Office of Clinical Pharmacology Review

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Submission Type	Pediatric efficacy supplement
Brand Name	Amitiza
Generic Name	Lubiprostone
Dosage Form and Strength	Capsule, 24 mcg, 8 mcg
Route of Administration	Oral
Proposed Indication	Pediatric functional constipation
Applicant	Sucampo
Associated IND	IND 059623
OCP Review Team	Sojeong Yi, PhD Justin Earp, PhD Insook Kim, PhD
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1. EXECUTIVE SUMMARY

Amitiza (lubiprostone capsule) 24 mcg BID was originally approved in adults for use in the treatment for chronic idiopathic constipation (CIC) on January 31, 2006, and subsequently for irritable bowel syndrome with constipation (IBS-C) in adult women and for use in patients with opioid-induced constipation (OIC) in non-cancer pain patients.

In this supplemental NDA, the applicant proposes to expand the use of Amitiza 24 mcg BID to the indication of pediatric functional constipation (PFC), which is regarded as pediatric equivalent CIC, in patients aged 10 to 17 years. To support the proposed indication, the applicant submitted the data from one double-blinded, placebo-controlled phase 3 trial (Study PFC-1131), one open-label phase 2 trial (Study SC-0641), and two long-term open-label extension studies (Study PFC-11S1 and SCMP-303) in pediatric patients with PFC.

The applicant proposes to fulfill the following post-marketing requirement under the Pediatric Research Equity Act (PREA):

> PMR 572-4: Conduct a safety and efficacy study in pediatric patients with chronic idiopathic constipation ages ≥ 6 Years to <18 Years

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the submission and found no clinical pharmacology issues that preclude the approval.

Since the efficacy of the product was not established in pediatric patients aged 10-17 years, labeling of the pertinent pharmacokinetic (PK) data in pediatric patients is not recommended.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Lubiprostone is **a locally acting** chloride channel activator that enhances a chloride-rich intestinal fluid secretion without altering sodium and potassium concentrations in the serum. In adult, lubiprostone has **low systemic availability** following oral administration and concentrations of lubiprostone in plasma are below the level of quantitation (10 pg/mL). Therefore, standard pharmacokinetic parameters of lubiprostone cannot be reliably calculated. However, the pharmacokinetic parameters of M3, which is only measurable active metabolite of lubiprostone, have been characterized. Lubiprostone is extensively metabolized primarily by carbonyl reductase that is ubiquitously expressed, but not hepatic cytochrome P450 system.

The PK of lubiprostone and M3 were studied in pediatric patients with PFC in three clinical trials (i.e., Study SC-0641, Study PFC-1131, and Study PFC-11S1). In pediatric patients ≥ 6 years, lubiprostone was not detected in any plasma samples collected from three clinical trials

consistently in adults. Due to sparsity of M3 concentration data from Study PFC-1131/PFC-11S1, reliable pharmacokinetic parameters of M3 were only available from Study SC-0641.

In Study SC-0641, The PK of M3 was studied in pediatric patients with PFC aged 4-17 years after a single dose of lubiprostone 12 mcg or 24 mcg using intensive PK blood sampling. In pediatric patients weighing < 36 kg, only the 12-mcg dose was studied whereas in patients weighing ≥ 36 kg both 12 and 24 mcg doses were studied. The following single dose PK data was based on the data from Study SC-0641.

Dose-proportionality: In pediatric patients weighing ≥ 36 kg, the mean C_{max} and AUC increased in a dose-proportional manner following a single dose of lubiprostone 12 mcg and 24 mcg.

Pediatric patients vs. adults: Following a single dose of lubiprostone 24 mcg, the C_{max} and AUC_{0-t} of M3 in pediatric patients weighing ≥ 36 kg mostly overlapped with those of healthy adults in a cross-study comparison. (i.e., the mean C_{max}: 41.8 pg/mL vs. 41.9 pg/mL; the mean AUC_{0-t}: 58.5 h*pg/mL vs. 59.1 h*pg/mL) (Figure 1).

Body weight: The mean AUC of M3 tended to be higher in pediatric patients as the body weight decreases. Following a single dose of lubiprostone 12 mcg, the mean AUC of M3 in patients weighing 12 to < 24 kg and 24 to < 36 kg were about 4-fold and 1.4-fold higher compared to patients weighing ≧ 36 kg, respectively (Figure 1).

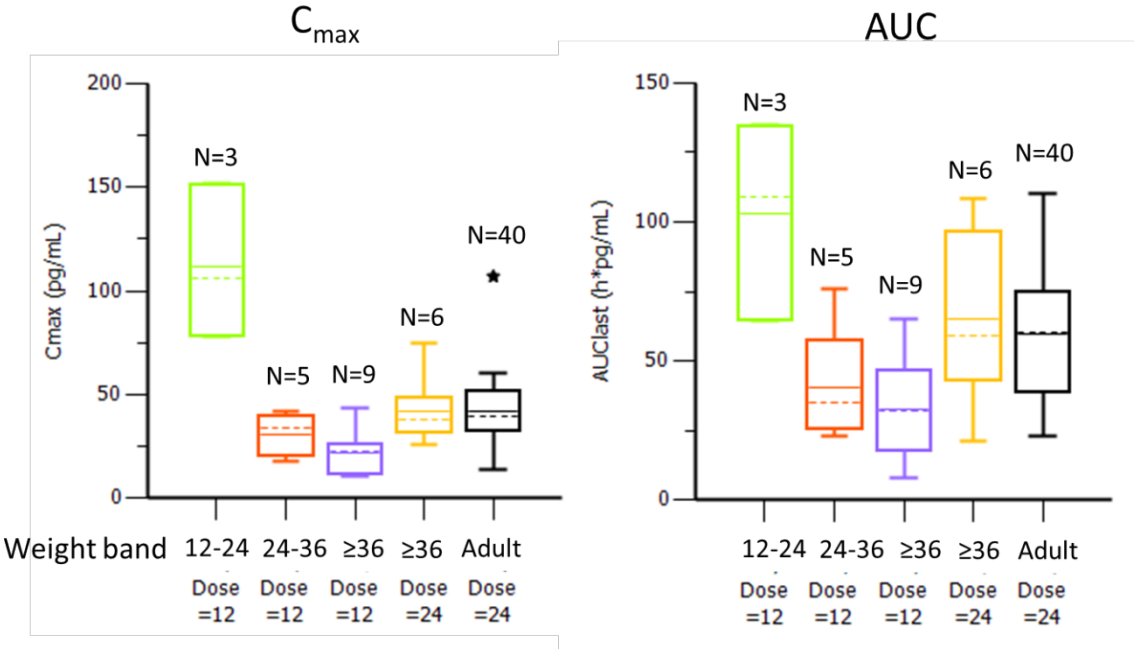


Figure 1. Comparison of C_{max} and AUC of M3 between pediatric patients with PFC aged 4 to 17 years (Study SC-0641) vs. healthy adults (Study SPI/0211SC-041) (Reviewer's plot)

Age: The AUC of M3 in adolescents is similar to adults (at lubiprostone 24 mcg) whereas children younger aged 6 to 11 years shows 2-fold higher AUC of M3 than adolescents aged 12 to 17 years (at lubiprostone 12 mcg) (Table 1). Comparison of PK across age groups following 24 mcg single dose is limited due to the small number of patients in each age group.

Table 1. Pharmacokinetic parameters of M3 in patients with PFC by age group (Reviewer's calculation)

Study	Population	Dose (mcg)	N	T_{max} (h) Median (Min-Max)	C_{max} (pg/mL) Mean ± SD	AUC_{0-t} (h•pg/mL) Mean ± SD
SC-0641	6-11 years	12	7	1 (0.5-2)	51.5 ± 50.1	62.3 ± 40.7
		24	2	0.5-1	57.25	50.7
	12-17 years	12	8	1 (0.5-6)	21.5 ± 12.0	27.9 ± 14.1
		24	4	1 (0.5-2)	34.1 ± 6.1	72.4 ± 38.1

In Study PFC-1131/PFC-11S1 in which sparse PK sampling was conducted, the highest M3 concentrations observed ranged from 50.1 to 238 pg/mL in pediatric patients weighing ≥ 50 kg following lubiprostone 24 mcg BID. The upper value of the range (i.e., 238 pg/mL) was approximately 2.5-fold higher than the upper value of C_{max} range (i.e., 13.8-107 pg/mL) in adults after a single dose of lubiprostone 24 mcg.

2.2 Dosing and Therapeutic Individualization

The clinical efficacy of lubiprostone in pediatric patients with PFC aged 6 to 17 years were primarily evaluated through the Phase 3 clinical study (Study PFC-1131) using overall spontaneous bowel movement (SBM) response rate as the primary endpoint. Based on the Phase 3 clinical study results, lubiprostone 24 mcg BID was proposed for PFC in patients aged 10 to 17 years. However, the efficacy of lubiprostone 24 mcg BID in pediatric patients aged 10 to 17 years was not established in this development program. Refer to the clinical and statistics review by Dr. Hart and Dr. Ling for the details. Therefore, we discuss the dose selection rationale in this review.

