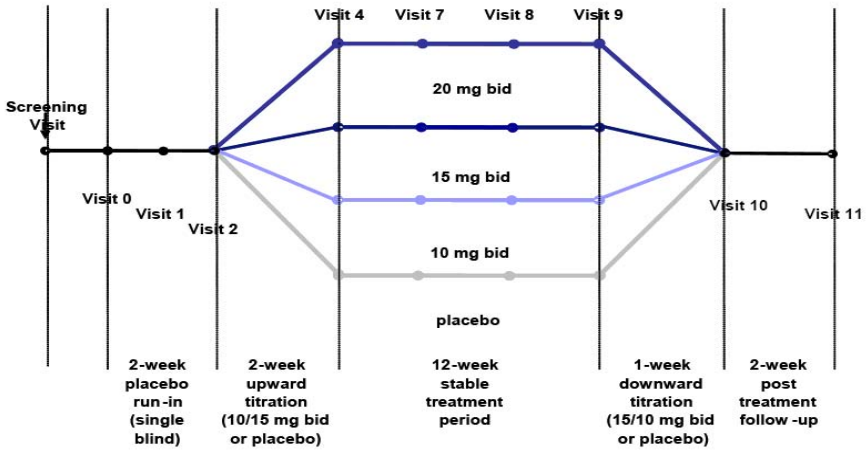
Overall, fampridine-SR seems to show the effectiveness based on both primary endpoint (responder analysis) and the percent change from the baseline in walking speed (ft/sec). However, the exposure-response relationship is flat within the studied exposure range and for most of the exposure range under 10 mg bid, the incidence rate of CNS-related AE is higher than that for the placebo group. Hence, a lower dose than 10 mg bid should be studied if the current safety profile including seizure incidence at 10 mg bid is not acceptable.

5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	**Description**	**Location in \\cdsnas\pharmacometrics**
Reviewer.sas	The reviewer's exposure-response analyses	

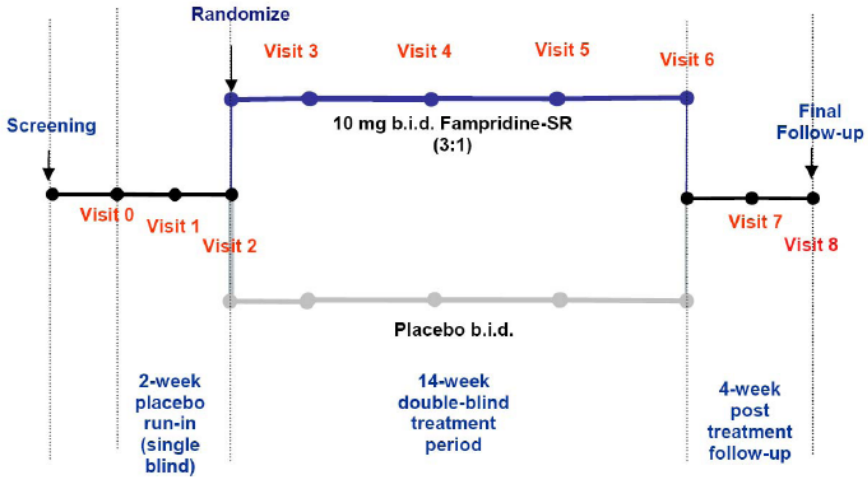
Appendix 1

1. The study design for the pivotal placebo-controlled studies and a supportive phase II study.
   - MS-F202

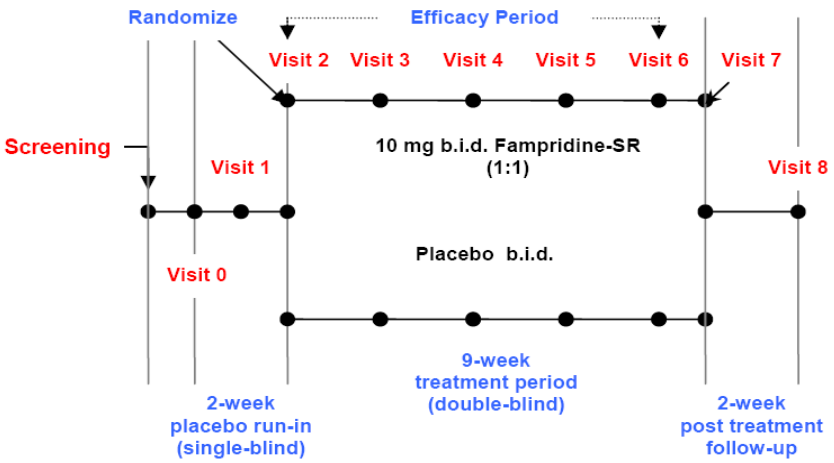


   - MS-F203

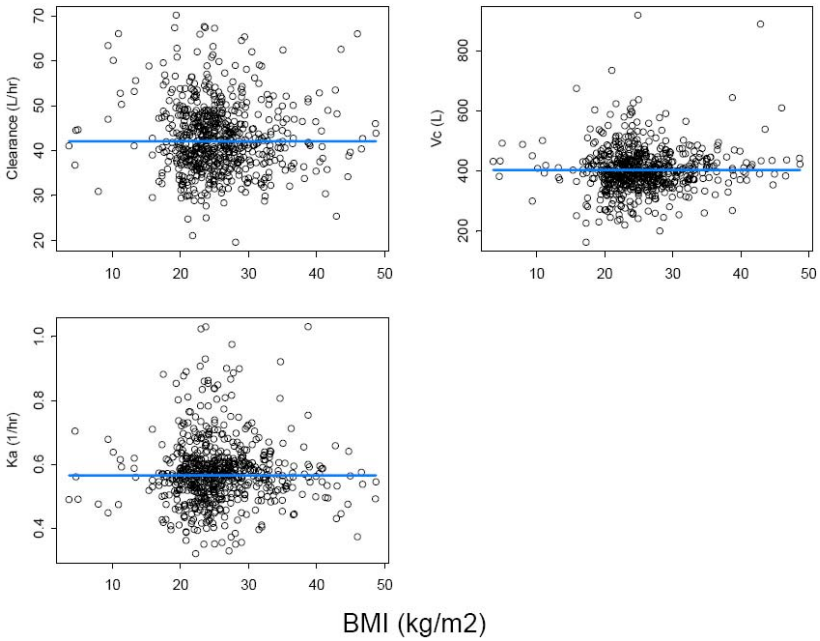
Figure 1 Study Treatment Schedule

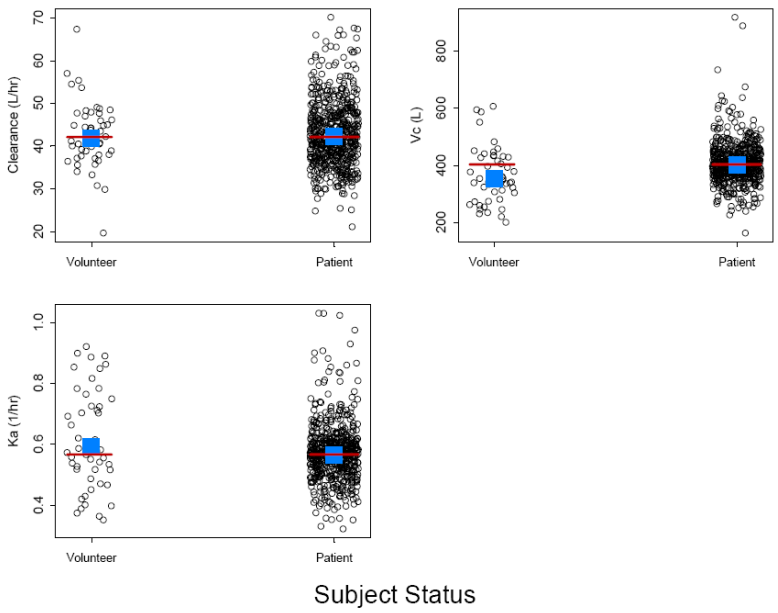


   - MS-F204



2. PK and covariates relationship





* The red line represents typical (population) predicted values and the blue square is the mean of observed (individual predicted) values. It is applied all plots below.

(b) (4)

(b) (4)

3. The parameter estimates from final population PK.

Parameter	Estimated	SE*	95% confidence interval		Estimated variability (%)**
			lower	upper	
θ_1 (CL intercept)	3.740	0.032	3.678	3.802	
θ_2 (V intercept)	6.000	0.046	5.909	6.091	
θ_3 (Ka intercept)	-0.569	0.059	-0.685	-0.453	
θ_4 (Tlag intercept)	-1.910	0.232	-2.365	-1.455	
θ_5 (FeFa25F-SR112003 on CL)	0.116	0.046	0.027	0.205	
θ_6 (RD10F-SR012004 on CL)	-0.294	0.063	-0.418	-0.170	
θ_7 (0195-001US on CL)	-0.091	0.050	-0.188	0.007	
θ_8 (AN751-102 on CL)	0.061	0.049	-0.034	0.156	
θ_9 (MS-F201 on CL)	0.057	0.062	-0.065	0.179	
θ_{10} (MS-F202 on CL)	0.153	0.042	0.071	0.235	
θ_{11} (MS-F203 on CL)	-0.076	0.036	-0.147	-0.005	
θ_{12} (MS-F204 on CL)	-0.049	0.039	-0.125	0.027	
θ_{13} (CrCl on CL)	0.507	0.049	0.412	0.602	
θ_{14} (Age on CL)	-0.166	0.058	-0.279	-0.053	
θ_{15} (Sex on CL)	-0.157	0.024	-0.203	-0.111	
θ_{16} (Sex on V)	-0.284	0.046	-0.374	-0.194	
θ_{17} (Food on Ka)	-0.597	0.095	-0.782	-0.412	
ω^2_{CL}	0.046	0.004			21
$\omega^2_{CL,V}$	0.009	0.007			
ω^2_{V}	0.106	0.021			33
ω^2_{Ka}	0.142	0.031			38
σ^2	0.070	0.004			26

