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APPLICATION NUMBER:

209363Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	209363
Link to EDR	\\CDSESUB1\evsprod\NDA209363\0000
Submission Date	1/17/2017
Submission Type	Priority
Proposed Brand Name	Solosec®
Generic Name	Secnidazole
Dosage Form and Strength	Oral granules, containing 2 g secnidazole, in a single-dose child resistant foil packet
Route of Administration	Oral, admixed with applesauce, yogurt, or pudding
Proposed Indication & Dosage Regimen	2 g single dose for treatment of bacterial vaginosis (BV)
Applicant	Symbiomix therapeutics LLC
Associated IND	IND No. 117,811
OCP Review Team	Sonia Pahwa, Ph.D., Reviewer Phil Colangelo, PharmD, PhD, Team Leader
OCP Final Signatory	John Lazor, PharmD, Director, DCP IV

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1. EXECUTIVE SUMMARY

This NDA 209363 is for Solosec®, which is 2 g secnidazole (SYM-1219) oral granules in a single-dose child resistant foil packet. The Applicant is seeking approval for the treatment of bacterial vaginosis (BV). The dose regimen is a single 2 g packet of granules taken once orally when admixed with applesauce, yogurt, or pudding. Secnidazole has been available for many years in Europe and other countries outside of the US as a single, oral 2 g dose, used as either monotherapy or in combination with other drugs. The drug has been used and investigated as a single or multiple dose regimen for the treatment of amebiasis, giardiasis, trichomoniasis and BV. The clinical pharmacology program was based on SYM-1219 being delivered orally as a single-dose to women in the US.

1.1 Recommendations

The Clinical Pharmacology information provided by the Applicant in this submission is acceptable, and the Clinical Pharmacology review team recommends that NDA 209363 for Solosec be approved for the treatment of bacterial vaginosis.

Table 1.1-1 Summary of OCP's Recommendations & Comments on Key Review Issues

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Substantial evidence of effectiveness was provided by the two registrational trials. The PK studies conducted by the applicant provide supportive evidence for exposure of secnidazole dosed as 2 g granules admixed with soft foods taken once orally.
Bridge between the to-be-marketed and clinical trial formulations	The Applicant switched the manufacturing site from (b) (4) to Catalent (Catalant, Inc. Somerset, NJ). One Pivotal study (SYM-1219-301) was conducted with the 'Cat' formulation which is the to-be-marketed formulation. Two PK studies and one efficacy study used the formulation developed by (b) (4). The Applicant demonstrated bioequivalence between the to-be-marketed (Cat) and (b) (4) formulation.
Labeling	The Reviewer's proposed labeling changes/recommendations in Section 2.3 will be forwarded to the Applicant.

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Secnidazole is a 5-nitroimidazole with antibacterial and antiprotozoal activity.

Absorption

Following a 2-g single oral granule dose, maximum secnidazole concentrations of approximately 45 µg/mL are achieved within approximately 1.5 hours after dosing. There is no impact of a high fat meal and no changes in the PK of secnidazole following administration in applesauce, yogurt, or pudding.

Distribution

According to the literature, the mean residence time (MRT) for secnidazole is 30 hours, and the apparent volume of distribution (V/F) is low (0.7 L/kg)[1], indicating that secnidazole is not extensively distributed to tissues. The plasma protein binding of secnidazole is <5%.

Elimination

The total body clearance of secnidazole is approximately 25 mL/min[1]. The renal clearance of secnidazole is approximately 3.9 mL/min. The plasma elimination half-life for secnidazole is approximately 17 hours.

Metabolism

In vitro reaction phenotyping studies conducted by the Applicant showed that secnidazole is slowly metabolized in human liver microsomes with generally ≤1% conversion to metabolites.

Secnidazole is metabolized via oxidation to one minor hydroxylated metabolite[1].

Excretion

Following a single 2 g oral dose of secnidazole granules, excretion of unchanged secnidazole in urine is 13-15% of the administered dose. The Applicant provided a literature reference in the NDA that reported approximately 50% of an administered 2 g dose of secnidazole is excreted in the urine as both unchanged and glucuronidated secnidazole[1].

[1]Frydman AM, Lemar M, Dow J, Djebbar F and Gaillot J. A review of the pharmacokinetics of secnidazole in man. 16th International Congress of Chemotherapy 1989; 2: 445.1- 445.3.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The Applicant's proposed dose for BV is a single 2-gram oral dose (one packet of granules) to be admixed with soft foods, i.e., applesauce, yogurt, or pudding. This dose regimen is supported by the efficacy and safety data submitted in the application.