Dose selection rationale

The doses for Study SC-0641 were selected by allometric scaling from the approved adult dose for CIC, i.e., 24 mcg BID ranging from 0.5 to 1.1 mcg/kg BID. In pediatric patients ≥ 36 kg, two dose levels were studied, i.e., 12 mcg BID and 24 mcg BID, while in pediatric patients 12 to < 24 kg and 24 to < 36 kg, one dose level was studied, i.e., 12 mcg QD and 12 mcg BID, respectively. Thus, a dose-response relationship could be observed in pediatric patients weighed ≥ 36 kg. Comparing weekly SBM frequency between 12 mcg BID and 24 mcg BID in patients ≥ 36 kg, the 24 mcg BID treatment resulted in numerically higher SBM frequency over 4 weeks

compared to 12 mcg BID (Figure 2). However, the difference in the mean change from baseline in weekly SBM frequency became smaller at Week 4 (1 vs. 1.4). As the open-label and uncontrolled design of Study SC-0641, and relatively short treatment duration (i.e., 4 week) limited the interpretability of the observed dose-response relationship for SBM.

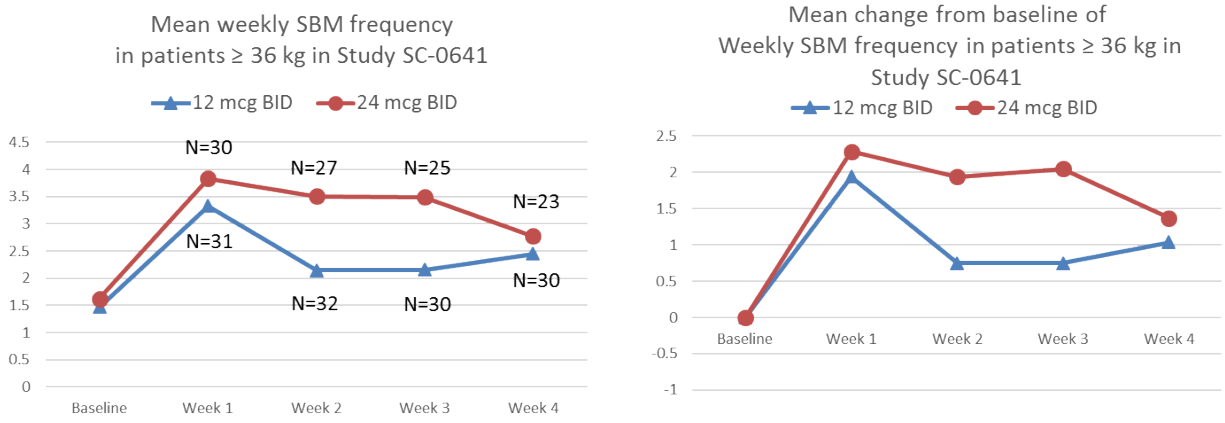


Figure 2. Comparison of weekly SBM frequency between treatment groups within pediatric patients with PFC weighed ≥ 36 kg in Study SC-0641 (Left: the mean weekly SBM frequency; Right: the mean change from baseline of weekly SBM frequency) (Reviewer's plot)

In the subsequent phase 3 trial (Study PFC-1131) in pediatric patients with PFC, only one dose level was studied in a body weight cohort, i.e., 12 mcg BID or 24 mcg BID based on body weight band, i.e., < 50 kg and ≥ 50 kg, respectively. A different body weight cut-off for dosing, i.e., 50 kg, was chosen based on the observation of higher treatment-related AE incidence at 24 mcg BID than at 12 mcg BID in patients ≥ 36 kg in Study SC-0641. However, following the 12-week treatment, neither of body weight cohorts did not show significant improvement in overall SBM response rate compared to placebo in Study PFC-1131. Any dose higher than 24 mcg BID was not studied in pediatric patients.

Of note, in the Phase 2 clinical study in adults with CIC at 24, 48 (24 mcg BID), and 72 mcg/day (24 mcg TID) doses for 3-week treatment (Study SPI/CTR02-004), no statistically significant improvement was observed beyond 24 mcg/day in terms of weekly SBM frequency. Based on the subsequent phase 3 trials in adults with CIC in which the dose of 48 mcg/day (24 mcg BID) had been chosen, 24 mcg BID was ultimately approved for CIC in adults.

2.3 Outstanding Issues

There is no clinical pharmacology issue identified.

2.4 Summary of Labeling Recommendations

Since the efficacy of the product was not established in pediatric patients aged 10-17 years, labeling of the pertinent PK data in pediatric patients is not recommended.

If the PK information of M3 in pediatric patients should be described in the label, we recommend using observed PK parameters of M3 only from Study SC-0641, but not including the PK data from Study PFC-1131 and Study PFC-1S11 which is unreliable due to the sparsity of the data.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Amitiza (lubiprostone capsule) was approved in adults for use in the treatment for CIC at 24 mcg BID on January 31, 2006, and for a treatment for IBS-C in adult women at 8 mcg BID on April 29, 2008. On April 19, 2013, Amitiza was also approved for use in patients with OIC due to the use of opioids for chronic, non-cancer pain at 24 mcg BID.

At the time of approval for CIC, a PREA PMR was established to evaluate the treatment of CIC in pediatric patients ages 0 to 17 years. Even though the original PREA requirement was to study in pediatric patients with CIC, the applicant proposed clinical studies in patients with PFC. The applicant subsequently conducted Study SC-0641 as a 4-week open-label study in children aged 3-17 years with PFC and submitted the study results as a labeling supplement NDA 21908/S-008 on May 29, 2009. However, the agency determined that it did not fulfill PMR as it was not placebo-controlled study and did not have long-term safety assessments. Further, through discussions between the agency and the applicant in March 2013 and August 2013, the agency agreed that the pediatric population with PFC is the pediatric equivalent of CIC in adult. In addition, a formal waiver for pediatric patients aged < 6 months were granted on February 11, 2016 based on no patients with CIC in the age group < 6 months. Thus, the PREA PMRs were revised to the following:

> PMR 572-4: Conduct a safety and efficacy study in pediatric patients with chronic idiopathic constipation ages ≥ 6 Years to <18 Years
>
> PMR 572-5: Conduct a safety and efficacy study in pediatric patients with chronic idiopathic constipation ages ≥ 6 Months to < 6 Years.

In fulfillment of PREA PMR 572-4, the applicant conducted following four clinical trials to evaluate the efficacy and safety of lubiprostone in patients aged ≥ 6 to < 18 years with PFC:

- Study SC-0641: An exploratory, 4-week, open-labeled, uncontrolled, safety, efficacy and pharmacokinetics study in PFC patients aged < 18 years

- Study PFC-1131: A pivotal, 12-week, double-blinded, placebo-controlled, efficacy, safety and pharmacokinetics study in PFC patients aged ≥ 6 to < 18 years
- Study PFC-11S1: A long-term extension, 36-week, open-labeled, uncontrolled, long-term safety, efficacy and pharmacokinetics study to which patients completing the preceding PFC-1131 were enrolled
- Study SCMP-303: An additional, 24-week, open-labeled, uncontrolled, long-term safety study in PFC patients aged ≥ 6 to < 18 years

In this supplemental NDA, the applicant proposes to expand the use of Amitiza 24 mcg BID to the indication of PFC in patients aged 10 to 17 years by describing the results of the clinical data obtained from the 4 studies above. The data was discussed with the Agency in pre-NDA meeting on February 8, 2017. The applicant also proposes to fulfill a PMR 572-4. Of note, to fulfill PMR 572-5 for patients aged 6 months to < 6 years, the clinical development program is ongoing with a separate age-appropriate formulation.

Additionally, for detailed review on the Study SC-0641, please refer to Clinical Pharmacology Review of a labeling supplement NDA 21908/S-008 dated November 9, 2009, by Christian Grimstein, PhD and Dennis Bashaw, PharmD.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Following is excerpted from the approved label of Amitiza.

Mechanism of Action

Lubiprostone is a locally acting chloride channel activator that enhances a chloride-rich intestinal fluid secretion without altering sodium and potassium concentrations in the serum. Lubiprostone acts by specifically activating ClC-2, which is a normal constituent of the apical membrane of the human intestine, in a protein kinase A–independent fashion. By increasing intestinal fluid secretion, lubiprostone increases motility in the intestine, thereby facilitating the passage of stool and alleviating symptoms associated with chronic idiopathic constipation.

Pharmacokinetics

Lubiprostone has low systemic availability following oral administration and concentrations of lubiprostone in plasma are below the level of quantitation (10 pg/mL). Therefore, standard pharmacokinetic parameters such as area under the curve (AUC), maximum concentration (C_{max}), and half-life ($t_{½}$) cannot be reliably calculated. However, the pharmacokinetic parameters of M3, which is only measurable active metabolite of lubiprostone, have been characterized in adults and in pediatric patients.

In adults, peak plasma levels of M3, after a single oral dose with 24 mcg of lubiprostone, occurred at approximately 1.10 hours. The C_{max} was 41.5 pg/mL and the mean AUC_{0-t} was 57.1

pg·hr/mL. M3 has a $t_{½}$ ranging from 0.9 to 1.4 hours. The AUC_{0-t} of M3 increases dose proportionally after single 24-mcg and 144-mcg doses of lubiprostone.