* SE = standard error of parameter estimate; ** Variability: between subject variability $=\sqrt{\omega^2}$; residual variability $=\sqrt{\sigma^2}$

4. Model diagnostics for population PK model

(b) (4)

5. The parameter estimates for the sponsor's exposure-response model.

- Exposure-efficacy analysis (responder analysis)

PK variable	Parameters	Estimated	90% CI	
			Lower	Upper
AUC (μg·hr/L)	E_0	-2.339	-2.805	-1.874
	Emax	4.117	2.830	5.403
	EC_{50} (μg·hr/L)	384.2	139.8	1055.4
Cp (μg/L)	E_0	-2.337	-2.81	-1.864
	Emax	3.146	1.676	4.617
	EC_{50} (μg/L)	22.15	11.32	43.35

- Exposure-safety analysis

AE type	Variable	Parameters	Estimated	95% CI		SE	CV (%)*
				Lower	Upper		
Total	Dose	E_0	7.516	4.414	10.62	1.582	21
		Slope	0.966	0.705	1.227	0.133	14
	AUC	E_0	0.293	0.178	0.408	0.059	20
		Slope	-1.906	-2.637	-1.175	0.373	20
CNS	Dose	E_0	12.35	6.312	18.39	3.080	25
		Slope	-2.458	-3.323	-1.593	0.441	18
	AUC	E_0	0.625	0.365	0.884	0.133	21
		Slope	7.516	4.414	10.62	1.582	21

* SE/Estimated*100%

4.3 THOROUGH QT STUDY REVIEW

Interdisciplinary Review Team for QT Studies Consultation: Thorough QT Study Review

IND	17,627
Brand Name	Fampridine SR
Generic Name	4-aminopyridine
Sponsor	Acorda Therapeutics, Inc.
Indication	Multiple Sclerosis
Dosage Form	Sustained Release Matrix Tablets
Drug Class	Potassium channel blocker
Therapeutic Dosing Regimen	Fampridine SR 10 mg bid for Multiple Sclerosis
Duration of Therapeutic Use	Chronic
Maximum Tolerated Dose	30 mg bid (increased risk for seizure at higher doses)
Submission Number and Date	SN 263, September 07, 2007
Review Division	DNP / HFD 120

1 SUMMARY

1.1 OVERALL SUMMARY OF FINDINGS

Based on the case report form, subject 109 completed 24-hour Holter recordings on both Day -1 and Day 5. However, QTc measurements were not provided in the sponsor's data set, are absent from Listing 16.2.6.1 of the study report, and day -1 waveforms were not submitted to the ECG warehouse. The sponsor should repeat the analysis including subject 109 or provide an explanation for why the data for this subject is missing.

No significant QT prolongation effect of fampridine SR (10 mg and 30 mg) was detected in this TQT study. The largest upper bounds of the 2-sided 90% CI for the mean difference between fampridine SR (10 mg and 30 mg) and placebo were below 10 ms, the threshold for regulatory concern as described in ICH E14 guidance. The largest lower bound of the two-sided 90% CI for the ΔΔQTcI for moxifloxacin was around 5 ms, and the moxifloxacin profile over time was adequately demonstrated in Figure 5, indicating that the assay sensitivity of the study was established.

In this double-blind, randomized, parallel study, 208 healthy subjects received 5 days dosing of fampridine SR 10 mg b.i.d., fampridine SR 30 mg b.i.d., placebo and moxifloxacin 400 mg. A total of 208 healthy subjects were enrolled and 198 subjects completed. Overall summary of findings is presented in Table 1.

Table 1: The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for Fampridine SR (10 mg and 30 mg) and the Largest Lower Bound for Moxifloxacin (FDA Primary Analysis)

Treatment	Time (hour)	ΔΔQTcI (ms)	90% CI (ms)
Fampridine SR 10 mg	0.5	3.4	(-0.5, 7.2)
Fampridine SR 30 mg	0.5	2.3	(-1.5, 6.0)
Moxifloxacin 400 mg*	3	9.8	(5.9, 13.6)

* Multiple endpoint adjustment was not applied. The largest lower bound after Bonferroni adjustment for 4 time points (hours 1, 2, 3, and 4) was 4.9 ms.

The supratherapeutic dose (30 mg) produces mean C_{max} values 3-fold higher than the mean C_{max} for the therapeutic dose (10 mg). These concentrations are above those for the predicted worst case scenario (administration to patients with severe renal impairment) and show that at these concentrations there are no detectable prolongations of the QT interval. It is expected that administration of fampridine in patients with severe renal impairment can result in as much as a 2-fold increase in mean fampridine C_{max} compared to the 10 mg dose.

2 BACKGROUND

Fampridine-SR is a potassium (K^+) channel blocker currently being evaluated clinically as a treatment for improving neurological function in patients with Multiple Sclerosis (MS) and chronic spinal cord injury.

2.1 MARKET APPROVAL STATUS

Fampridine-SR is not approved for marketing in any country.

2.2 PRECLINICAL INFORMATION

Source: IB, March 1, 2007

"Acorda Study hERG4AP102003: "Effects of 4-aminopyridine on Cloned hERG Channels Expressed in Human Mammalian Cells"

In study hERG4AP102003, hERG channels expressed in a human embryonic kidney (HEK293) cell line was used as the assay. 4-aminopyridine produced a concentration dependent inhibition of hERG current (Mean ± SEM): 8.2 ± 0.5% (n=3), 31.8 ± 1.9% (n=4), 67.6 ± 1.8% (n=4) and 76.7 ± 1.8% (n=3) at 0.3, 1, 10, and 30 mM, respectively. The estimated IC_{50} was 3.83 mM with a Hill coefficient of 0.69. Application of the vehicle control (0.1% sterile water in HB-PS) resulted in no change in hERG current amplitude (0.3 ± 0.1% block, n=3). The positive control, terfenadine at 60 nM resulted in 82.8 ± 1.4% (n=3) block of hERG current.

"Acorda Study Purk4AP102003: "Effect of 4-aminopyridine on Action Potential Parameters in Isolated Dog Purkinje Fibers"

In study Purk4AP102003, four Purkinje fibers obtained from four male Beagle dogs were perfused with Tyrode's solution for baseline control followed by 0.5, 5, 50 and 500 μM concentrations of 4-aminopyridine. At 0.5, 5.0 and 50 μM concentrations, 4- aminopyridine did not alter action potential parameters significantly and this indicates that these concentrations have a relatively low

probability of producing QT prolongation under *in vivo* conditions. The data showed a dose dependent increase in action potential duration at 30% repolarization (APD_{30}) at 5.0 and 50 µM concentrations which may be attributed to 4-aminopyridine's effects on transient outward potassium current and would not be expected to induce any significant influence on QT interval. At 500 µM concentration, 4-aminopyridine prolonged ADP at all three repolarization levels examined, indicating its potential to inhibit the rapidly activating component of the delayed rectifier current, I_{Kr} (hERG) at this concentration and prolong QT interval under *in vivo* conditions. However, the 4-aminopyridine-induced prolongation of APD_{50} (action potential duration at 50% repolarization) and APD_{90} did not show reverse rate dependence indicating that blockade of ion channels other than I_{Kr} (hERG) may also be responsible for the 4-aminopyridine-induced prolongation of APD at 500 µM.

"4.2.3. Nonclinical Safety Pharmacology: Cardiovascular Effects of 4-Aminopyridine in Beagle Dogs (Study TPS 486A-501-510-93)(27)

A total of 2 male and 3 female purpose-bred beagle dogs received fampridine 0.5, 1, or 1.5 mg/kg body weight *via* the cephalic vein at an infusion rate of 1.0 ml/minute for 15 minutes. One animal received a dose of 0.5 mg/kg and 2 animals each received doses of 1.0 or 1.5 mg/kg. Each animal was re-dosed 2 hours after the first infusion. The dogs were anesthetized with pentobarbital, intubated, and a Lead II ECG attached. The femoral artery was cannulated for direct blood pressure measurements. Recordings of blood pressure, heart rate, ECG, and respirations were made prior to the first infusion and every 15 minutes for 2 hours following each infusion in this terminal evaluation.

"No consistent or meaningful treatment related effects were noted in the dog given fampridine 0.5 mg/kg. Changes in heart rate and respiratory rate were attributed to the pharmacological action of the anesthetic and the duration of anesthesia. At doses of 1.0 and 1.5 mg/kg, muscle twitching and slight to moderate elevation in blood pressure were the only consistent effects. There was no effect on the ECG."

2.3 PREVIOUS CLINICAL EXPERIENCE

Source: IB, March 1, 2007

"Approximately 1,450 people have been exposed to Fampridine in the course of the clinical development program. More than 1,300 individuals have been exposed to the current Fampridine-SR formulation at doses of 10mg or higher. The Fampridine-SR tablet formulation has been evaluated in 617 people with MS, including 305 people for more than 6 months, 144 for more than a year and 129 for more than two years. The ongoing extension studies, MS-F202 EXT and MS-F203 EXT have recruited a total of 445 patients, 245 of whom remain active (01 March 2007). In addition to this long-term exposure in MS, 62 people with SCI have been exposed for more than 6 months and 41 for more than a year in open label extension studies (SCI-F201 EXT and SCI-F300 EXT), in addition to their exposure in previous double-blind studies.

"The most common adverse events in the MS population reported for controlled clinical trials of doses up to 80 mg/day include dizziness, accidental injury, asthenia, insomnia, headache, paresthesia, urinary tract infection, pain, and nausea.

"In the MS studies including the open-label trials, serious adverse events that were considered by the Investigator to be possibly or probably related to study drug include seizure, altered mental state, agitation, confusion, tremor, worsening of neuralgia, tachycardia, chest pain, leg weakness and manic episode. Except for seizures, these treatment-related serious adverse events occurred in one subject each.