2.2.2 Therapeutic individualization

No

2.3 Outstanding Issues

The fate of an administered 2 g oral dose of secnidazole was not characterized via an ADME study by the NDA Applicant. The need to conduct such a study was discussed between the Applicant and the Clinical Pharmacology review team prior to NDA submission, and it was agreed upon that an ADME study was not needed since secnidazole will be given as a one-time single 2 g dose and secnidazole granules have been used extensively outside of the US for treatment of BV without any significant safety concerns. As stated above in Section 2.1, the Applicant provided a literature reference in the NDA that reported approximately 50% of an administered 2 g dose of secnidazole is excreted in the urine as both unchanged and glucuronidated secnidazole[1]. In the Phase 1 NDA Study SYM-1219-101 conducted by the Applicant, 13-15 % of an administered 2 g dose is excreted as unchanged secnidazole in the urine. Thus, although approximately 50% of a 2 g dose of secnidazole was not accounted for, there remains no concern from the Clinical Pharmacology review team for the aforementioned reasons stated here.

[1]Frydman AM, Lemar M, Dow J, Djebbar F and Gaillot J. A review of the pharmacokinetics of secnidazole in man. 16th International Congress of Chemotherapy 1989; 2: 445.1- 445.3.

2.4 Summary of Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling comments be included in the final package insert (Table 2.4-1).

Table 2.4-1 Summary of Labeling Issue Identification and Recommendations

Section/heading	Acceptable to OCP?			Comment
	A	AWE	NA	
Section 12.2/ PD	☐	☒	☐	Revise wording regarding the (b) (4) QT

				prolongation result
12.2/PD	☐	☐	☒	According to 21 CFR 201.57(c)(13)(i)(B), include a statement such as 'Secnidazole exposure-response relationships and the time course of pharmacodynamics response is unknown' in subsection.
12.3/Absorption	☐	☒	☐	Add a description of the meal with respect to the calories and fat content, per guidance *Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products – Considerations, Content, and Format*.
12.3/ Pharmacokinetics	☐	☒	☐	• Remove (b) (4) • (b) (4) is not generally understood by Health Care Providers. SD or Min/Max is suggested per guidance *Clinical Pharmacology Section of Labeling for Human Prescription Drug and Biological Products – Content and Format.*
12.3/ Elimination	☐	☒	☐	• Two subheadings "Metabolism" and "Excretion" were added. • Wording was rearranged based on the new subheadings
12.3/DDI	☐	☒	☐	Condense and/or revise the wording for clarity; add language in Section 7.

A = Acceptable; AWE=Acceptable with minor edits; NA=not acceptable/substantive disagreement (must provide comment)

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Solosec is a granule formulation in a (b) (4) administered for the treatment of BV as a single oral 2 g dose. Secnidazole was granted Qualified Infectious Disease Product (QIDP) and Fast Track status. During the clinical development program, Applicant participated in a number of Type B and Type C guidance meetings with the Division of Anti-infective Products including Pre-IND, Clinical Pharmacology, EOP2 Clinical, EOP2 CMC, and preNDA meetings.

In a Type C Meeting held on 21 January, 2015 the Agency agreed to the Applicant's proposal that an ADME study is not needed as a part of this submission. The Applicant also asked to obtain Agency's feedback about studies needed to be performed to label dosing instructions for secnidazole granules orally after adding granules into soft food, such as applesauce, yogurt, or pudding. The Agency indicated that a relative BA study was needed to evaluate the systemic exposure to secnidazole when the 2 g granules were admixed with these soft foods.

Type B End-of-Phase 2 Meeting was held on March 18, 2015 to obtain Agency's feedback on manufacturing site changes during clinical development of SYM-1219. Because of the manufacturing site change, a clinical bioequivalence study (Study SYM-1219-102) was conducted to compare the (b) (4) to the Catalent formulation.