In vitro protein binding studies indicate lubiprostone is approximately 94% bound to human plasma proteins. Lubiprostone is rapidly and extensively metabolized by 15-position reduction, α-chain β-oxidation, and ω-chain ω-oxidation. These metabolisms are not mediated by the hepatic cytochrome P450 system but rather appear to be mediated by the ubiquitously expressed carbonyl reductase. M3 makes up less than 10% of the dose of radiolabeled lubiprostone. Systemic exposure to M3 increase 2- and 5-fold in moderate and severe hepatic impairment, respectively, compared to normal liver function. After a single oral dose of 72 mcg of ^{3}H-labeled lubiprostone, 60% of total administered radioactivity was recovered in the urine within 24 hours and 30% of total administered radioactivity was recovered in the feces by 168 hours. The mean elimination half-life of total radioactivity in plasma was 3 hrs. Pharmacokinetic parameters of total radioactivity following 72-mcg dose at fed status demonstrated that C_{max} decreased by 55% while $AUC_{0-\infty}$ was unchanged when lubiprostone was administered with a high-fat meal.

3.3 Clinical Pharmacology Review Questions

3.3.1 Is there a dose-response relationship on efficacy or safety for lubiprostone in pediatrics?

Efficacy

Ultimately, the efficacy of lubiprostone 24 mcg BID in pediatric patients was not established in this development program. Nonetheless, by our exploratory visual investigation, we found some tendency of dose-dependent increase between 12 mcg BID and 24 mcg BID in weekly SBM frequency which was one of secondary endpoints, but it was neither significant nor consistent across studies. Any dose higher than 24 mcg BID was not studied in pediatric patients, therefore it is unknown if a higher dose might have been efficacious.

Dose-response relationship could be evaluated between 12 mcg BID and 24 mcg BID in Study SC-0641 for patients ≥ 36 kg whereas dose-response analysis in Study PFC-1131 and PFC-11S1 was impractical because only one dose level was studied in a body weight cohort, i.e., 12 mcg BID for patients < 50 kg and 24 mcg BID for patients ≥ 50 kg. Thus, for Study PFC-1131, we tried comparison between the dose escalation group (< 50 kg, 24 mcg BID) and the other dose groups (< 50 kg, 12 mcg BID; ≥ 50 kg, 24 mcg BID), instead.

Reviewer's comment: *For the reason mentioned above, the exposure-efficacy/response analysis that the applicant provided (Report SAG-CTR17-001) is deemed inadequate to evaluate dose-response relationship or exposure-response relationship with the integrated dataset not taking 'weight-based dosing' into account. Further detail, see* *Appendix 4.2. Dose-Response Analyses.*

In **Study SC-0641**, 61 pediatric patients were ≥ 36 kg out of 124 participants with PFC. Subsequently, 31 were assigned to 12 mcg BID and 30 were assigned to 24 mcg BID. Comparing the mean weekly SBM frequency between 12 mcg BID and 24 mcg BID within patients ≥ 36 kg, 24 mcg BID showed higher numbers of SBM frequency over 4 weeks compared to 12 mcg BID but it was not statistically significant between two dose groups (Figure 2, See Page 6). However, the open-label and uncontrolled design of Study SC-0641, and relatively short treatment duration (i.e., 4 week) limited the interpretability of the observed dose-response relationship for weekly SBM frequency.

In **Study PFC-1311,** a total 120 out of 223 pediatric patients (54.6%) who were initially assigned to 12 mcg BID escalated dose to 24 mcg BID at Week 1 (mITT1 population) due to insufficient efficacy (i.e., < 3 SBMs up to Day 7). The distribution of dose escalating group was relatively even across the different age group (Table 7, See Page 19). Within the dose escalation group, the mean weekly SBM frequency was slightly increased at Week 2 compared to those at Week 1 (Figure 3), but it was unclear whether the increase in SBM frequency was attributed to either dose escalation or cumulative drug effect over 2 weeks. In addition, the mean weekly SBM frequency of the dose escalation group was lower than both the placebo group and 12 mcg BID treatment without dose escalation throughout 4 weeks, and it turned similar to the placebo group at Week 4 (Figure 3). This exploratory analysis indicates that the insufficient drug response at Week 1 did not necessarily resolved by the dose escalation. In other words, the insufficient drug response was not solely caused by the insufficient dose to show efficacy.

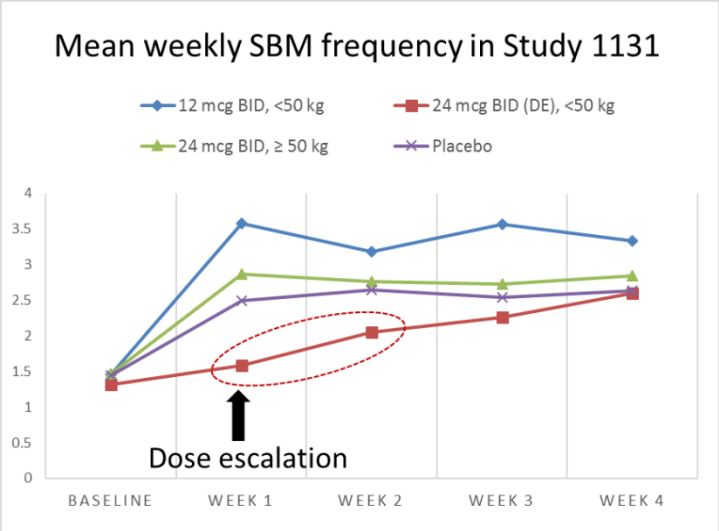
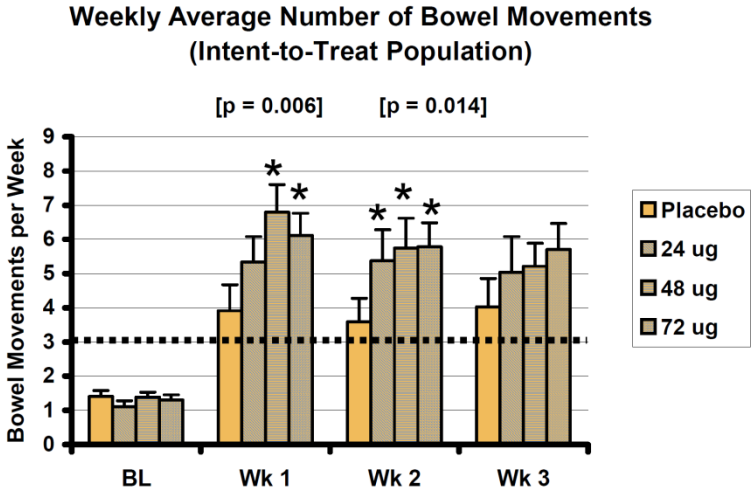


Figure 3. Mean weekly SBM frequency in Study PFC-1131 by treatment group (mITT1 population) (Reviewer's plot)

Of note, in adults with CIC at lubiprostone 24, 48, and 72 mcg/day over 3 -week treatment (Study SPI/CTR02-004, a phase 2 dose-finding clinical study), the doses greater than 24 mcg/day did not show statistically significant improvement in weekly SBM frequency (Figure

4). The dose of 48 mcg/day (24 mcg BID) was chosen for the subsequent phase 3 trials in CIC and ultimately approved for CIC based on Phase 3 studies. For detailed review on dose-response relationship in adult patients with CIC from the Study SPI/CTR02-004, refer to Clinical Pharmacology Review of original NDA 21908 dated January 5, 2006, by Suliman Al-fayoumi, PhD and Dennis Bashaw, PharmD.



[ ] = statistically significant overall p-value based on a Cochran-Mantel Haenszel (CMH) test using modified ridit scores, controlling for site, and using Shaffer's modified sequentially rejective multiple test procedure.
* = statistically significant pairwise comparison based on a Cochran-Mantel Haenszel (CMH) test comparing placebo to active drug using modified ridit scores, controlling for site and using Shaffer's modified sequentially rejective multiple test procedure.

Figure 4. Weekly SBM frequency in adults with CIC (Study SPI/CTR02-004)

Safety

In adults with CIC, a dose-dependent increase in gastrointestinal adverse events (GI-related AEs) were observed (refer to Clinical Review of original NDA 21908 dated December 5, 2005 by Dr. Kristen Buck and Dr. Ruyi He). Compared to adult with CIC, in pediatric patients with PFC vomiting and abdominal pain was more frequent whereas diarrhea and nausea was less. Refer to Clinical Review by Dr. Hart for the review of safety.

Since either 24 mcg BID or 12 mcg BID was studied in pediatric patients ≥50 kg or < 50 kg, a dose-dependency could not be evaluated without confounding effect by body weight. Nevertheless, a dose-dependent increase in incidence of GI-related AEs (e.g. diarrhea, vomiting, nausea, and abdominal pain) was observed within patients < 50 kg. A dose-response relationship for AEs was explored between 12 mcg BID group and the dose-escalation group from 12 mcg to 24 mcg in patients < 50 kg based on the incidence of 'most common adverse events' (i.e.,

reported by >4% of patients treated with lubiprostone 24 mcg BID, and at an incidence rate greater than observed for the placebo group) in the 'overall safety cohort' (i.e., all safety data from four clinical trials submitted) (Table 2). Even though there was no clear dose-dependent increase in overall AE incidence, the incidences of GI-related AEs appeared higher with 24 mcg BID compared to that with 12 mcg BID in the patients < 50 kg. This observation is in line with the finding in adults with CIC from Phase 2b study (Study SPI/CTR02-004); the incidences of GI-related AEs were evidently dose-related with lubiprostone administration with placebo, 12 mcg BID, 24 mcg BID, or 24 mcg TID.