"In the Spinal Cord Injury (SCI) studies (including the open-label trials), serious adverse events that were considered by the Investigator to be possibly or probably related to study drug include seizure, loss of consciousness, memory impairment, anxiety, nausea, somnolence, bowel obstruction, decreased motor function, hyperesthesia, pancytopenia, bradycardia and hypotension exacerbation. Except for seizures, these treatment-related serious adverse events occurred in one subject each.

"Fampridine-SR should not be administered to patients who have a history of seizures or have received therapy for a seizure disorder. Patients should be advised to exercise caution when driving or operating heavy machinery while being treated with Fampridine-SR."

Reviewer's Comments: Seizures have been observed in the MS and SCI program although the sponsor states that published studies indicate that the incidence of seizure in the MS population is higher than that in the unaffected population. Syncope has been reported in the clinical program, although not associated with QT prolongation. Deaths have been due to aortic rupture and suicide. There are no reports of ventricular arrhythmias.

2.4 CLINICAL PHARMACOLOGY

Appendix 5.1 summarizes the key features of Fampridine-SR's clinical pharmacology.

3 SPONSOR'S SUBMISSION

3.1 OVERVIEW

The sponsor submitted the study report for fampridine, including electronic datasets and waveforms to the ECG warehouse.

3.2 TQT STUDY

3.2.1 Title

A Double-Blind Randomized Parallel Trial to Define the ECG Effects of Fampridine-SR Using a Clinical and a Supratherapeutic Dose Compared to Placebo and Moxifloxacin (a Positive Control) in Healthy Men and Women: A Thorough ECG Trial

3.2.2 Protocol Number

TQTc-F-SR001 (PRACS R06-1598)

3.2.3 Study Dates

17 October 2007 - 03 December 2007

3.2.4 Objectives

***Primary*:** To evaluate the effects of fampridine on ECG intervals and morphology with emphasis on cardiac repolarization (QTcI).

***Secondary*:** To determine if there is a pharmacodynamic relationship between the duration of the QTc intervals and the plasma concentration of fampridine.

3.2.5 Study Description

3.2.5.1 Design

Study TQTc-F-SR001 was a single-center, double-blind, *double-dummy*, randomized, placebo-controlled, four-arm parallel design study. The study was conducted in a Phase I unit. All treatment included a 24-hr baseline and then all the subjects received treatment to steady state for 5 days.

3.2.5.2 Controls

The Sponsor used both placebo and positive (moxifloxacin) controls.

3.2.5.3 Blinding

All treatment arms were administered in a double-blind fashion. Note that moxifloxacin was over-encapsulated in this trial.

3.2.6 Treatment Regimen

3.2.6.1 Treatment Arms

Two hundred and eight (208) healthy subjects were randomized in a 1:1:1:1 ratio, stratified by gender, to 1 of the following 4 treatment regimens:

A. Fampridine-SR 10 mg q12h: Subjects received one Fampridine-SR 10 mg tablet and two matching Fampridine placebo tablets q12h from Day 1 to Day 5. On the morning of Day 5, subjects also received a moxifloxacin-placebo capsule.

B. Fampridine-SR 30 mg q12h: Subjects received three 10 mg Fampridine-SR tablets q12h from Day 1 to Day 5. On the morning of Day 5, subjects also received a moxifloxacin-placebo capsule.

C. Placebo matched to Fampridine-SR: Subjects received three Fampridine placebo tablets q12h from Day 1 to Day 5. On the morning of Day 5, subjects also received a moxifloxacin-placebo capsule.

D. Moxifloxacin 400 mg: Subjects received three Fampridine placebo tablets q12h from Day 1 to Day 5. On the morning of Day 5, subjects also received an over-encapsulated moxifloxacin 400 mg tablet.

There were 47, 62, 48 and 51 subjects in treatments A, B, C and D, respectively.

3.2.6.2 Sponsor's Justification for Doses

"There appears to be an increased risk of seizure with higher doses, particularly above 30 mg q12h. Seizures have been observed in patients with multiple sclerosis at doses as low as 10 mg q12h, but the incidence here is not readily distinguishable from the rate expected in the MS population generally. For practical purposes, a dose of 20 mg q12h should be well tolerated. A supra-therapeutic dose of 30 mg q12h would be expected to be tolerated by the majority of subjects, though dizziness, nausea, insomnia and other side effects may lead to a number of discontinuations at this dose level. A dose of 40 mg q12h would be unlikely to be tolerated by the majority of subjects without prolonged dose-titration, and this dose appears to be associated with a significant risk of seizures as an adverse effect even in populations with no known predisposition to seizure.

"The currently available dosage form is the 10 mg sustained-release tablet. The trial design selected for this study included the therapeutic dose level 10 mg q12h and the supra-therapeutic dose, 30 mg q12h.

"The potential risk of escalation to the next higher dose of 40 mg q12h did not appear to be acceptable."

Reviewer's Comment: The supratherapeutic dose of 30 mg b.i.d. is 3-fold higher than the therapeutic dose of 10 mg b.i.d.. The increase in exposure over the clinical dose is shown in Table 4. *The current expected high clinical exposure scenario is severe renal impairment which would increase the C_{max} by 2-fold and exposure by 3-fold. The increase in C_{max} observed in this study easily meets this scenario. The increase in exposure observed in this study just meets this scenario. Given the clinical limitations in exposing subjects to doses greater than 30 mg b.i.d., the dose is acceptable.*

3.2.6.3 Instructions with Regard to Meals

Doses will be administered within 30 minutes after completion of a meal. Meals are to be consumed and doses taken at the same time on each occasion.

Reviewer's Comment: Administration with food is expected to increase C_{max} by 12.7%. Therefore administration of fampridine within 30 minutes of a meal is acceptable.

3.2.6.4 ECG and PK Assessments

ECG measurements were obtained on Day -1 and Day 5 at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18 and 23.5 hours post-dose. Blood samples for measurement of plasma fampridine concentration were obtained pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18 and 23.5 hours post-dose and immediately after ECG assessment.

Reviewer's Comment: The timing of ECG and PK assessments is acceptable.

3.2.6.5 Baseline

Time-matched baseline assessments took place the day prior to dosing (Day -1) at specified time points for each treatment.

3.2.7 ECG Collection

ECGs were obtained digitally using the H12+ ECG continuous 12-lead digital recorder. ECGs were stored on a flash card (about every 10 seconds) and were not available for review until the card was received by the central ECG laboratory and analyzed.

Four 12-lead ECGs were downloaded from the H-12 flash card within about 5 minutes (providing 4 ECGs for each time point) at baseline (Day -1), and then again on Day 5 at the time points specified above (3.2.6.4.)

ECGs used in the analysis were selected by pre-determined time points and were read centrally using a high-resolution, manual, on-screen caliper, semiautomatic method with annotations.

ECG analysis was conducted in Lead II and, when not analyzable, in Lead V5, or most appropriate lead. ECG readers were blinded to subject identifiers, treatment and visit. Readers employed by (b) (4) were Cardiac Safety Specialists (CSSs). The initial measurements were derived by a software program and subsequently adjudicated by the CSSs. The measurements may have been corrected by a QC specialist and/or a Cardiologist as part of the (b) (4) QC and review process.

All ECGs for a given subject were analyzed by the same reader. Quality Assurance reports for inter- and intra-observer variability were produced by (b) (4) and provided to the Sponsor.

Standard digital 12-lead ECGs were recorded at screening, Day -2, and then again approximately 2 to 3 hours after dosing on Days 1 and 3 of treatment to detect any immediate ECG effects for subject safety.

3.2.8 Sponsor's Results

3.2.8.1 Study Subjects

A total of 208 subjects (95 females, 113 males), 18-45 years of age, with a normal baseline ECG and BMI between 19-28 kg/m^2 participated in this trial and 198 subjects completed. Of the 10 (4.8%) patients who prematurely discontinued, 9 subjects (#303, 311, 324, 332, 347, 350, 358, 391 and 392, all assigned to treatment B) were withdrawn due to adverse events, and 1 subject (#148, assigned to treatment A) withdrew consent due to a schedule conflict.

3.2.8.2 Statistical Analyses

The primary analysis, a time-matched analysis with the primary endpoint "placebo-corrected change from baseline to steady state (Day 5) in QTcI interval", was based on a mixed effect model (<u>fixed effects</u>: time, treatment, time×treatment, baseline QTcI; <u>random effect</u>: subject). General Satterthwaite approximation was used to compute denominator degrees of freedom for the tests of fixed effects.

Table 2: Point Estimates and Upper Bounds from Sponsor's Time-Matched and Time-Averaged Analyses on ΔΔQTcI

Analysis Type	Time (hr)	Fampridine-SR 10 mg		Fampridine-SR 30 mg		Moxifloxacin 400 mg	
		Estimate [1]	Upper Bound [2]	Estimate [1]	Upper Bound [2]	Estimate [1]	Upper Bound [2]
Time-Matched	0.5 Hr	3.3	6.6	1.6	4.7	-0.5	4.1
	1 Hr	3.6	7.0	1.6	4.7	2.1	6.7
	2 Hr	3.4	6.7	-0.1	3.0	5.5	10.1
	3 Hr	3.8	7.1	0.9	4.0	9.4	14.0
	4 Hr	3.1	6.4	0.5	3.6	8.2	12.8
	5 Hr	2.3	5.6	1.1	4.2	7.4	12.0
	6 Hr	1.3	4.6	1.1	4.2	6.4	11.0
	8 Hr	1.2	4.5	-0.7	2.4	4.9	9.5
Time-Matched	10 Hr	2.5	5.9	-0.2	2.9	7.3	11.9
	12 Hr	1.7	5.0	-2.4	0.7	5.4	10.0
	14 Hr	0.6	3.9	-1.3	1.8	4.3	9.0
	18 Hr	0.6	3.9	-1.4	1.7	4.7	9.3
	23.5 Hr	-0.5	2.8	-1.7	1.4	2.2	6.8
Time-Average	Daily-Mean	0.6	2.1	0.6	2.0	5.1	6.5

[1] Mixed Model ANOVA is fit for placebo-corrected change from baseline and includes terms for: treatment, gender, time, and a time by treatment interaction.
[2] Upper Bound = upper one-sided 95% ANOVA model based confidence limit.