The design and analysis of the two pivotal Studies SYM-1219-201 and SYM-1219-301 were discussed and agreed upon with the Agency. Applicant's proposal to use the SYM-1219-201 and SYM-1219-301 studies as the two adequate and well-controlled studies was submitted in the pre-NDA meeting briefing package; the study results, Integrated Summary of Efficacy (ISE), Integrated Summary of Safety (ISE)and statistical analysis plan (SAP), were also included. The Division provided responses on May 1, 2016 and agreed that the clinical program and the SAP were acceptable for filing the NDA.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Secnidazole is a 5-nitroimidazole with antibacterial and antiprotozoal activity. 5-nitroimidazoles enter the microbial cell as inactive prodrugs where the nitro group is reduced to toxic radical anions and nitrite ions, both of which are believed to be responsible for antimicrobial activity
QT Prolongation	At the therapeutic dose of 2g there was no significant QTc prolongation effect observed in the thorough QT Study SYM-1219-105. The study failed to exclude 10-ms at the supra-therapeutic dose of 6g. The largest upper bound of the 2-sided 90% CI for the mean difference between SYM-1219 2 g and placebo was below 10 ms; the largest upper bound for comparison between SYM-1219 6 g and placebo was 11 ms, above the regulatory threshold of 10 ms as described in ICH E14 guidelines. Both the QTc analysis and PK/QTc analysis demonstrate that secnidazole does not have a clinically relevant effect on the QTc interval and corresponds to a negative TQT study, as defined by the ICH E14 guidance (Refer to Thorough QT Study Review by Interdisciplinary Review Team for QT Study Consultation).
General Information	
Bioanalysis	Validated HPLC/MS/MS methods to determine secnidazole concentrations in human plasma and urine (Refer to **Appendix**

<table>
<tr><td></td><td colspan="3">4.1)</td></tr>
<tr><td>Range of effective dose</td><td colspan="3">2 g single oral dose</td></tr>
<tr><td>Dose Proportionality</td><td colspan="3">Secnidazole C_{max} following single oral administration generally increased in a dose-proportional manner from 1 g to 6 g. Across studies, mean (%CV) maximum concentrations were approximately 22.6 (12.7) µg/mL following 1 g, 45 (16.8) µg/mL following 2 g, and 113.1 (24.6) µg/mL following 6 g of secnidazole and exhibited proportional increases with increasing dose.
The pharmacokinetics of secnidazole following single dose administration of 1 g to 6 g demonstrates dose proportionality.</td></tr>
<tr><td colspan="4">Absorption</td></tr>
<tr><td rowspan="2">Food effect
(Fed/fasted)
Geometric Mean % [90% CI]</td><td>$AUC_{0-\infty}$</td><td>C_{max}</td><td>T_{max}</td></tr>
<tr><td>97.77
(93.40, 102.35)</td><td>97.36
(94.21, 100.62)</td><td>Fasted: Median T_{max} (range) =4 hours (3 to 6 hours)
Fed: Median T_{max} (range) = 6 hours (4 to 8 hours)</td></tr>
<tr><td colspan="3">There is no effect of high fat meal[a] on the pharmacokinetics of secnidazole granules following a single oral dose of 2 g when admixed with applesauce.</td></tr>
<tr><td colspan="4">Distribution</td></tr>
<tr><td>Volume of Distribution</td><td colspan="3">Vd/F is reported to be 0.7 L/kg[1].</td></tr>
<tr><td>Plasma Protein Binding</td><td colspan="3"><5%</td></tr>
<tr><td colspan="4">Elimination</td></tr>
<tr><td>Terminal Elimination half-life</td><td colspan="3">Mean (SD) =17.5 (2.8) hours</td></tr>
<tr><td colspan="4">Metabolism</td></tr>
<tr><td>% metabolized [in vitro]</td><td colspan="3"><1%</td></tr>
<tr><td colspan="4">Excretion</td></tr>
<tr><td>Primary excretion pathways</td><td colspan="3">Total body clearance of secnidazole has been reported to be ~25 mL/min in literature[1]. The mean (SD) renal clearance of secnidazole is 3.9 (1.05) mL/min.
Excretion of unchanged secnidazole in urine is 13-15% of the administered dose.</td></tr>
<tr><td colspan="4">In vitro interaction liability (Drug as perpetrator)</td></tr>
<tr><td>Inhibition/Induction of metabolism</td><td colspan="3">Secnidazole induces CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19 at 5000 µM and 10000 µM concentrations. These concentrations are substantially higher than what is observed after the clinical dose regimen of 2 g as a single dose.
Secnidazole exhibited direct inhibition of CYP2C19 and CYP3A4, with IC_{50} values ranging from 3722 to 4306 µM. All</td></tr>
</table>

	other CYP enzymes exhibited IC_{50} values > 5000 µM. No evidence of time-dependent inhibition was observed for any enzyme. Secnidazole is not likely to inhibit or induce CYP enzymes at concentrations following the clinical dose regimen of 2 g as a single dose.
Inhibition/Induction of transporter systems	Secnidazole is not a significant inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 at the clinically relevant concentration.
In vitro interaction liability (Drug as victim)	
Substrate of metabolic enzymes	Secnidazole is metabolized *in vitro* by human hepatic CYP450 enzyme system with ≤1 % conversion to metabolites. The likelihood of CYP inhibitors or inducers affecting the metabolism of secnidazole is minimal.
Substrate transporter systems	Secnidazole is not a substrate of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2.