Additionally, at 24 mcg BID dose, the incidences of GI-related AEs were higher in patients < 50 kg compared to patients ≥ 50 kg but it might be confounded by age effect. For further detail about 'Dose-Safety (Adverse Events) Analysis', see Appendix 4.2. Dose-Response Analyses.

Table 2. Incidence of Most Common Adverse Events – Overall Safety Cohort[1)]

N (%)	Placebo (N=195)	12 mcg BID (N=306)	24 mcg BID (n=124)	24 mcg BID (n=288)	Adults with CIC 24 mcg BID (N=1113)[2)]
		Body weigh < 50 kg	Body weigh < 50 kg	Body weigh ≥ 50 kg	
Treatment-related AE	105 (53.8)	107 (35.0)	66 (53.2)	121 (42.0)	646 (58.0)
Diarrhea	6(3.1)	24 (7.8)	12 (9.7)	32 (11.1)	147 (13.2)
Vomiting	12 (6.2)	45 (14.7)	32 (25.8)	28 (9.7)	51 (4.6)
Nausea	13 (6.7)	40 (13.1)	28 (22.6)	49 (17.0)	346 (31.1)
Abdominal pain/ Abdominal pain upper	23 (11.8) 6 (3.1)	28 (9.2) 14 (4.6)	18 (14.5) 13 (10.5)	38 (13.2) 13 (4.5)	75 (6.7) 24 (2.2)
Headache	11 (5.6)	24 (7.8)	15 (12.1)	34 (11.8)	147 (13.2)
Dizziness	6 (3.1)	4 (1.3)	8 (6.5)	14 (4.9)	46 (4.1)
Nasopharyngitis	8 (4.1)	2 (7.4)	7(5.6)	17 (5.9)	32 (2.9)

1) Overall Safety (OS) Cohort: includes pooled data from studies PFC-1131, PFC-11S1, SCMP-303 and SC-0641. Placebo, 12 mcg QD, 12 mcg BID, and 24 mcg BID doses are represented (with 24 mcg BID group combining all patients who were initially assigned to 24 mcg BID or who titrated from 12 mcg to 24 mcg BID at Week 1 in study PFC-1131). (Modified from 'Integrated Summary of Safety Table 2.4.8.2.')

2) General Safety cohort: studies SC9921, SC0131, SC01S1, SC0232, SC01S2 (both study periods), and SC02S3 were pooled submitted in original NDA 21908.

3.3.2 Is drug exposure in pediatrics aged 6-17 years comparable to those in adults?

Even though plasma concentrations of M3 were measured in three clinical trials (i.e., Study SC-0641, Study PFC-1131, and Study PFC-11S1), reliable pharmacokinetic parameters of M3 were only available from Study SC-0641 due to sparsity of M3 concentration data from Study PFC-1131/PFC-11S1 (Refer to Appendix 4.1. Individual Study Reviews). In Study PFC-1131/PFC-

11S1, plasma samples were sparsely collected and 97% samples from patients who received lubiprostone were below the level of quantitation.

Dose-proportionality between 12 mcg and 24 mcg

In pediatric patients ≥ 36 kg, C_{max} and AUC_{0-t} of M3 dose-proportionally increased after a single dose of 12 mcg and 24 mcg (i.e., C_{max}: 19.5 ± 12.8 pg/mL and 41.8 ± 17.0 pg/mL; AUC_{0-t}: 23.0 ± 19.2 h*pg/mL and 58.5 ± 30.6 h*pg/mL at 12 mcg and 24 mcg, respectively) (**Error! Reference source not found.**). This finding is in line with the observation in adults in Study SPI/0211SC-041 indicating dose-proportional increase in AUC_{0-t} of M3 after a single dose of 24 mcg and 144 mcg.

***Reviewer's comment:** For the 24-mcg dose, the approved Amitiza 24 mcg capsule was used whereas the 12- mcg lubiprostone capsule was neither approved nor studied in adults. No relative bioavailability was conducted comparing 24-mcg and 12-mcg formulations.*

Comparison between pediatrics and adults.

At a single dose of lubiprostone 24 mcg, the range of C_{max} and AUC_{0-t} of M3 in pediatric patients weighing ≥ 36 kg was mostly overlapped with those of healthy adults from Study SPI/0211SC-0411 (i.e., C_{max}: 41.8 ± 17.0 pg/mL vs. 41.9 ± 15.6 pg/mL; AUC_{0-t}: 58.5 ± 30.6 h*pg/mL vs. 59.1 ± 21.8 h*pg/mL) (Figure 1, Page 4; **Error! Reference source not found.**).

Table 3. PK parameters of M3 in pediatrics patients with PFC aged 4 to 17 years by body weight group compared healthy adults

Study	Population	Dose (mcg)	N	T_{max} (h) Median (Min-Max)	C_{max} (pg/mL) Mean ± SD	AUC_{0-t} (h•pg/mL) Mean ± SD
0211SC-041	Adult	24	40	1 (0.5-3.0)	41.9 ± 15.6	59.1 ± 21.8
SC-0641	12 kg ≤ BW< 24 kg	12	3	0.5	112.0 ± 37.4	103 ± 35.6
	24 kg ≤ BW< 36 kg	12	5*	1 (0.5-1.0)	26.4 ± 17.5	32.6 ± 24.0
	BW ≥ 36 kg	12	9	1.25 (0.5-6.0)	19.5 ± 12.8	23.0 ± 19.2
	BW ≥ 36 kg	24	6	1.0 (0.5-2.0)	41.8 ± 17.0	58.5 ± 30.6

*A total 6 patients were studied but since two samples of one patient were excluded due to sample labeling error, PK parameters were only calculated for N=5.

In contrast, in Study PFC-1131/PFC-11S1, in patients ≥ 50 kg who took 24 mcg BID, the highest M3 concentrations observed ranged from 50.1 to 238 pg/mL, which was approximately 2.5-fold higher than the range of C_{max} in adults after a single dose of 24 mcg, i.e., 13.8 to 107 pg/mL (Figure 5, Table 4). Since no pharmacokinetic data is available for adults following multiple doses of 24 mcg BID, a head-to-head comparison between pediatrics and adults could not be done. Nonetheless, this finding implies that M3 concentration may be potentially higher in pediatrics than adults after multiple doses considering that M3 is unlikely to be significantly

accumulated in the body following multiple doses based on short elimination half-life of M3, 0.9-1.4 hours.

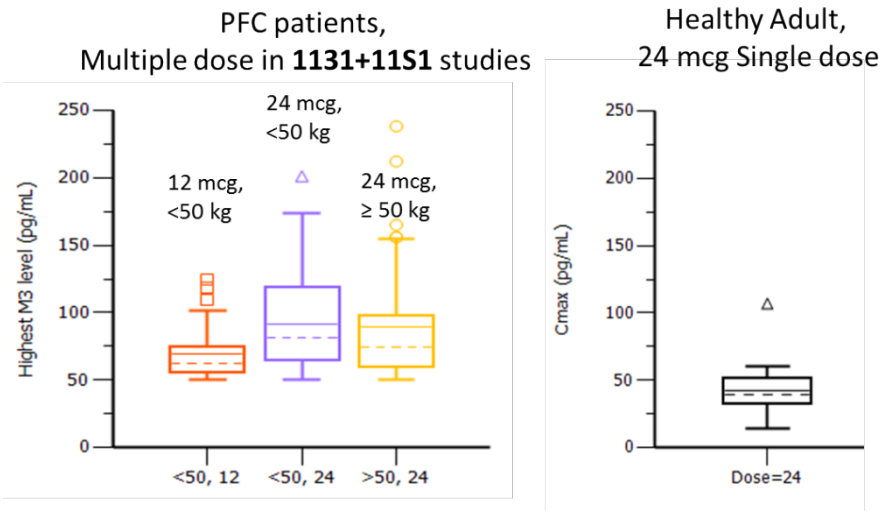


Figure 5. The highest M3 concentrations observed in pediatric patients with PFC aged 6 to 17 years (Study PFC-1131/PFC-11S1) compared to healthy adults (Study SPI/0211SC-041) (Reviewer's plot)

Table 4. The M3 concentration observed in pediatric patients with PFC aged 6 to 17 years compared to healthy adults (Reviewer's calculation) *

	Study PFC-1131/PFC-11S1 in 6-17-year-old children with PFC			Study SPI/0211SC-041 in healthy adult
Dose administration	12 mcg BID	24 mcg BID	24 mcg BID	24 mcg single dose
Body weight	< 50 kg	< 50 kg	≥ 50 kg	-
The mean (min, max) of the highest M3 concentration observed (pg/mL)	69.6 (50.1, 126)	94.3 (50.3, 201)	89.6 (50.1, 238)	41.5 (13.8, 107)
The proportion of patients who showed measurable M3 levels	33/306 (10.8%)	24/86 (27.9%)	42/224 (18.8%)	-

* Data was extracted from 'LubERS04OCT2017.csv' submitted in SDN 1570

Comparison between different body weight cohorts

Body weight tends to affect the systemic exposure of M3, especially for body weight < 24 kg. Following a single dose of 12 mcg, the mean AUC of M3 in patients weighing 12 to < 24 kg and 24 to < 36 kg were about 4-fold and 1.4-fold higher compared to patients ≥ 36 kg, respectively

(Figure 1, Page 4; **Error! Reference source not found.**). It is unclear as to why the systemic exposure to M3 in patients weighing 12 to < 24 kg was substantially higher than patients ≥ 24 kg although it needs to be noted that patient group weighing 12 to < 24 kg only consisted of N=3.