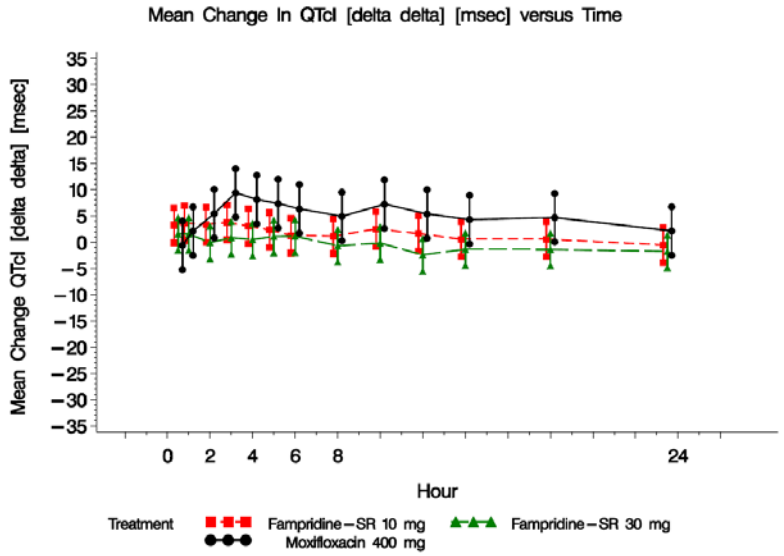
(Source: Table 14.2.3-16, Cardiovascular Electrocardiographic Data Report, Page 21)

As shown in Table 2, there was no upper CI > 10 mg for either of the fampridine dose groups and the estimated means were all < 4 ms. Only the point estimates and the upper confidence intervals were provided by the sponsor.

Reviewer's comments: This reviewer calculated the point estimates as well as the lower confidence intervals of the mean differences of moxifloxacin and placebo after baseline adjustment at each time point. Results are presented in 4.2.1.1.

Figure 1 displays the QTcI duration for two fampridine dose groups and moxifloxacin group at each time point, placebo corrected:

Figure 1: Sponsor's LS Mean and 90% CI ΔΔQTcI Timecourse



(Source: Figure 14.5.1-1; Cardiovascular Electrocardiographic Data Report; Page 23)

Analysis results by gender are provided in Table 3. No upper bounds crossed the 10 ms boundary for each fampridine dose group. The study drug induced QTcI change by gender is not obvious; however, it appears moxifloxacin induced QTcI effect between males and females is quite different.

Table 3: Sponsor's Gender Analysis Results

Table 14.2.3-19
Placebo-Corrected Change from Baseline - Estimates from Mixed Model ANOVA [1]
Gender Effects - QTc Individual (msec)
Electrocardiographic Analysis Set

Table 4-3		Fampridine-SR 10 mg				Fampridine-SR 30 mg				Moxifloxacin 400 mg			
		Female		Male		Female		Male		Female		Male	
Analysis Type	Time (hr)	Est-imate [1]	Upper Bound [2]	Est-imate [1]	Upper Bound [2]	Est-imate [1]	Upper Bound [2]	Est-imate [1]	Upper Bound [2]	Est-imate [1]	Upper Bound [2]	Est-imate [1]	Upper Bound [2]
Time-Matched	0.5 Hr	3.1	7.2	3.0	6.8	0.9	4.8	2.2	5.6	4.8	10.7	-3.8	1.2
	1 Hr	2.6	6.7	4.1	7.8	-0.0	3.9	2.8	6.2	6.6	12.5	-0.5	4.5
	2 Hr	2.3	6.4	3.8	7.5	-1.6	2.3	1.1	4.6	9.9	15.8	2.8	7.9
	3 Hr	3.5	7.5	3.7	7.5	-0.0	3.9	1.6	5.0	14.5	20.5	6.3	11.3
	4 Hr	2.4	6.5	3.1	6.9	-0.7	3.2	1.3	4.8	13.0	18.9	5.1	10.2
	5 Hr	1.2	5.3	2.7	6.5	-0.5	3.3	2.3	5.8	11.7	17.6	4.7	9.8
	6 Hr	1.3	5.3	1.0	4.7	0.6	4.5	1.6	5.0	11.8	17.7	2.9	8.0
	8 Hr	0.8	4.8	1.1	4.9	-1.6	2.3	0.1	3.5	10.0	15.9	1.8	6.9
	10 Hr	1.6	5.7	2.8	6.6	-1.6	2.3	0.9	4.4	11.9	17.8	4.5	9.6
Time-Matched	12 Hr	0.3	4.4	2.4	6.1	-4.3	-0.4	-0.9	2.5	9.5	15.4	3.0	8.1
	14 Hr	-0.0	4.0	0.7	4.5	-2.5	1.4	-0.4	3.0	9.2	15.1	1.4	6.5
	18 Hr	-0.0	4.0	0.7	4.5	-2.5	1.4	-0.5	3.0	9.6	15.5	1.8	6.8
	23.5 Hr	-0.5	3.6	-0.9	2.9	-2.2	1.6	-1.3	2.2	7.7	13.6	-1.2	3.8
Time-Average	Daily-Mean	1.4	3.6	0.1	2.1	0.0	2.1	1.0	2.8	7.7	9.9	3.3	5.2

1] Mixed Model ANOVA is fit for placebo-corrected change from baseline and includes terms for:
treatment, gender, time (categorical), time by treatment and gender by treatment interactions, and baseline value.
Baseline value adjustment is gender and time specific.

[2] Upper Bound = upper one-sided 95% ANOVA model based confidence limit.
Data source: ecg_all2.sas7bdat Program source: CalCI-ECG-corrected-Gender.sas Listing source: 16.2.6

Reviewer's Comments: The reviewer performed an independent analysis and the results were consistent with the sponsor's results.

3.2.8.3 Safety Analysis

There were no deaths in the study.

One serious adverse event (SAE) of hallucinations was reported over the course of the trial in a female subject randomized to Treatment B (Fampridine-SR 30 mg q12h) one day after completing the study. She had experienced adverse events during the study that she did not report including dizziness, euphoria, nausea, headache, insomnia and paranoia.

Nine (9) subjects were discontinued prematurely due to adverse events that were mild to moderate in severity including nausea, dizziness, headache, chills, vomiting, insomnia and heart racing.

3.2.8.4 Clinical Pharmacology

3.2.8.4.1 Pharmacokinetic Analysis

The PK results are presented in Table 4. C_{max} and AUC values in the thorough QT study were 3-fold higher following administration of 30 mg b.i.d. fampridine compared with 10 mg b.i.d. fampridine, the intended clinical dose.

Table 4: Pharmacokinetic Parameters for Fampridine (0-12 hours) – Arithmetic Mean (±SD)

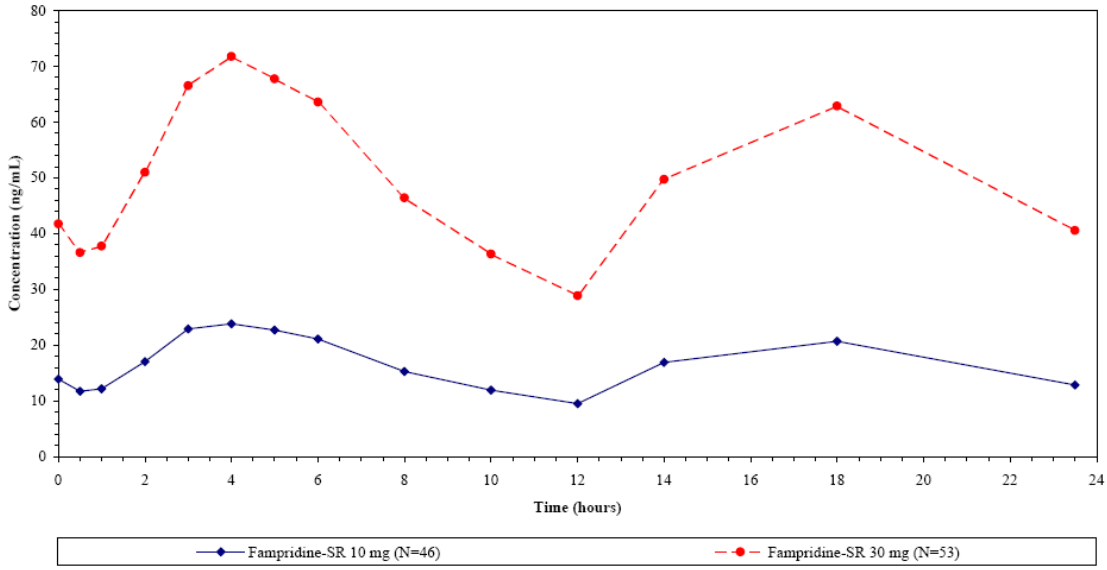
PK Parameter (units)	Fampridine-SR 10 mg	Fampridine-SR 30 mg
$AUC_{0-\tau}$ (ng-hr/mL)	200.50 (±30.65)	603.90 (±98.50)
$C_{max\ (ss)}$ (ng/mL)	25.31 (±4.04)	75.00 (±12.11)
$C_{min\ (ss)}$ (ng/mL)	9.34 (±1.76)	28.22 (±6.78)
$C_{av\ (ss)}$ (ng/mL)	16.71 (±2.55)	50.32 (±8.21)
Fluctuation Index	0.96 (±0.15)	0.94 (±0.19)
% Peak to Trough Fluctuation	95.78 (±15.18)	93.82 (±18.44)
Peak to Trough Swing	15.97 (±3.40)	46.77 (±9.87)
$T_{max\ (ss)}$ (hr)	3.85 (±0.84)	3.98 (±0.99)

Source: Clinical Study Report P-5.