[1]Frydman AM, Lemar M, Dow J, Djebbar F and Gaillot J. A review of the pharmacokinetics of secnidazole in man. 16th International Congress of Chemotherapy 1989; 2: 445.1- 445.3.
[a]Approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

Two Phase 3 clinical trials (Studies SYM-1219-201 and 1219-301) were conducted in female patients with BV to provide evidence of safety and effectiveness of secnidazole oral granules when given as a single 2 g dose admixed in applesauce. The available clinical pharmacology information provides an estimate of PK exposure of secnidazole when dosed once as 2 g granules. No exposure response evaluation was performed in the NDA.

A clinical dose response was assessed in Study SYM-1219-201 which evaluated two dose levels of SYM-1219 (1 g and 2 g) compared to placebo. Results from this study showed that efficacy across the endpoints was greater with either dose level of SYM-1219 compared to placebo and that efficacy was consistently greater with the 2 g dose than with SYM-1219 1 g. The greater efficacy seen with SYM-1219 2 g than with SYM-1219 1 g is consistent with the higher exposure seen in the pharmacokinetics study in healthy volunteers (See Study SYM-1219-101, Part A Appendix 4.2.1).

In keeping with the standard set by FDA for other BV products, no interpretive criteria are proposed for secnidazole MIC values and BV-associated pathogens.

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

Yes, the proposed dosing regimen is appropriate based on results from pivotal studies SYM-1219-201 and SYM-1219-301, which demonstrated the efficacy of SYM-1219 2 g for treatment of women with BV. In Study SYM-1219-201 the primary endpoint, Clinical Outcome Responder rate at Test of cure/ End of study (TOC/EOS) visit, was compared between each active treatment group and the placebo group using a Cochran-Mantel-Haenszel (CMH) statistical test adjusted for BV strata (number of BV episodes in prior 12 months ≤ 3 and ≥ 4). In Study SYM-1219-301, the statistical analysis of both Clinical Outcome Responder and Cure used a CMH test adjusted for BV strata and race (Black/African American or Other). In both studies, a greater percentage of patients receiving a single oral administration of SYM-1219 2 g achieved clinical response compared to placebo, i.e., 67.7% and 17.7%, respectively in SYM-1219-201 and 53.3% and 19.3%, respectively in SYM-1219-301 at the TOC/EOS visit. The treatment effect was statistically significant, $p < 0.001$ (adjusted CMH test) when compared to placebo.

In addition, a greater percentage of patients receiving SYM-1219 2 g achieved normal Nugent score and Therapeutic Response compared to placebo in the two studies at the TOC/EOS visit. Normal Nugent scores were reported in 40.3% and 6.5% of patients treated with SYM-1219 2 g and placebo, respectively, in Study SYM-1219-201 and 43.9% and 5.3%, respectively, in Study SYM-1219-301. Therapeutic Outcome response for secnidazole oral granules and placebo (i.e., a patient with both Clinical Outcome Response and bacteriological cure) was 40.3% and 6.5%, respectively in Study SYM-1219-201, and 34.6% and 3.5% in Study SYM-1219-301. For both endpoints, the difference was statistically significant in the two studies, $p < 0.001$ (adjusted CMH test). Please refer to clinical review by Dr. Mayurika Gosh for details.

Secnidazole demonstrated *in vitro* activity against numerous anaerobic bacterial and microaerophilic species that are associated with BV including recent vaginal isolates of *Anaerococcus tetradius*, *Atopobium vaginae*, *Finegoldia magna*, *Gardnerella vaginalis*, *Mageeibacillus indolicus*, *Mobiluncus* spp., *Peptoniphilus* spp., *Bacteroides* spp., *Porphyromonas* spp., *Prevotella* spp., and *Megasphaera*-like bacteria. After a single oral 2-g dose of SYM-1219, concentrations of secnidazole were measurable in plasma to approximately 144 hours after dosing. Efficacy and safety studies along with microbiological profile and pharmacokinetic properties for secnidazole, support the use of a single 2-g dose to treat bacterial vaginosis in women.

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

The pharmacokinetics of secnidazole are not significantly impacted by race and ethnicity.