However, in Study PFC-1131/PFC-11S1, at the same dose of lubiprostone 24 mcg BID, the range of the highest M3 concentrations observed was not significantly different between pediatric patients < 50 kg and ≥ 50 kg (i.e., 50.3-201 pg/mL vs. 50.1-238 pg/mL) (Figure 6, Table 4). There is still a possibility that the difference in M3 concentrations between body weight groups could not be captured due to the sparsity of the data.

Comparison between different age groups

The systemic exposure of M3 in adolescents is similar to adults (at lubiprostone 24 mcg) whereas children younger than 12 years shows higher exposure than adolescents (at lubiprostone 12 mcg).

As the applicant claimed the indication for pediatrics aged 10-17 years old, C_{max} and AUC_{0-t} of M3 from Study SC-0641 were re-categorized by the age group, i.e.,10-17 years and 4-9 years. The pharmacokinetic parameters of M3 at 24 mcg in patients aged 10-17 years were in line with those in adults. However, the patients aged 4-9 years taking 12 mcg BID showed 3.5-fold C_{max} and 2.6-fold AUC_{0-t} compared to the patients aged 10-17 years taking the same dose (Table 5).

Additionally, we re-categorized C_{max} and AUC_{0-t} of M3 by 6 to 11 years and 12 to 17 years which is aligned with PMR 572-4 (i.e., safety and efficacy in pediatrics aged 6 to 17 years). At 24 mcg, C_{max} and AUC_{0-t} of M3 were comparable between adolescents aged 12 to 17 years and adults. However, at 12 mcg, C_{max} and AUC_{0-t} of M3 were approximately 2.5-fold higher in children aged 6 to 12 years compared to adolescents aged 12 to 17 years (Table 5).

Table 5. PK parameters of M3 in pediatrics patients with PFC by age group compared healthy adults

Study	Population	Dose (mcg)	N	T_{max} (h) Median (Min-Max)	C_{max} (pg/mL) Mean ± SD	AUC_{0-t} (h•pg/mL) Mean ± SD
0211SC-041	Adult	24	40	1 (0.5-3.0)	41.9 ± 15.6	59.1 ± 21.8
SC-0641 (Reviewer's calculation)	4-9 years	12	6	0.5 (0.5-1.0)	82.3 ± 38.9	83.9 ± 41.6
		24	1	1.0	39.7	50.4
	10-17 years	12	11	1.0 (0.5-6.0)	23.2 ± 11.4	32.3 ± 16.8
		24	5	1.0 (0.5-2.0)	42.3 ± 18.9	68.1 ± 34.4
	6-11 years	12	7	1.0 (0.5-2.0)	51.5 ± 50.1	62.3 ± 40.7
		24	2	0.5-1.0	57.25	50.7
	12-17 years	12	8	1.0 (0.5-6.0)	21.5 ± 12.0	27.9 ± 14.1
		24	4	1.0 (0.5-2.0)	34.1 ± 6.1	72.4 ± 38.1

4. APPENDICES

4.1 Individual Study Reviews

In this submission, the clinical efficacy and safety of lubiprostone in pediatrics with PFC aged 6-17 years were primarily evaluated through the pivotal Phase 3 clinical study (Study PFC-1131 and Study PFC-11S1). Study SC-0641 was only supportive given relatively short treatment duration (i.e., 4 week) and the open-label and uncontrolled design. SCMP-303 evaluated long-term safety but not clinical efficacy.

Among the four clinical trials, pharmacokinetics of M3 metabolite was assessed in three clinical trials, i.e., SC-0641, PFC-1131, and PFC-11S1 as follows:

Table 6. The submitted clinical studies with pharmacokinetic assessment

Study ID	Trial Design/ Endpoint	Dose and Duration	Population/ Number of Patients	PK sampling scheme
Study SC-0641	Open-label, uncontrolled, multicenter Efficacy, Safety & PK Primary endpoint: the frequency of SBMs at Week 1.	< 24 kg: 12 mcg QD 24 to < 36 kg: 12 mcg BID ≥ 36 kg: 24 mcg or 12 mcg BID (1:1 randomization) 4 weeks	PFC patients aged 4-17 years Treated: 124 PK subset: 24	Day 1: pre-dose, 0.5, 1, 1.5, 2, 4, and 6 hrs post-dose
Study PFC-1131	Double-blind, Randomized, Multicenter, Placebo-controlled Efficacy, Safety & PK Primary endpoint: the overall SBM response	< 50 kg: 12 mcg BID or matching placebo (Dose escalation to 24 mcg BID based on insufficient efficacy at Week 1) ≥ 50 kg: 24 mcg BID or matching placebo 12 weeks	PFC patients aged 6-17 years Randomized: 606 Treated: 595 PK: 508	Day 1: pre-dose, between 0.5 and 1.5 hrs post-dose Day 29: between 2 and 6 hrs post-dose
Study PFC-11S1	Open-label, Multi-Center extension study Efficacy, Safety & PK Primary endpoint: the overall SBM response	Roll over the dose in PFC-1131 For patients who received placebo: < 50 kg: 12 mcg BID ≥ 50 kg: 24 mcg BID 36 weeks	PFC patients aged 6-17 years Treated: 418 PK: 414	Day 1: pre-dose, between 0.5 and 1.5 hrs post-dose

Study SC-0641 (Full PK profile)

In Study SC-0641 which provided full PK profiles of M3 in pediatrics aged 4-17 years, pharmacokinetic samples from a total of 24 male and female patients aged 4-17 years were taken at pre-dose, 0.5, 1, 1.5, 2, 4, and 6 hours after intake of a single 12 mcg or 24 mcg lubiprostone capsule.

The mean plasma M3 concentration-time profiles in Study SC-0641 was plotted with those in adults at single dose 24 mcg from Study SPI/0211SC-0411 which was conducted to evaluate cardiac safety at single dose of 24 mcg and 144 mcg in a total 44 healthy male and female adults (Figure 6).

Mean plasma M3 concentration-time profiles in pediatrics by different weight band were generally overlapped with the adult data (Figure 6, right), aside from 3 patients, who were weighed 12 to < 24 kg and received 12 mcg, showing much higher concentrations than children ≥ 24 kg and adults (Figure 6, left). Of note, the age of those who were weighed 12 to < 24 kg ranged from 4 to 8 years.

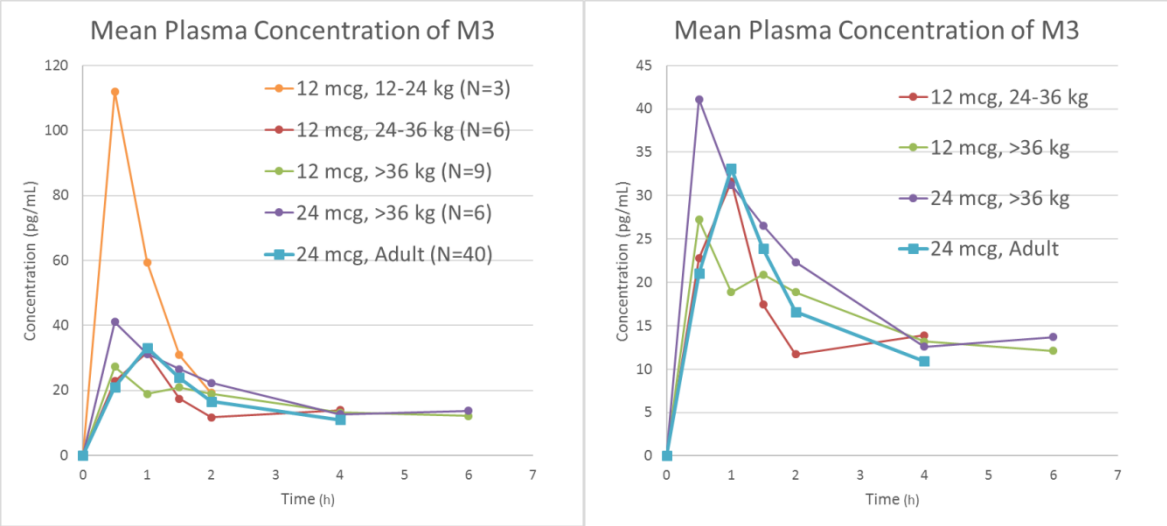


Figure 6. Mean plasma M3 concentration-time profile in pediatric patient with PFC aged 4 to 17 years (Study SC-0641) compared to healthy adults (Study SPI/0211SC-0411) (Left: all age group included; Right: population weighed < 24 kg was excluded) (Reviewer's plot)

Study PFC-1131/PFC-11S1 (Sparse PK)

In Study 1131 in pediatric patients with PFC aged 6 to 17 years, lubiprostone 12 mcg BID or matching placebo was assigned to patients weighing <50 kg, and lubiprostone 24 mcg BID or matching placebo was assigned to patients weighing ≥50 kg for 12 weeks. For those originally

assigned to the 12 mcg BID group, the dose was increased to 24 mcg BID at Visit 3 (Week 1) based on pre-defined efficacy assessment (i.e., < 3 SBMs up to Day 7). In the subsequent extension Study PFC-11S1, patients who continued participating in the extension study received 12 mcg or 24 mcg BID as they had received in Study PFC-1131. For patients who had received placebo during Study PFC-1131, assignment of dose was based on body weight (50 kg cut-off) at the time of roll-over to Study PFC-11S1. Of note, the distribution of dose escalating group was relatively even across the different age groups (Table 7).