The mean concentration time profiles of fampridine for the 10 mg b.i.d. and 30 mg b.i.d. dose regimens are shown in

Figure **2**.

Figure 2: Fampridine mean concentration-time profile (ng/ml) on Day 5 following 10 mg b.i.d. and 30 mg b.i.d. dosing.



Source: Clinical Study Report P-STATISTICS-14.

3.2.8.4.2 Exposure-Response Analysis

The relationship between change from baseline QTc and plasma concentrations of fampridine was explored with a linear mixed model with plasma concentrations of fampridine as a fixed effect and subjects as random effect. The results of the modeling analysis are in Table 5 and a plot of ΔQTcI vs. drug concentration is presented in Figure 3.

Table 5: Parameter Estimates of Linear Mixed Model of Change from Baseline Corrected QT vs. Fampridine Plasma Concentration

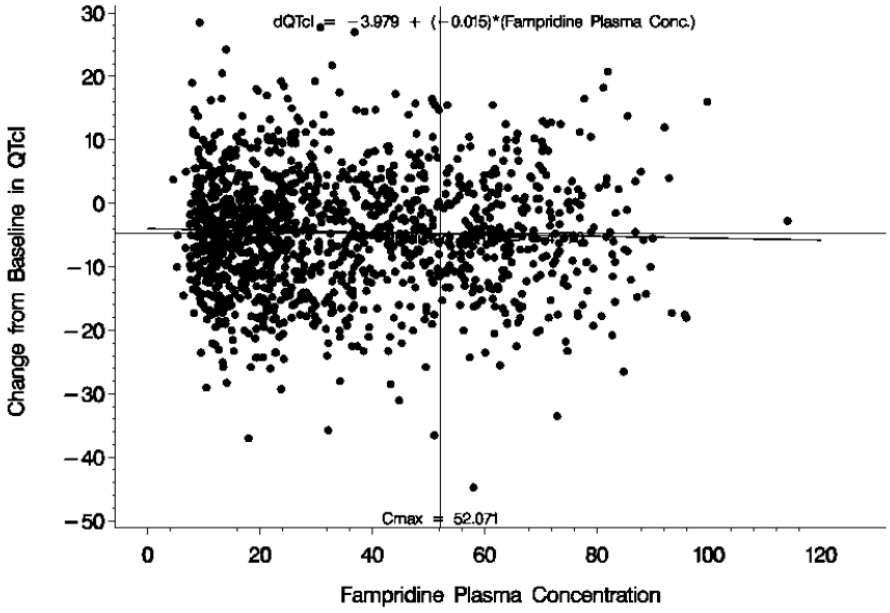
QT Parameter	Slope of Plasma Concentration	Standard Error of Plasma Concentration	p-value	Predicted QTc at Median C_{max}	One-Sided Upper 95% Confidence Bound of Predicted QTc [2]	Overall Model Fit
QTcI	-0.0152	0.0158	0.3360	-4.8596	-3.7150	<.0001
QTcF	-0.0168	0.0159	0.2918	-4.3850	-3.2228	<.0001
QTcB	-0.0242	0.0213	0.2555	-1.7725	-0.3576	<.0001

[1] Linear Mixed Model if fit for change from baseline (not placebo-correct) versus the plasma concentration as a fixed effect with subject included in the model as a random effect.

[2] Upper Bound = upper one-sided 95% linear mixed model based confidence limit.

Source: Clinical Study Report P-52, Table 12.1.

Figure 3: QTcI Change from Baseline vs. Fampridine Plasma Concentration



4 REVIEWERS' ASSESSMENT

4.1 EVALUATION OF THE QT/RR CORRECTION METHOD

The observed QT-RR interval relationship is presented in Figure 4 together with the Bazett's (QTcB), Fridericia (QTcF), and individual correction (QTcI).

We also evaluated the linear relationships between different correction methods (QTcB, QTcF, QTcI) and RR. We used the mean sum of squared slopes as the criterion. The smaller this value is, the better the correction. Based on the results listed in the following table, it appears that both QTcF and QTcI performed equally well. QTcI has been used for the primary statistical analysis.

The observed QT-RR interval relationship is presented in Figure 4 together with the Bazett's (QTcB), Fridericia (QTcF), and individual correction (QTcI).

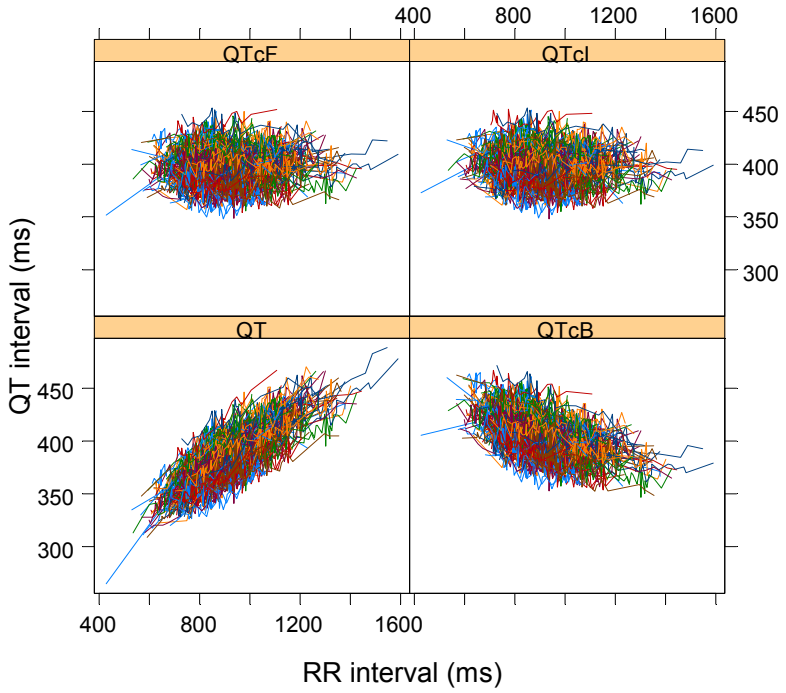
Table 6: Mean Sum of Squared Slopes* for Different QT-RR Correction Methods

		Treatment								
		Fampridine 10 mg		Fampridine 30 mg		Placebo		Moxifloxacin 400mg		All Trt
		n	msss	n	msss	n	msss	n	msss	msss
method	sex									
QTcB	F	21	0.0043	32	0.0045	21	0.0040	21	0.0050	0.0044
	M	25	0.0046	30	0.0038	27	0.0052	30	0.0041	0.0044
	All	46	0.0044	62	0.0042	48	0.0047	51	0.0045	0.0044
QTcF	F	21	0.0011	32	0.0014	21	0.0016	21	0.0012	0.0013
	M	25	0.0006	30	0.0011	27	0.0014	30	0.0007	0.0010

		Treatment								
		Fampridine 10 mg		Fampridine 30 mg		Placebo		Moxifloxacin 400mg		All Trt
		n	msss	n	msss	n	msss	n	msss	msss
	All	46	0.0008	62	0.0013	48	0.0015	51	0.0009	0.0011
QTcI	F	21	0.0013	32	0.0015	21	0.0014	21	0.0015	0.0015
	M	25	0.0010	30	0.0008	27	0.0013	30	0.0009	0.0010
	All	46	0.0011	62	0.0012	48	0.0013	51	0.0011	0.0012

***Mean sum of squared slopes (msss): $a_x = \Sigma\, b_x(i)^2/n$, where b_x is the estimated slopes for correction x.**

Figure 4: QT, QTcB, QTcF, and QTcI vs. RR (Each Subject's Data Points are Connected with a Line)



4.2 STATISTICAL ASSESSMENTS

4.2.1 QTc Analysis

4.2.1.1 The Primary Analysis for the Study Drug (Fampridine)

The statistical reviewer used an ANCOVA model to analyze the data at each time point to compare ΔQTcI between each fampridine dose group and placebo after baseline adjustment. The ANCOVA model includes TREATMENT, SEX as factors and baseline ΔQTcI as covariate. The largest upper bounds of the 2-sided 90% CI for the mean difference between fampridine SR 10 mg and placebo, and between fampridine SR 30 mg and placebo were 7.2 and 6.0 ms, respectively. Gender effect had a significant impact on

ΔQTcI at hours 3, 4, 5, 6, and 8 with p-values ≤ 0.0004. The analysis results are listed in the following tables.

Note: Subject #109 (assigned to treatment A) was not included in our analysis.