Effects of Race

The influence of race on the PK of secnidazole across the Phase 1 PK studies was examined. The Phase 1 studies included 142 subjects that were White, 52 subjects that were Black, and 4 subjects that were "Other". There were no apparent differences in systemic drug exposure following a single oral 2-g dose of SYM-1219 when subjects of different races were compared.

Effects of Ethnicity

The influence of ethnicity on the PK of secnidazole across the Phase 1 PK studies was examined. The Phase 1 studies included 99 subjects that were Hispanic or Latino and 99 subjects that were Not Hispanic or Latino. There were no apparent differences in systemic drug exposure following a single oral 2-g dose of SYM-1219 when subjects of different ethnicities were compared.

Renal Impairment

SYM-1219 is a single dose treatment. Therefore, specific population studies in subjects with renal impairment are not needed as per FDA Guidance for Pharmacokinetics in Patients with Impaired Renal Function.

Hepatic Impairment

SYM-1219 is a single dose treatment. Therefore, specific population studies in subjects with hepatic impairment are not needed as per FDA Guidance for Pharmacokinetics in Patients with Impaired Hepatic Function.

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

Food effect

The applicant conducted a food effect study (See Study SYM-1219-102 Appendix 4.2.2) designed to evaluate the PK of a single dose of secnidazole 2 g oral granules when admixed with applesauce and administered under fasted and fed conditions (Table 3.3.4-1).

Table 3.3.4-1 Summary of Secnidazole Plasma Pharmacokinetic Parameters by Treatment

Parameter	SYM-1219-Cat Fasted (N=23)	SYM-1219-Cat Fed (N=23)
C_{max} (µg/mL)		
Mean (SD)	41.21 (5.489)	40.13 (4.871)
%CV	13.32	12.14
Median	39.90	40.90
Min, Max	32.7, 56.2	31.0, 47.7
T_{max} (h)		
Median	4.000	6.000
Min, Max	3.00, 6.00	4.00, 8.00
AUC_{0-t} (h*µg/mL)		
Mean (SD)	1224.06 (217.138)	1214.36 (272.581)
%CV	17.74	22.45
Median	1169.20	1197.00
Min, Max	868.7, 1621.7	754.5, 1673.3
AUC_{0-inf} (h*µg/mL)		
Mean (SD)	1261.47 (236.505)	1248.15 (291.629)
%CV	18.75	23.36
Median	1203.70	1237.30
Min, Max	874.3, 1750.4	762.0, 1769.4

Source: CSR SYM-1219-102, Table 14.2.6 and Listings 16.2.14.1 – 16.2.14.3

Table 3.3.4-2 shows that the SYM-1219-Cat Fasted and SYM-1219-Cat Fed comparisons for C_{max}, AUC_{0-t}, and AUC_{0-inf} demonstrated that the administration of secnidazole 2g oral granules with a high fat meal did not have an impact on the pharmacokinetics of secnidazole and indicated that secnidazole oral granules can be administered without regard to meals. These data support the labeling- "(b) (4)".

Table 3.3.4-2 Statistical evaluation of the effect of food on the C_{max}, AUC_{0-t}, and AUC_{0-inf} of secnidazole

Parameter Treatment	Comparison	% Ratio: 100*Test/Reference Geometric LSMean % Ratio (SE)	90% C.I.
C_{max} (µg/mL)			
SYM-1219-Cat Fasted (B) SYM-1219-Cat Fed (C)	Test (C) / Reference (B)	97.36 (1.905)	(94.21, 100.62)
AUC_{0-t} (h*µg/mL)			
SYM-1219-Cat Fasted (B) SYM-1219-Cat Fed (C)	Test (C) / Reference (B)	98.10 (2.617)	(93.79, 102.60)
$AUC_{0-\infty}$ (h*µg/mL)			
SYM-1219-Cat Fasted (B) SYM-1219-Cat Fed (C)	Test (C) / Reference (B)	97.77 (2.659)	(93.40, 102.35)

Source: CSR SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

Administration with Soft foods

The oral administration of secnidazole 2 g oral granules admixed in pudding or yogurt has the comparable bioavailability to that of secnidazole granules admixed in applesauce (See Study SYM-1219-103 Appendix 4.2.3). The summary of the plasma secnidazole PK parameters following a single administration of SYM-1219 granules admixed with pudding, yogurt, and applesauce is presented in Table 3.3.4-3. Rate and extent of secnidazole absorption following administration of SYM-1219 granules with 4 oz. of chocolate pudding, 6 oz. of low-fat, vanilla yogurt, was comparable to secnidazole when admixed with 4 oz. of unsweetened applesauce. Based on the results of this study, secnidazole granules can be admixed in pudding, yogurt, or applesauce (Tables 3.3.4-4, 3.3.4-5).