Table 7. Number of patients who were < 50 kg in Study 1131 (mITT1 population)

	Stayed at 12 mcg BID (N=103)	Dose escalation to 24 mcg BID at Week 1 (N=120)	Total (N=223)
6 <= Age <= 9	65	67	132
10 <= Age <= 13	34	46	80
14 <= Age <= 17	4	7	11

*mITT1 population: sites # 1064 and #1082 excluded

In Study PFC-1131, pharmacokinetic blood samples from 508 patients were taken at pre-dose and between 30 to 90 min after the first dose and between 2 to 6 hours on Day 29. In Study PFC-11S1, pharmacokinetic blood samples were taken in 414 patients who were rolled over from Study 1131 at pre-dose and between 30 to 90 min after the first dose (Figure 7).

Plasma M3 concentrations were measurable merely in 110 out of all collected 3431 samples (i.e., 3.2%) from patients who received lubiprostone and the most samples were below the level of quantitation (BLQ). Since the proportion of patients with any measurable M3 concentrations was only 22.8% (91 out of 400), the pharmacokinetic parameters of M3 were not able to be adequately assessed. Therefore, the highest observed M3 concentrations were only assessed as being representative of exposure, instead of C_{max} or AUC.

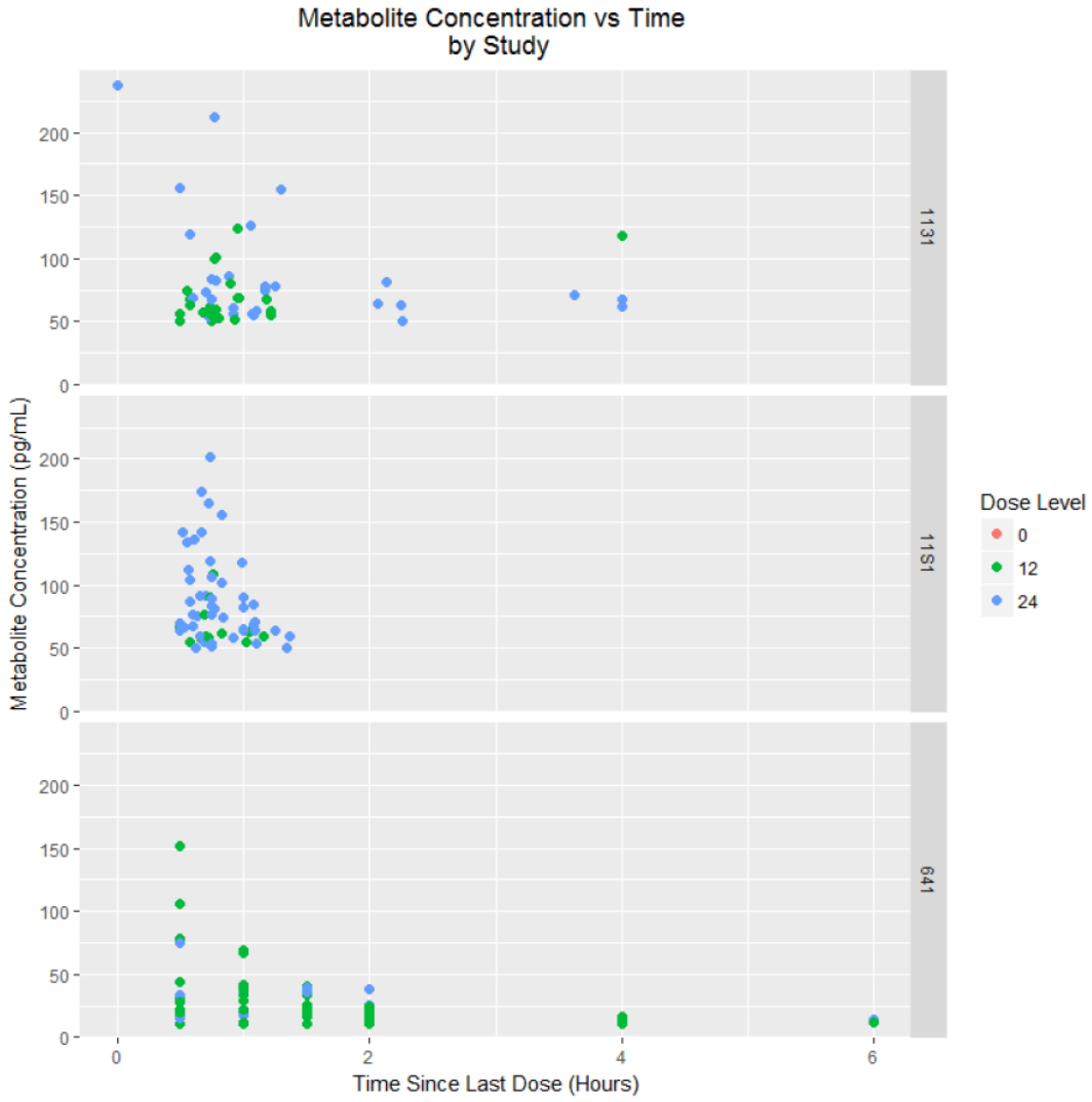


Figure 7. M3 concentration-time plots since last dose by dose level and panelled by study (Upper: Study PFC-1131; Middle: Study PFC-11S1; Bottom: Study SC-0641) (Report SAG-CTR17-001)

Report SAG-CTR17-001 (Population PK)

The applicant performed population PK and exposure-efficacy/response analyses (Report SAG-CTR17-001) based on the pooled M3 concentration data from the three clinical trials with PK assessment (Table 6). In the applicant's analysis, to supplement the few measured M3 concentrations in PFC-1131/PFC-11S1, lubiprostone concentrations were imputed for the BLQ values in the dataset (e.g., 50 pg/mL) to calculate AUC. For the purposes of this analysis, this

was not acceptable given the large fraction of the dataset that values were imputed for (approximately 60%). After excluding BLQ values, only a limited number of concentration values remained (approximately 10%). Thus, estimated PK parameters such as C_{max} and AUC were not considered reliable. Subsequently, the exposure-response analysis based on the unaccepted C_{max} and AUC values was deemed inadequate.

In response to IR dated 10/2/2017, the applicant updated the PK report (SAG-CTR17-001, version 2) which included a table with PK parameters by age groups in SDN 1570 as below:

Table 2 Comparison of Lubiprostone PK Parameters by Age Group for 24 mcg dosed subjects

	Imputation Method 1			Imputation Method 2		
Population	AUC (pg*hr/mL)	Cmax (pg/ml)	N	AUC (pg*hr/mL)	Cmax (pg/ml)	N
Adults	59.1 (21.8)	41.5 (15.8)	40	-	-	-
10-17 year olds	119.5 (139.5)	79 (39.1)	40	230.6 (259.4)	78.2 (47.6)	154
4-9 year olds	50.7 (1.0)	79.3 (56.1)	2	114.2 (91.2)	84.2 (39.6)	7

Source: \Sucampo\Analysis\ERSAnalysis21DEC2016_v5.Rmd
Note: Imputation Method 1 involved excluding M3 PPK samples below the level of quantification. Imputation Method 2 involved imputing M3 PPK samples listed as below the level of quantification to the lower limit of quantification value listed for the imputed BQL PK sample record.

However, the summarized PK parameters provided did not reflect the fact that two different doses were administered to patients based on body weight cut-off. In other word, within the same age group, PK parameters were just averaged up regardless of the dose administered. In addition, the applicant included the imputed AUC values for BLQ data from Study PFC-1131 and Study PFC-11S1 which has substantial portion of BLQ data. Therefore, the summary of PK parameters provided by age group was deemed inadequate to compare the values between age groups.

4.1.1. Is the proposed dose supported in 10-17 years-old patients regardless of body weight < 50 kg or ≥ 50 kg?

The target population was proposed based on age cut-off (i.e., pediatric patients aged 10-17 years), even though the dose administered in Study SC-0641 and Study PFC-1131/PFC-11S1 was determined based on body weight cut-off (i.e., < 50 kg vs. ≥ 50 kg).

To evaluate whether the proposed dose, 24 mcg BID for 10-17-year-old patients is acceptable regardless of body weight which is unlike the way that clinical trials were conducted, M3 exposure in 10-17 years-old patients was compared between two different weight bands, i.e., < 50 kg vs. ≥ 50 kg at the same 24 mcg BID dose.

Among 5 patients aged 10-17 years old from Study SC-0641, there was no significant fference in plasma M3 concentration-time profiles between patients < 50 kg vs. ≥ 50 kg following a single dose of 24 mcg (Figure 8, left).