Table 7: Analysis Results of ΔQTcI and ΔΔQTcI for Fampridine SR 10 mg

	ΔQTcI: Fampridine			ΔQTcI: placebo			ΔΔQTc	
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	90% CI
0.5	45	-1.4	9.6	48	-4.8	8.5	3.4	(-0.5, 7.2)
1	45	-6.3	8.3	48	-8.5	7.4	2.7	(-1.1, 6.4)
2	45	-4.9	9.8	48	-7.7	9.0	3.0	(-1.0, 7.1)
3	45	-4.8	9.7	48	-7.5	10.0	3.0	(-1.0, 6.9)
4	45	-4.9	9.4	48	-5.9	10.1	1.7	(-2.2, 5.6)
5	45	-4.7	9.9	48	-5.3	10.1	1.1	(-2.8, 5.1)
6	45	-3.7	10.2	48	-2.4	9.7	-0.7	(-4.6, 3.3)
8	45	-4.6	7.3	48	-4.1	8.0	0.0	(-3.5, 3.5)
10	44	-4.7	8.5	48	-5.4	7.5	1.2	(-2.5, 5.0)
12	45	-2.6	9.2	47	-1.8	7.4	0.1	(-3.9, 4.1)
14	45	-6.3	8.0	48	-5.7	8.0	0.0	(-3.9, 3.8)
18	44	-7.2	10.2	48	-6.0	9.9	-0.9	(-5.1, 3.2)
23.5	44	-3.2	8.6	47	-1.4	7.3	-1.3	(-5.1, 2.4)

Table 8: Analysis Results of ΔQTcI and ΔΔQTcI for Fampridine SR 30 mg

	ΔQTcI: Fampridine			ΔQTcI: placebo			ΔΔQTc	
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	90% CI
0.5	53	-2.4	10.4	48	-4.8	8.5	2.3	(-1.5, **6.0**)
1	53	-6.3	9.6	48	-8.5	7.4	2.0	(-1.6, 5.7)
2	53	-6.9	10.1	48	-7.7	9.0	0.7	(-3.2, 4.6)
3	52	-6.1	8.6	48	-7.5	10.0	1.4	(-2.4, 5.2)
4	52	-5.0	10.2	48	-5.9	10.1	1.1	(-2.6, 4.9)
5	52	-4.4	9.6	48	-5.3	10.1	1.2	(-2.7, 5.0)
6	52	-0.5	7.4	48	-2.4	9.7	2.0	(-1.8, 5.7)
8	52	-3.9	7.8	48	-4.1	8.0	0.1	(-3.2, 3.4)
10	52	-4.2	9.0	48	-5.4	7.5	1.2	(-2.4, 4.8)
12	53	-4.0	10.6	47	-1.8	7.4	-2.0	(-5.8, 1.8)
14	53	-6.0	10.9	48	-5.7	8.0	-0.4	(-4.1, 3.3)
18	53	-6.2	9.1	48	-6.0	9.9	-0.3	(-4.3, 3.7)
23.5	53	-1.9	9.8	47	-1.4	7.3	-0.5	(-4.0, 3.1)

4.2.1.2 Assay Sensitivity Analysis

The statistical reviewer used the same ANCOVA model to analyze moxifloxacin and placebo data. The results are presented in Table 9. The largest unadjusted 90% lower

confidence interval was 5.9 ms. Since the peak effect of moxifloxacin on QT/QTc interval generally occurs during the first four hours, the adjusted CIs were calculated using Bonferroni adjustment for 4 time points (hours 1, 2, 3 and 4). The largest adjusted lower confidence interval was 4.9 ms at hour 3. It was around the threshold of regulatory concern (5 ms), suggesting the expected moxifloxacin effect can be detected in this study.

Table 9: Analysis Results of ΔQTcI and ΔΔQTcI for Moxifloxacin

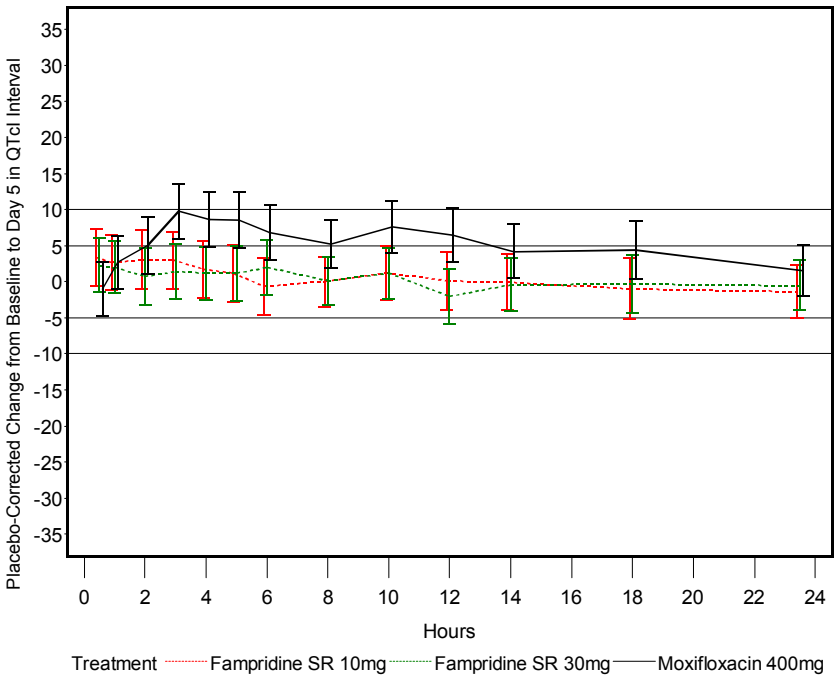
	ΔQTcI: Moxifloxacin			ΔQTcI: placebo			ΔΔQTc		
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	Unadjusted 90% CI	Adjusted* 90% CI
0.5	50	-6.2	8.9	48	-4.8	8.5	-1.0	(-4.8, 2.7)	(-5.8, 3.8)
1	51	-5.8	11.2	48	-8.5	7.4	2.7	(-1.0, 6.3)	(-2.0, 7.3)
2	51	-3.1	10.0	48	-7.7	9.0	5.1	(1.1, 9.0)	(0.0, 10.1)
3	51	2.0	9.8	48	-7.5	10.0	9.8	(**5.9**, 13.6)	(**4.9**, 14.6)
4	51	2.3	8.7	48	-5.9	10.1	8.6	(4.9, 12.4)	(3.8, 13.4)
5	49	3.3	9.5	48	-5.3	10.1	8.6	(4.7, 12.5)	(3.7, 13.5)
6	50	3.9	9.9	48	-2.4	9.7	6.8	(3.0, 10.6)	(2.0, 11.6)
8	51	0.8	10.0	48	-4.1	8.0	5.2	(1.9, 8.6)	(1.0, 9.5)
10	51	1.9	10.2	48	-5.4	7.5	7.6	(4.0, 11.2)	(3.0, 12.1)
12	51	4.6	10.2	47	-1.8	7.4	6.5	(2.7, 10.3)	(1.6, 11.3)
14	50	-1.9	10.0	48	-5.7	8.0	4.2	(0.5, 7.9)	(-0.5, 8.9)
18	51	-1.8	9.7	48	-6.0	9.9	4.4	(0.4, 8.4)	(-0.7, 9.5)
23.5	51	-0.3	8.8	47	-1.4	7.3	1.5	(-2.0, 5.1)	(-3.0, 6.1)

* Bonferroni method was applied for multiple endpoint adjustment for 4 time points: hours 1, 2, 3 and 4.

4.2.1.3 Graphs of ΔΔQTcI and QTcI Over Time

Figure 5 displays the time profile of ΔΔQTcI for different treatment groups.

Figure 5: LS Mean and 90% CI ΔΔQTcI Timecourse



4.2.1.4 Categorical Analysis

Table 10 lists the number of subjects as well as the number of observations whose absolute QTcI values were ≤ 450 ms and between 450 ms and 480 ms. No subject's QTcI was above 480 ms.

Table 10: Categorical Analysis for QTcI

	Total N		Value<=450 ms		450 ms<Value<=480 ms	
Treatment Group	**# Subj.**	**# Obs.**	**# Subj. (%)**	**# Obs. (%)**	**# Subj. (%)**	**# Obs. (%)**
Placebo	48	624	46 (95.8%)	619 (99.2%)	2 (4.2%)	5 (0.8%)
Moxifloxacin	51	663	51 (100%)	663 (100%)	0 (0%)	0 (0%)
Fampridine SR 10mg	45	631	45 (100%)	631 (100%)	0 (0%)	0 (0%)
Fampridine SR 30mg	62	751	62 (100%)	751 (100%)	0 (0%)	0 (0%)

Table 11 lists the categorical analysis results for ΔQTcI. No subject's change from baseline was above 30 ms.

Table 11: Categorical Analysis of ΔQTcI

	Total N		Value<=30 ms	
Treatment Group	**# Subj.**	**# Obs.**	**# Subj.**	**# Obs.**
Moxifloxacin	51	663	51 (100%)	663 (100%)

	Total N		Value<=30 ms	
Treatment Group	**# Subj.**	**# Obs.**	**# Subj.**	**# Obs.**
Fampridine SR 10mg	45	631	45 (100%)	631 (100%)
Fampridine SR 30mg	62	751	62 (100%)	751 (100%)

4.2.2 PR Analysis

The same statistical analysis was performed based on PR interval. The point estimates and the 90% confidence intervals are presented in Table 12 and Table 13. The largest upper limits of 90% CI for the PR mean differences between fampridine SR 10 mg and placebo and fampridine SR 30 mg and placebo were 3.3 and 5.5 ms, respectively.