Table 3.3.4-3 Summary of Mean Plasma Secnidazole Pharmacokinetic Parameters Following Treatment A - Pudding (Test), Treatment B – Yogurt (Test), and Treatment C - Applesauce (Reference) (PK Population)

Pharmacokinetic Parameters	Treatment A (Pudding) (N=23)	Treatment B (Yogurt) (N=24)	Treatment C (Applesauce) (N=24)
AUC0-t (µg*hr/mL)[a]	1370 (19.6)	1390 (19.9)	1430 (20.8)
AUCinf (µg*hr/mL)[a]	1410 (21.9)	1440 (22.2)	1480 (23.6)
AUC%extrap (%)[b]	3.09 ± 2.46	3.47 ± 2.69	3.76 ± 2.99
Cmax (µg/mL)[a]	45.4 (10.8)	43.1 (11.7)	43.9 (10.3)
Tmax (hr)[c]	4.00 (3.99, 6.01)	4.00 (4.00, 8.00)	4.00 (3.02, 6.14)
t1/2 (hr)[b]	17.6 ± 4.41	18.1 ± 4.73	18.5 ± 4.85

Treatment A = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of pudding (test)
Treatment B = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of yogurt (test)
Treatment C = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of applesauce (reference)

[a] Presented as geom. mean (geom. CV%)
[b] Presented as mean ± SD
[c] Presented as median (minimum, maximum)

Source: CSR SYM-1219-103, Tables 14.2.1.4, 14.2.1.5, 14.2.1.6

Table 3.3.4-4 Summary of Statistical Comparisons of Plasma Secnidazole Pharmacokinetic Parameters Following SYM-1219 granules Administration in Pudding Relative to Applesauce (Treatment A Versus Treatment C, PK Population)

	Geometric LS Means			
Parameters	Treatment A (Pudding) (Test) (N=23)	Treatment C (Applesauce) (Reference) (N=24)	GMR(%)	90% Confidence Interval
AUC0-t (µg*hr/mL)	1381	1427	96.80	93.51 - 100.22
AUCinf (µg*hr/mL)	1428	1483	96.31	92.82 - 99.92
Cmax (µg/mL)	45.29	43.86	103.25	100.33 - 106.24

Treatment A = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of pudding (test)
Treatment C = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of applesauce (reference)
Parameters were ln-transformed prior to analysis.
Geometric least-squares (LS) means were calculated by exponentiating the LS means from the mixed-effects model
% Geometric mean ratio (GMR) = 100 × (test/reference)

Source: CSR SYM-1219-103, Table 14.2.1.14

Table 3.3.4-5 Summary of Statistical Comparisons of Plasma Secnidazole Pharmacokinetic Parameters Following SYM-1219 granules Administration in Yogurt Relative to Applesauce (Treatment B Versus Treatment C, PK Population)

Parameters	Geometric LS Means Treatment B (Yogurt) (Test) (N=24)	Treatment C (Applesauce) (Reference) (N=24)	GMR(%)	90% Confidence Interval
AUC0-t (µg*hr/mL)	1393	1427	97.66	94.39 - 101.05
AUCinf (µg*hr/mL)	1444	1483	97.37	93.90 - 100.96
Cmax (µg/mL)	43.09	43.86	98.22	95.50 - 101.03

Treatment B = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of yogurt (test)
Treatment C = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of applesauce (reference)
Parameters were ln-transformed prior to analysis.
Geometric least-squares (LS) means were calculated by exponentiating the LS means from the mixed-effects model
% Geometric mean ratio (GMR) = 100 × (test/reference)

Source: CSR SYM-1219-103, Table 14.2.1.15

Drug-Drug Interactions

DDI study with Oral Contraceptive

The Applicant conducted an open-label, randomized, 2-period study that evaluated the effect of a single dose of secnidazole 2 g oral granules on the pharmacokinetics of single doses of the combination oral contraceptive (Ortho Novum®) containing 35 µg ethinyl estradiol (EE) and 1 mg norethindrone (NET) in 54 healthy female subjects between the ages of 18 and 45 years, inclusive (See Study SYM-1219-101 Appendix 4.2.1).