In Study PFC-1131/PFC-11S1, the highest M3 concentrations observed were compared between subpopulation < 50 kg and ≥ 50 kg, within the 10-17-year-old subgroup at 24 mcg BID. The distribution of the highest M3 concentrations observed were similar between body weight groups, i.e., < 50 kg vs. ≥ 50 kg (Figure 8, right).

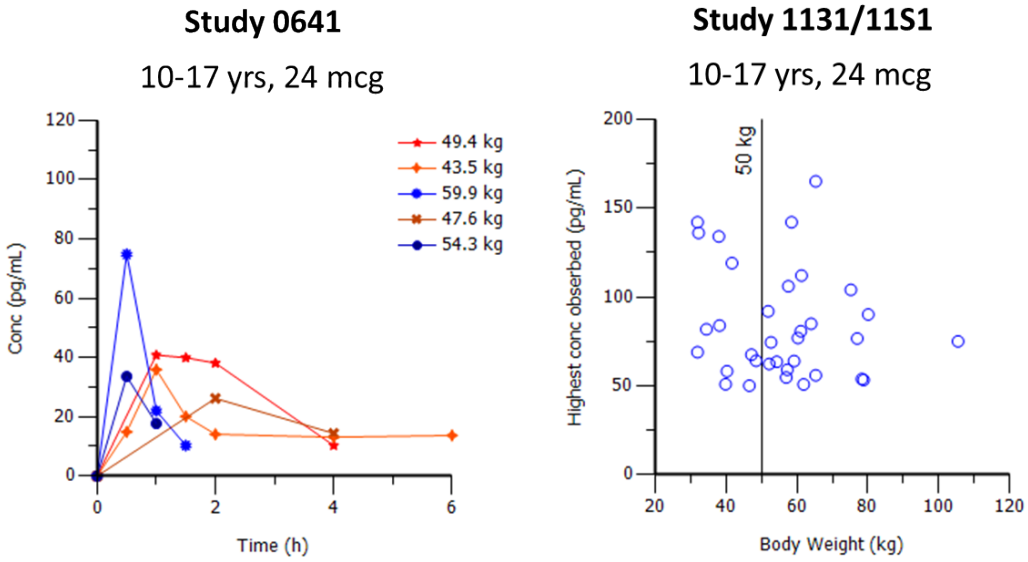


Figure 8. Comparison of M3 concentrations in patients aged 10-17 years between body weight < 50 kg vs. ≥ 50 kg following lubiprostone 24 mcg BID (Left: M3 concentration-time profiles in Study SC-0641; Right: the highest M3 concentrations observed in Study PFC1131/PFC-11S1) (Reviewer's plot)

Additionally, as the review team regarded that the proposed age cut-off, i.e., 10-17 years, was not clinically justified and 12-17 years of age would be more likely to be relevant to adults, the same comparison between body weight groups < 50 kg vs. ≥ 50 kg was applied to subpopulation aged 12-17 years. Subsequently, within patients aged 12-17 years of Study PFC-1131/PFC-11S1, no significant difference was observed between two body weight groups in terms of the highest M3 concentrations observed at the same 24 mcg BID dose (Figure 9).

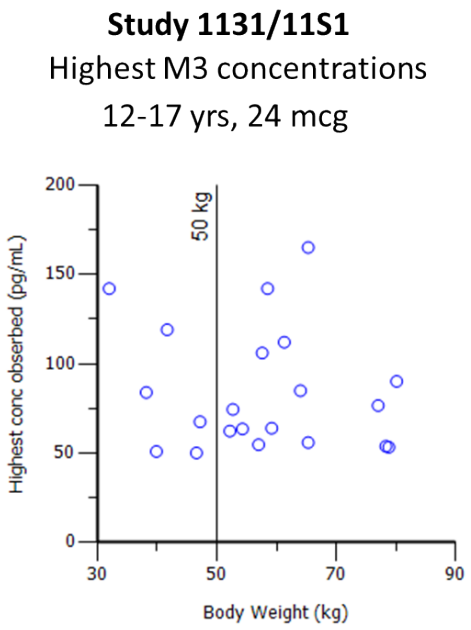


Figure 9. Comparison of the highest M3 concentrations observed in patients aged 12-17 years between body weight < 50 kg vs. ≥ 50 kg following lubiprostone 24 mcg BID in Study PFC-1131/PFC-11S1 (Reviewer's plot)

In other word, within either 10-17-year-old or 12-17-year-old groups, 24 mcg BID seems to lead to similar M3 concentration regardless of body weight groups, either < 50 kg or ≥ 50 kg. It suggests that the proposed dose, lubiprostone 24 mcg BID, is acceptable for 10-17-year-old or 12-17-year-old based on the comparable M3 exposure only if safety and efficacy is adequately supported for PFC patients.

4.2 Dose-Response Analyses

A dose-response analysis was performed in light of the limited PK data. The applicant's analysis suggested there was no clear dose-response relationship for either efficacy or safety. This may be in part due to the fact that Study PFC-1131/PFC-11S1 evaluated only one dose level in the same weight band, i.e., 12 mcg BID for patients < 50 kg and 24 mcg BID for patients ≥ 50 kg, ultimately making dose-response assessment impractical. Further, the applicant conducted dose-response analysis by using the fixed dose and not taking 'weight-based dosing' into account. Given that body weight appears to influence the PK of lubiprostone, the provided results of dose-response analysis may be confounded by not taking exposure into account.

Dose-Efficacy Response Analysis

Dose-response for efficacy (average weekly SBM frequency, straining score, stool consistency score, and overall SBM responder status) analysis was evaluated with the pooled data from SC-

0641, PFC-1131, and PFC-11S1. The applicant concluded the analysis results indicate pronounced placebo effects on all efficacy endpoints in addition to slightly positive trends in efficacy by dose increase as shown in Figure 10. However, the results of the applicant's dose-response analysis may have been confounded by body weight, because the applicant analyzed as if there were three dose levels, i.e., placebo, 12 mcg, and 24 mcg, without consideration that patients were assigned to 12 mcg or 24 mcg based on body weight band, < 50 kg and ≥ 50 kg.

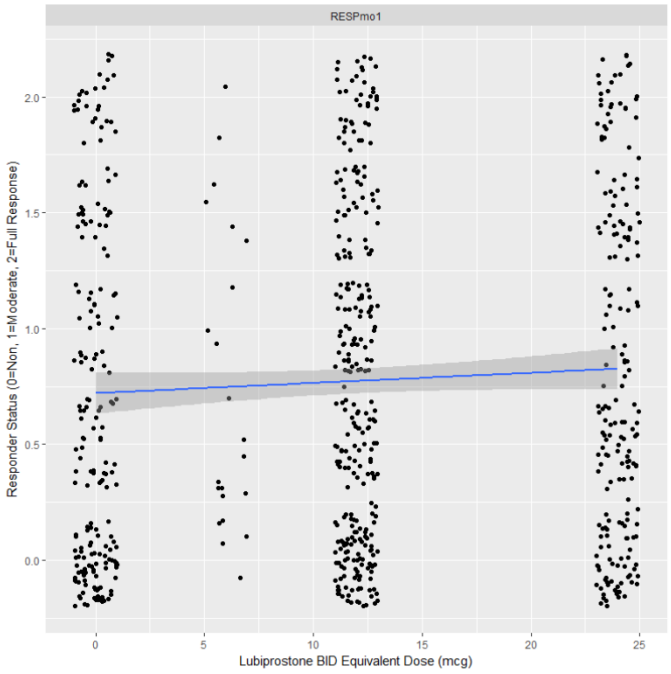
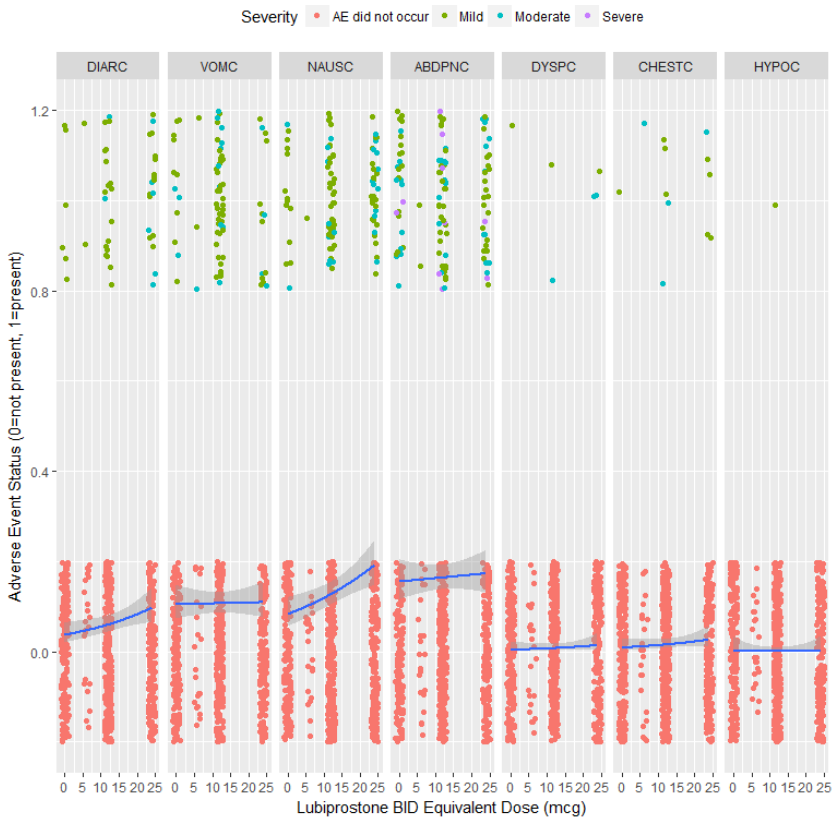


Figure 10. Observed SBM responder status incidence by dose (linear smooth in blue) (Study Report SAG-CRT17-001)

Dose-Safety (Adverse Events) Analysis

Dose-AE analysis was performed by the applicant with the pooled data from SC-0641, PFC-1131, and PFC-11S1 primarily focusing on the most commonly reported AEs including GI-related AEs such as diarrhea, vomiting, nausea, and abdominal pain in addition to dyspnea, chest pain, and hypotension (Figure 11). The incidence of GI-related AEs appeared to increase slightly with increasing dose.