Table 12: Analysis Results of ΔPR and ΔΔPR for Fampridine SR 10mg

	ΔPR: Fampridine			ΔPR: Placebo			ΔΔPR	
Time/(hr)	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**LS Mean**	**90% CI**
0.5	45	-2.3	10.4	48	0.7	8.4	-2.1	(-5.8, 1.6)
1	45	-4.2	10.8	48	-1.1	9.1	-1.7	(-5.6, 2.3)
2	45	-1.3	9.1	48	-2.9	8.9	2.2	(-1.6, 6.0)
3	45	-5.2	8.7	48	0.6	9.1	-4.7	(-8.4, -0.9)
4	45	-1.8	9.3	48	-1.1	8.0	-0.2	(-3.8, **3.3**)
5	45	-1.6	10.1	48	-0.4	6.4	-0.7	(-4.5, 3.1)
6	45	-0.3	10.8	48	1.8	8.0	-1.5	(-5.1, 2.2)
8	45	-1.1	8.5	48	1.3	9.2	-1.8	(-5.4, 1.8)
10	44	-2.1	10.9	48	0.5	6.5	-2.0	(-5.6, 1.6)
12	45	-1.5	8.9	47	0.9	8.7	-1.8	(-5.3, 1.7)
14	45	-3.3	7.5	48	0.0	8.6	-2.8	(-6.3, 0.6)
18	44	-4.3	9.5	48	-2.0	10.5	-1.7	(-5.9, 2.5)
23.5	44	-3.1	10.2	47	0.2	7.4	-2.7	(-6.5, 1.1)

Table 13: Analysis Results of ΔPR and ΔΔPR for Fampridine SR 30mg

	ΔPR: Fampridine			ΔPR: Placebo			ΔΔPR	
Time/(hr)	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**LS Mean**	**90% CI**
0.5	53	-1.7	9.0	48	0.7	8.4	-2.3	(-5.8, 1.3)
1	53	-1.2	9.9	48	-1.1	9.1	-0.4	(-4.2, 3.4)
2	53	-0.5	10.3	48	-2.9	8.9	1.9	(-1.7, **5.5**)
3	52	-1.9	9.4	48	0.6	9.1	-2.3	(-5.9, 1.3)
4	52	-2.2	9.0	48	-1.1	8.0	-1.2	(-4.6, 2.2)
5	52	0.0	9.8	48	-0.4	6.4	0.3	(-3.3, 4.0)
6	52	0.0	8.4	48	1.8	8.0	-1.8	(-5.3, 1.7)

	ΔPR: Fampridine			ΔPR: Placebo			ΔΔPR	
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	90% CI
8	52	-0.4	7.9	48	1.3	9.2	-1.8	(-5.3, 1.7)
10	52	-1.1	7.6	48	0.5	6.5	-1.7	(-5.1, 1.8)
12	53	-2.6	7.8	47	0.9	8.7	-3.5	(-6.9, -0.2)
14	53	-1.7	9.1	48	0.0	8.6	-1.9	(-5.2, 1.4)
18	53	-1.7	9.2	48	-2.0	10.5	-0.2	(-4.2, 3.8)
23.5	53	0.6	9.3	47	0.2	7.4	0.3	(-3.3, 3.9)

4.2.3 QRS Analysis

The same statistical analysis was performed based on QRS interval. The point estimates and the 90% confidence intervals are presented in Table 14 and Table 15. The largest upper limits of 90% CI for the QRS mean differences between fampridine SR 10 mg and placebo and fampridine SR 30 mg and placebo were 3.5 and 2.6 ms, respectively.

Table 14: Analysis Results of ΔQRS and ΔΔQRS for Fampridine SR 10mg

	ΔQRS: Fampridine			ΔQRS: Placebo			ΔΔQRS	
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	90% CI
0.5	45	0.4	3.2	48	-0.5	3.3	0.8	(-0.5, 2.1)
1	45	0.5	2.9	48	-1.3	4.5	1.6	(0.1, 3.1)
2	45	0.7	3.1	48	-1.1	4.2	1.7	(0.2, 3.2)
3	45	1.3	3.0	48	-0.2	4.1	1.4	(-0.1, 2.9)
4	45	0.9	3.2	48	-0.1	4.6	1.0	(-0.5, 2.5)
5	45	0.8	3.5	48	-0.5	3.5	1.2	(-0.2, 2.7)
6	45	0.2	3.5	48	-0.4	4.2	0.8	(-0.7, 2.3)
8	45	0.2	2.6	48	0.6	3.5	-0.2	(-1.5, 1.1)
10	44	0.7	3.3	48	-0.1	3.1	0.9	(-0.5, 2.4)
12	45	-0.1	3.5	47	-0.6	3.5	0.6	(-0.8, 1.9)
14	45	0.6	3.1	48	-1.5	3.7	2.0	(0.6, **3.5**)
18	44	0.3	2.5	48	-0.6	2.9	0.9	(-0.4, 2.2)
23.5	44	1.1	3.4	47	0.1	3.0	0.9	(-0.4, 2.2)

Table 15: Analysis Results of ΔQRS and ΔΔQRS for Fampridine SR 30mg

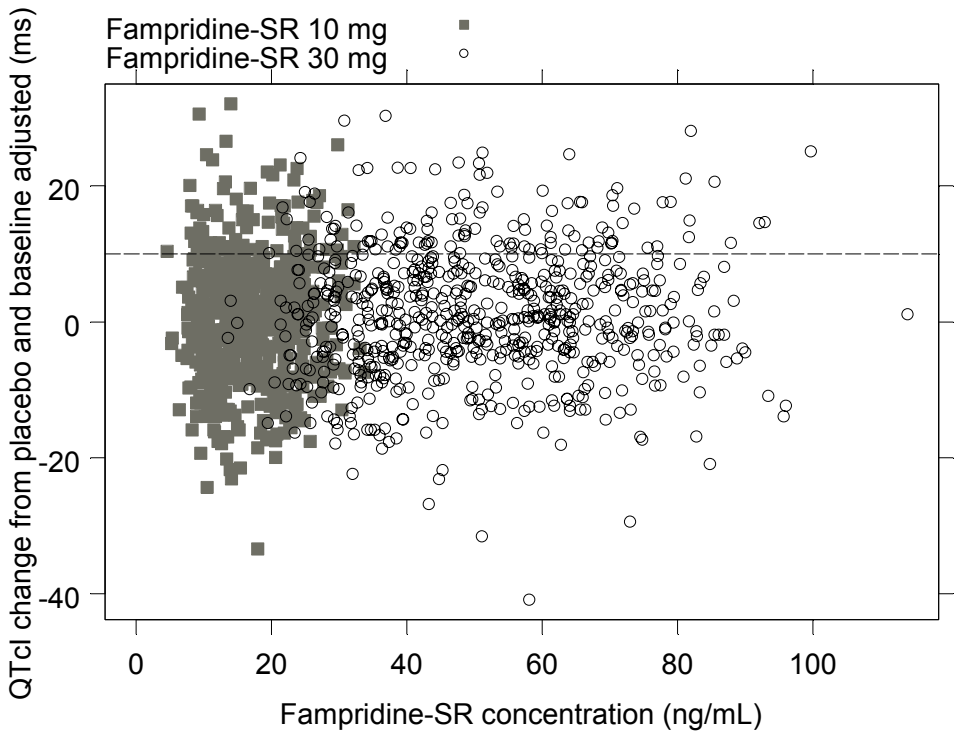
	ΔQRS: Fampridine			ΔQRS: Placebo			ΔΔQRS	
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	90% CI
0.5	53	0.0	3.4	48	-0.5	3.3	0.6	(-0.6, 1.9)
1	53	-0.2	3.7	48	-1.3	4.5	1.2	(-0.3, **2.6**)
2	53	-0.3	3.6	48	-1.1	4.2	0.9	(-0.6, 2.3)
3	52	-0.5	3.8	48	-0.2	4.1	-0.1	(-1.5, 1.3)
4	52	-0.8	3.0	48	-0.1	4.6	-0.4	(-1.9, 1.0)
5	52	-0.1	3.2	48	-0.5	3.5	0.5	(-0.9, 1.9)

	ΔQRS: Fampridine			ΔQRS: Placebo			ΔΔQRS	
Time/(hr)	N	Mean	SD	N	Mean	SD	LS Mean	90% CI
6	52	-0.2	3.3	48	-0.4	4.2	0.5	(-1.0, 1.9)
8	52	-0.4	3.2	48	0.6	3.5	-0.7	(-1.9, 0.5)
10	52	-0.4	3.9	48	-0.1	3.1	0.0	(-1.4, 1.4)
12	53	-0.9	3.5	47	-0.6	3.5	-0.0	(-1.3, 1.3)
14	53	-1.4	4.0	48	-1.5	3.7	0.2	(-1.1, 1.6)
18	53	-1.3	3.7	48	-0.6	2.9	-0.5	(-1.8, 0.7)
23.5	53	-0.9	3.2	47	0.1	3.0	-0.9	(-2.1, 0.4)

4.3 CLINICAL PHARMACOLOGY ASSESSMENTS

The relationship between ΔΔQTcI and fampridine concentrations is visualized in Figure 6 with no evident exposure-response relationship.

Figure 6: ΔΔ QTcI vs. Fampridine concentration



4.4 CLINICAL ASSESSMENTS

4.4.1 Safety assessments

None of the events identified to be of clinical importance per the ICH E 14 guidelines i.e. syncope, seizure, ventricular arrhythmias or sudden cardiac death, occurred in this study.

4.4.2 ECG assessments

Waveforms from the ECG warehouse were reviewed. According to ECG warehouse statistics over 96% of the ECGs were annotated in the primary lead II, with less than 0.1% of ECGs reported to have significant QT bias, according to the automated algorithm. Overall ECG acquisition and interpretation in this study appears acceptable.

4.4.3 PR and QRS intervals

Fampridine SR did not exert any significant effects on the PR or QRS intervals. The largest upper limits of 90% CI for the PR mean differences between fampridine SR 10 mg and placebo and fampridine SR 30 mg and placebo were 3.3 and 5.5 ms, respectively.

The largest upper limits of 90% CI for the QRS mean differences between fampridine SR 10 mg and placebo and fampridine SR 30 mg and placebo were 3.5 and 2.6 ms, respectively.