The results demonstrate there is a 29% decrease in Cmax for EE following concomitant administration of 2g of secnidazole oral granules with EE/NET which is deemed to not be clinically relevant. Co-administration of secnidazole 2g oral granules and EE/NET, either on the same day or 1 day apart, had no clinically relevant effects on the PK of EE or NET (Table 3.3.4-6). These results indicate that contraceptive efficacy should be maintained during co-administration of SYM-1219 oral granules and EE/NET. These data support the labeling -

(b) (4)

Table 3.3.4-6 EE and NET Plasma Pharmacokinetic Parameters Following Administration of EE/NET and SYM-1219

Parameter	Period 1 (EE/NET) Mean (%CV)	Period 2 (SYM-1219 + EE/NET) Mean (%CV)	GMR (90% CI)
EE			
Group B1* (N=26)			
C_{max}, pg/mL	81 (27)	60 (42)	71 (63–80)
AUC_{0-t}, h•pg/mL	644 (24)	616 (31)	94 (90–99)
AUC, h•pg/mL	911 (29)	954 (29)	105 (98–114)
Group B2* (N=25)			
C_{max}, pg/mL	89 (36)	93 (35)	105 (98–112)
AUC_{0-t}, h•pg/mL	681 (29)	667 (26)	99 (95–103)
AUC, h•pg/mL	995 (29)	937 (34)	93 (87–100)
NET			
Group B1* (N=26)			
C_{max}, ng/mL	9 (56)	10 (44)	113 (100–128)
AUC_{0-t}, h•ng/mL	50 (70)	54 (45)	116 (106–126)
AUC, h•ng/mL	58 (75)	62 (48)	116 (106–127)
Group B2* (N=25)			
C_{max}, ng/mL	10 (56)	11 (48)	117 (103–131)
AUC_{0-t}, h•ng/mL	61 (93)	61 (67)	111 (104–118)
AUC, h•ng/mL	75 (109)	72 (72)	109 (102–116)

*During period 1, all subjects in groups 1 and 2 received a single dose of EE/NET on day 1. Study drug administration during period 2 was as follows: subjects in group 1 received EE/NET followed immediately by 2 g SYM-1219 on day 1; subjects in group 2 received 2 g SYM-1219 on day 1 followed by EE/NET on day 2.

AUC=area under the curve; AUC_{0-t}=area under the curve from time zero to the last measurable concentration; CI=confidence interval; C_{max}=maximum plasma concentration; CV=coefficient of variation; EE=ethinyl estradiol; GMR=geometric mean ratio; NET=norethindrone.

Source: Applicant's Summary of Clinical Pharmacology Studies, Page 63, Table 20

In vitro Inhibition of human recombinant Aldehyde Dehydrogenase-2(ALDH2)

Secnidazole exhibited an apparent IC_{50} of 503 µM in a reversible inhibition assay. Secnidazole at concentrations up to 100 µM, the highest concentration that could be tested due to assay method limitation, exhibited no substantial evidence of time or concentration-dependent inhibition. The mean plasma C_{max} following a 2 g single oral granule dose of secnidazole is 245 µM, which indicates likelihood of inhibition of ALDH2 is minimal. These data support the labeling Section

12.3– “ (b) (4) ” (See In vitro Individual study reviews Appendix 4.2.4).

CYP enzyme mediated DDIs

Secnidazole was found to be an *in vitro* inducer of CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19 in human hepatocytes at concentrations of secnidazole (5000-10000 µM) which are significantly higher (approximately 20-40-fold higher) than the observed clinical C_{max} for secnidazole 245 µM (~45 µg/mL) and therefore induction with a single 2 g dose of secnidazole is not likely. Secnidazole exhibited direct inhibition of CYP2C19 and CYP3A4, with IC_{50} values ranging from 3722 to 4306 µM; other CYP enzymes tested exhibited IC_{50} values >5000 µM. These IC_{50} values are much higher than clinically relevant concentrations. Thus, secnidazole is not deemed by the reviewer to be a significant inducer or inhibitor of CYP450.

Transporter-mediated DDIs

Secnidazole is not a substrate of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2. Secnidazole is not a significant inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 (See Report 13SYMBP1R1, in vitro Individual study reviews Appendix 4.2.4).

3.3.5 Is the to-be-marketed formulation the same as the clinical trial formulation, and if not, are there bioequivalence data to support the to-be-marketed formulation?

The to-be-marketed formulation of SYM-1219 as well as the formulation used in the Phase 3 program and the later PK/BA studies (SYM-1219-103, SYM-1219-104, SYM-1219-105, SYM-1219-301 and SYM-350) were developed with the assistance of Catalent, Inc. (Somerset, NJ).