Since the dose-safety analysis was also evaluated without considering that the administered dose was determined based on body weight, this exposure-response assessment is limited by not including body weight. Nonetheless, the observed dose-dependent tendency in GI-related AEs were in line with the clinical safety data.



DIARC = diarrhea; VOMC = vomiting; NAUSC = nausea; ABDPNC = abdominal pain; DYSPC = dyspnea; CHESTC = chest pain; HYPOC = hypotension

Figure 11. Adverse event incidence and severity by dose (linear smooth in blue) (Study Report SAG-CRT17-001)

Additionally, statistical analysis with a simple logistic model was performed by the applicant for evaluating the dose-safety relationship for each GI-related AE with considerations of covariates including age and body weight. The results indicated that lubiprostone dose had statistically significant effect on the incidence of nausea, vomiting, and diarrhea whereas it was not significant for abdominal pain and the other non-GI AEs such as dyspnea, chest pain, and hypotension. The logistic model for vomiting described that body weight was the best descriptor of vomiting occurrence ($P \sim 10^{-6}$), and lubiprostone dose level was also significant to a lower extent. The applicant interpreted that this finding was expected given younger children are more prone to vomiting than older children. It might partially explain that the reason why PFC patients showed more frequent vomiting than adult CIC patients (Table 2, Page 12). However, the effects of drug dose and age contributing to vomiting cannot be distinguished because the doses were given based on body weight which is closely correlated to age.

4.3 Summary of Bioanalytical Method Validation and Performance

In three studies (i.e., SC-0641, PFC-1131, and PFC-11S1) in which pharmacokinetic blood samples were collected, lubiprostone (also called RU-0211) and 15-Hydroxy-Lubiprostone acid (also called M3 or U-E232) were measured in plasma using validated bioanalytical assay methods based on LC-MS/MS.

The plasma samples obtained in Study SC-0641 were analyzed in [redacted] in [redacted] at [redacted] and then the laboratory for bioanalysis was changed to [redacted] at [redacted] for

In support of bioanalytical assay, the applicant submitted following bioanalytical method validation reports. Plasma bioanalytical method validation reports and final analytical reports were submitted as follows:

- For Study SC-0641 (appended at Section 16.4. Analytical Report of Study Report SPI/0211SC-0641):
  - ✓ Validation report: Validation Study of an LC/MS/MS Method for the Determination of RU-0211 and U-E232 in Human Plasma (Study Report #SPI/SR05-017)
  - ✓ Analytical report: Determination of lubiprostone (RU-0211) and M3 (U-E232) concentrations in human plasma obtained from "A Multi-center, Open-labeled Study of the Safety, Efficacy, and Pharmacokinetics of Lubiprostone in Pediatric Patients with Constipation (Clinical Protocol No.: SPI/0211SC-0641) – Appendix 16.4 of CSR
- For Study PFC-1131, and PFC-11S1:
  - ✓ Validation report: Quantitative Determination of Lubiprostone and 15-Hydroxy-Lubiprostone in Human Plasma (Sodium Heparin) by LC/MS/MS ([redacted])
  - ✓ Analytical report: Determination of Lubiprostone and 15-Hydroxy-Lubiprostone in Human Plasma with Tandem Mass Spectrometric Detection

A method was validated for measuring lubiprostone and M3 in human plasma in sodium heparin tube. Plasma samples were extracted by a solid-phase extraction procedure followed by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The accuracy and precision for each analyte was acceptable. Please see Table 8 and

Table 9.

In Study SC-0641, lubiprostone concentrations from 8 out of 161 samples were detectable, but 7 samples from one patient (ID (b) (6)) seemed to have been contaminated given the endogenous peak with the same retention time as lubiprostone was detected in those samples. The other one sample showed detectable level of lubiprostone (15.8 pg/mL) was taken at 0.5 hr post-dose from a patient weighed 18.6 kg.

In Study PFC-1131 and PFC-11S1, lubiprostone concentrations of all samples were reported below the LLOQ whereas some samples showed detectable level of M3. The bioanalytical methods applied required 750 µL for extraction process but for some study samples, less than 750µL of plasma was collected and therefore dilutions were performed to have sufficient volume for extraction. It resulted in higher LLOQ multiplying dilution factor to the original LLOQ (50 pg/mL). For example, if a sample was diluted twice, the LLOQ becomes 100 pg/mL for the diluted sample (i.e., 50 pg/mL * 2 = 100 pg/mL). Even though it does not seem desirable approach for the samples with insufficient volume from the reviewer's viewpoint, it does not affect the overall study results as the approximately 80% of samples showed below the LLOQ and diluting the sample might be the only wat to generate a reportable value.

Table 8. Plasma Bioanalytical Method Validation Summary-1 ((b) (4))

Attribute	**Analyte**	
	lubiprostone	M3
Linear Range	10–1000 pg/mL	10–1000 pg/mL
LLOQ	10 pg/mL	10 pg/mL
ULOQ	1000 pg/mL	1000 pg/mL
Selectivity, Precision (% CV), accuracy (% difference)	10 pg/mL; % difference = -7.0 to 0.4% at LLOQ; -7.5% to 9.0% at the other concentrations % CV = 2.5% at LLOQ; 3.3 to 6.9% at the other concentrations	1 pg/mL: % difference = -2.3 to 1.0% at LLOQ; -12.9 to 14.0% at the other concentrations % CV = 1.7% at LLOQ; 2.7 to 10.2% at the other concentrations
QC Concentration	10.0, 200, 800 pg/mL	10.0, 200, 800 pg/mL
Intra-Day Precision (% CV)	(1.7, 8.9)	(0.7, 7.5)
Intra-Day Accuracy (% difference)	(-0.8, 4.5)	(-13.3, -2.2)
Inter-Day Precision (% CV)	(3.6, 7.5)	(8.8, 10.4)
Inter-Day Accuracy (% difference)	(3.0, 5.0)	(-3.0, 0.0)
Stability		
Freeze and Thaw Stability	3 cycles at -70°C	3 cycles at -70°C
Storage Stability	95 d at -20 °C (plasma) 2 h on ice bath (plasma)	95 d at -20 °C (plasma) 2 h on ice bath (plasma)
Auto-sampler Stability of Processed Samples	48 h at 4°C	48 h at 4°C

CV = coefficient of variation; LLOQ = lower limit of quantitation; QC = quality control; ULOQ = upper limit of quantitation.

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Table 9. Plasma Bioanalytical Method Validation Summary-2 (b) (4))

Attribute	Analyte	
	lubiprostone	M3
Linear Range	50.0–5000 pg/mL	50.0–5000 pg/mL
LLOQ	50.0 pg/mL	50.0 pg/mL
ULOQ	5000 pg/mL	5000 pg/mL
Selectivity, Precision (% CV), accuracy (% difference)	50.0 pg/mL: % CV = 1.7 to 6.0 % difference = -2.0 to 2.0	50.0 pg/mL: % CV = 4.4 to 7.8 % difference = -5.8 to 4.2
QC Concentration	50.0, 150, 400, 2000 and 3750 pg/mL	50.0, 150, 400, 2000 and 3750 pg/mL
Intra-Day Precision (% CV)	(3.1, 12.4)	(1.8, 12.0)
Intra-Day Accuracy (% difference)	(-8.6, 7.5)	(-9.3, 10.7)
Inter-Day Precision (% CV)	(0.0, 6.3)	(-3.5, 7.3)
Inter-Day Accuracy (% difference)	(-1.6, 1.5)	(0.0, 4.3)
Stability		
Master Stock Solution Stability in Solvent	29 h at room temperature 145 d at -20°C 37 d at -20°C	29 h at room temperature 145 d at -20°C 37 d at -20°C
Freeze and Thaw Stability	5 cycles at -70°C	5 cycles at -70°C
Dilution Integrity	188000 pg/mL diluted 100×	188000 pg/mL diluted 100×
Storage Stability	61 d at -20°C and -70°C (plasma) 2 h at room temperature (blood) 27 h on ice (plasma)	61 d at -20°C and -70°C (plasma) 2 h at room temperature (blood) 27 h on ice (plasma)
Auto-sampler Stability of Processed Samples	166 h at 1-8°C	166 h at 1-8°C

CV = coefficient of variation; LLOQ = lower limit of quantitation; QC = quality control; ULOQ = upper limit of quantitation.

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