5 APPENDIX

5.1 HIGHLIGHTS OF CLINICAL PHARMACOLOGY

TOPIC	ACORDA'S RESPONSE	FAMPRIDINE REFERENCE
Therapeutic dose	10 mg, bid is the proposed therapeutic dose and the only dose strength studied in Phase 3 multiple sclerosis (MS) studies with Fampridine-SR (sustained release) tablets.	
Maximum tolerated dose	The overall experience with Fampridine-SR, as well as with adverse events in earlier published studies with immediate-release oral or intravenous formulations of 4-aminopyridine, indicates that the maximum tolerated dose varies for individual subjects. The tolerability and incidence of dropouts may also relate to the speed of dose escalation. **There appears to be an increased risk of seizure with higher doses, particularly above 30 mg bid.** Seizures have been observed in patients with MS at doses as low as 10 mg bid, but the incidence here is not readily distinguishable from the rate expected in the MS population generally. For practical purposes, a dose of 20 mg bid should be well tolerated. A supra-therapeutic dose of 30 mg bid would be expected to be tolerated by the majority of subjects, though dizziness, nausea, insomnia and other side effects may lead to a number of discontinuations at this dose level. A dose of 40 mg bid would be unlikely to be tolerated by many subjects, particularly with a rapid dose-escalation, and this dose is associated with a significant risk of seizure as an adverse effect event in populations with no known pre-disposition to seizure. The currently available dosage form is the 10 mg sustained-release tablet. The trial design selected for this study includes the therapeutic dose level 10 mg bid and the supra-therapeutic dose, 30 mg bid. The potential risk of escalation to the next higher dose of 40 mg bid does not appear to be acceptable.	Studies SCI-103, SCI-F201
Principal adverse events	Dizziness, nausea, parasthesias, seizures at high doses	Investigator Brochure

TOPIC	ACORDA'S RESPONSE	FAMPRIDINE REFERENCE
Maximum dose tested	Single Dose: 25 mg Multiple Dose: 60 mg, bid after 14 weeks of gradual dose titration from 20 mg, bid and with 31% dropout rate (patients with chronic spinal cord injury)	Studies SCI-101, MS-F200, FeFa25F-SR112003 Study SCI-103
Exposures achieved at maximum tested dose	Single Dose (25mg) Cmax = 55.8 ng/mL (CV 23.6%); AUC = 553.0 ng.hr/mL (CV 24.6%) Multiple Dose (60 mg, bid) Cmax = 152.0 ng/mL (CV 16.6%); AUC = 1075 mg.hr/mL (CV 15.2%)	Study FeFa25F-SR112003, Investigator Brochure Table 11. Study SCI-103
Range of linear PK	Linear within the ranges tested: 10-25 mg and 10-60 mg, bid (steady state)	
Accumulation at steady state	Fampridine –SR 20 mg bid in MS patients. R (based on Cmax) = 1.33 (CV 21.8%), time to steady state approximately 5 days	AN751-102 (Tables I and III, Appendix B)
Metabolites	Clinical studies have shown that Fampridine is metabolized less than 10% after an oral dose to humans. Two significant human Fampridine metabolites were identified as 3-hydroxy-4-aminopyridine and its sulfate conjugate (3-hydroxy-4-aminopyridine sulfate). An in vitro reaction phenotyping study was conducted with human liver microsomes and indicated that the primary CYP450 enzyme responsible for fampridine metabolism is CYP2E1. A similar metabolite profile was observed in the toxicology species (rat and dog) but the same two metabolites are produced at higher rates in rats and dogs.	Investigator brochure; Studies 0496002; ELN 14/972-921; RD10F-SR012004; ELN 9/960528; TPS 486A-501-510-93; XT064039
Absorption	Absolute/Relative Bioavailability Fampridine is well absorbed from the GI tract as indicated by the human ^{14}C Fampridine mass balance study. In this study, radiolabeled Fampridine administered as a single 15 mg oral solution to healthy subjects showed that excretion of radioactivity in the urine was virtually complete (greater than 94% total recovery of radioactivity) by 24 hours post-dose. Unchanged Fampridine	Study 0496002
	accounted for approximately 90% of the administered dose was excreted in the urine with metabolites accounting for only 10% of the total administered dose. Fecal excretion accounted for less than 1% of the administered dose.	

TOPIC	ACORDA'S RESPONSE	FAMPRIDINE REFERENCE
Distribution	Vd/F or Vd Vd approximately 179 L (female) 234 L (male) % free (not bound, in human plasma, 3 hr, 50 ng/mL) Mean percent free (%CV) = 97.5% (2.0%)	Population PK Report GM08-R97003 (Table 9) Study 6379-103 (Table 1)
Elimination	Route Fampridine and metabolites are primarily excreted in urine (~94%). A very small amount of the administered dose (<1%) is recovered in feces. Terminal t½ Mean (%CV) for parent = 6.45 h (20.4%) Mean (%CV) for metabolites = 7.61 h (38.1%) 3-hydroxy-4-aminopyridine sulfate (not measurable for 3-hydroxy-4-aminopyridine). CL/F or CL Mean (%CV) for parent: 22.2 L/h (26.7%); Mean (%CV) for metabolites: 23.9 L/h (73.2%) (3-hydroxy-4-aminopyridine); 7.61 L/h (39.6%) (3-hydroxy-4-aminopyridine sulphate)	Study 0496002; Investigator brochure, section 5.1. Study RD10F-SR012004 (Tables 11.4.1.1:1; 11.1.1.3:1) Study RD10F-SR012004 (Tables 11.4.1.1:1; 11.1.1.2:1; 11.1.1.3:1)

TOPIC	ACORDA'S RESPONSE	FAMPRIDINE REFERENCE
Intrinsic factors	<u>Age</u> Apparent clearance decreased linearly with age (slope -0.38) <u>Sex</u> Vd for women approximately 74% that for men (independent of weight difference). <u>Specify mean changes in Cmax and AUC</u>: (Normalized to 5mg dose, in multiple sclerosis patients, mean, 95%CI) • *Cmax*: Males 9.9 ng/mL (9.4,10.4); Females 14.6 ng/mL (13.8, 15.4). • *AUC*: Males 113 ng.hr/mL(106, 120); Females 163 ng.hr/mL (152, 175) <u>Race</u> Not studied. <u>Hepatic & Renal Impairment</u> • Hepatic Impairment Not studied • Renal Impairment Changes in pharmacokinetic parameters, Cmax and AUC were observed in renal impairment patients. The changes included: Based on geometric ratios, when compared to normal subjects, the peak and extent of exposure were 67% and 75% higher in mildly impaired subjects, 60% and 105% higher in moderately impaired subjects, 100% and 299% higher in severely impaired subjects, respectively.	Population PK Report GM08-R97003. Study AN751-101 (Table VIII, Appendix B) Study RD10F-SR012004 (Table 11.4.1.1:2)

TOPIC	ACORDA'S RESPONSE	FAMPRIDINE REFERENCE
Extrinsic factors	Drug interactions Treatment of cultured human hepatocytes with fampridine had little or no effect on CYP1A2, 2B6, CYP2C9, CYP2C19, CYP2E1, or CYP3A4/5 enzyme activities. In vitro studies with human liver microsomes revealed that there was no inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 at 4-aminopyridine concentrations up to 30 μM. There was evidence of direct inhibition of CYP2E1 by 4-aminopyridine at 30 μM (approximately 12% inhibition); however, the IC50 value for CYP2E1 was reported as greater than 30 μM which is approximately 100 times the average plasma 4-aminopyridine concentration measured for the 10-mg Fampridine-SR tablet. No clinically significant drug interactions identified from clinical studies. Two studies: Baclofen – no significant change in Cmax or AUC for fampridine or baclofen with co-administration Interferon beta – no significant change of fampridine Cmax or AUC with co-administration (interferon beta PK not studied) Food Effects Increase of Cmax of 12.7%, decrease of AUC 3.7% with high fat meal compared to fasted state (Fampridine-SR, 25 mg single dose).	XT073070 XT075077 Study 0194002 Study 1194-001US Investigator Brochure, Tables 15, 16. Study FeFa25F-SR112003; Investigator Brochure, Table 11
Expected high clinical exposure scenario	Other than accidental or deliberate 4-aminopyridine overdose, the greatest increase in exposure would be expected in patients with renal impairment. In the case of patients with moderate renal impairment, the single dose Cmax increases approximately 60% and the AUC is approximately doubled. Based upon the results from the renal impairment study severely renally impaired MS patients are excluded from the Phase 3 Fampridine-SR studies.proposed. It may be expected that patients with severe renal deficiency, based on an increase of 100% in Cmax and 299% in AUC, which would greatly increase the potential for central nervous system side effects, particularly convulsion, with these elevated plasma levels. For the same reason, the supratherapeutic dose to be studied is restricted to 30 mg bid in the Thorough QT study.	

5.2 TABLE OF STUDY ASSESSMENTS

Trial Phase	Screening	Day of Admission	Baseline	Treatment Phase		Discharge[e]
Day	-30 through Day -2[#]	-2	-1	1-4	5	6
Obtain informed consent	X					
Medical history	X	X[a]				X
Prior/concomitant medication assessment	X	X[a]				
Standard EEG	X					
Safety laboratory	X	X				X
Serum pregnancy test	X	X		X (day 3 only)		X
Drugs of abuse	X	X				
Serology status defined	X	X				
Physical examination	X	X[b]		X[b]	X[b]	X[b]
Routine vital signs	X	X		X	X	X
Safety digital ECGs at Site	X	X		X* (days 1&3 only)		X
12 lead ECG from H-12 (flash card placed in AM of Days-1,5)			X[c]		X[c]	
PK Blood samples					X[d]	
AE Assessment	X	X	X	X	X	X
Randomization			X[#]			
Dispense investigational drug				X	X	

* At 2-3 hours after dose; [#] Randomization could be performed at the end of Day -1 or on Day 1 immediately before dosing; [a] brief update; [b] Problem-based physical examination, only; [c] ECGs at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, 23.5 hours post dose on Days -1 and 5; [d] predose (trough level), and 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, and 23.5 hours post dose (Plasma samples were obtained from all subjects on all treatments to maintain comparable trial conditions and the blind, but were only analyzed for subjects in the Fampridine-SR groups.); [e] or at time of premature discontinuation.

Source: Table 9.1 (Study Schematic) from the CSR for TQTc-F-SR001

Linked Applications	Sponsor Name	Drug Name
IND 17627	ACORDA THERAPEUTICS INC	4-AMINOPYRIDINE

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12/04/2008

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12/05/2008
Dr. Yang yang was the primary statistical reviewer for this QT study.

SUCHITRA M BALAKRISHNAN
12/05/2008

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12/05/2008

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22250	ORIG-1	ACORDA THERAPEUTICS INC	FAMPRIDINE TABLETS

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