The drug product granules used in the early clinical studies (SYM-1219-101, SYM-129-102, SYM-1219-201) were developed with the assistance of (b) (4) a (b) (4) pharmaceutical development company who performed the manufacturing of supplies for early clinical use. The formulations are very similar in final composition although the process was changed in switching from (b) (4) to Catalent (refer to Biopharmaceutics review by Dr. Banu Zolnik).

A bioequivalence study (See Study SYM-1219-102 Appendix 4.2.2) compared PK of drug products produced by the 2 manufacturing sites for SYM-1219 (Table 3.3.5-1). The results showed that the secnidazole granules produced by (b) (4) and Catalent are bioequivalent (Table 3.3.5-2).

Table 3.3.5-1 Summary of Secnidazole Plasma Pharmacokinetic Parameters by Treatment

Parameter	SYM-1219-(b)(4) Fasted (A) (N=23)	SYM-1219-Cat Fasted (B) (N=23)
C_{max} (µg/mL)		
Mean (SD)	43.58 (5.701)	41.21 (5.489)
%CV	13.08	13.32
Median	45.20	39.90
Min, Max	30.8, 52.5	32.7, 56.2
T_{max} (h)		
Median	4.000	4.000
Min, Max	3.00, 4.00	3.00, 6.00
AUC_{0-t} (h*µg/mL)		
Mean (SD)	1321.62 (195.043)	1224.06 (217.138)
%CV	14.76	17.74
Median	1275.50	1169.20
Min, Max	983.8, 1714.4	868.7, 1621.7
AUC_{0-inf} (h*µg/mL)		
Mean (SD)	1364.74 (212.690)	1261.47 (236.505)
%CV	15.58	18.75
Median	1301.70	1203.70
Min, Max	997.9, 1817.4	874.3, 1750.4

Source: Clinical Study Report (CSR) SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

Table 3.3.5-2 Statistical Evaluation of C_{max}, AUC_{0-t}, and AUC_{0-inf} for the SYM-1219 2 g (b)(4) and Catalent Treatments

Parameter Treatment	Comparison	% Ratio: 100*Test/Reference	
		Geometric LSMean % Ratio (SE)[a]	90% C.I.[a]
C_{max} (µg/mL)			
SYM-1219-(b)(4) Fasted (A)			
SYM-1219-Cat Fasted (B)	Test (B) / Reference (A)	95.15 (1.862)	(92.07, 98.33)
AUC_{0-t} (h*µg/mL)			
SYM-1219-(b)(4) Fasted (A)			
SYM-1219-Cat Fasted (B)	Test (B) / Reference (A)	93.15 (2.485)	(89.06, 97.42)
AUC_{0-inf} (h*µg/mL)			
SYM-1219-(b)(4) Fasted (A)			
SYM-1219-Cat Fasted (B)	Test (B) / Reference (A)	92.94 (2.528)	(88.78, 97.29)

Source: CSR SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

The bioanalytical assay validation, sample stability, QC accuracy and precision in each clinical pharmacology study for secnidazole were reported in the individual bioanalytical reports and reviewed by the Clinical Pharmacology reviewer. All the bioanalytical methods were validated according to the criteria outlined in FDA guidance. Please refer to individual clinical pharmacology study reports for details (Appendix 4.2).

For the secnidazole Study SYM-1219-102, the following validated assays were used for quantification of the analyte.

Table 4.1-1 Validation Summary of Analytical Methods for Quantification of secnidazole in Human Plasma (Adapted from [redacted] Study Report [redacted] 10889-01)

Validation Summary	[redacted] Validation Study [redacted] 10889-01
Analyte	SYM-1219
Internal Standard (IS)	[redacted]
Method Description	Protein precipitation with analysis/detection by LC-MS/MS
Limit of Quantitation (µg/mL)	0.200 µg/mL
Average Recovery of Drug (% Mean)	80% at 0.600 µg/mL 85% at 3.00 µg/mL 81% at 37.5 µg/mL
Average Recovery of IS (% Mean)	81%
Standard Curve Concentrations (µg/mL)	0.200, 0.400, 1.00, 2.00, 4.00, 10.0, 20.0, 40.0, and 50.0 µg/mL
QC Concentrations (µg/mL)	LLOQ QC, 0.600, 3.00, and 37.5 µg/mL
QC Intra-Batch Precision Range (% CV)	0.7 to 6.2%
QC Intra-Batch Accuracy Range (% Bias)	-4.5 to 4.2%
QC Inter-Batch Precision Range (% CV)	3.0 to 5.3%
QC Inter-Batch Accuracy Range (% Bias)	-1.6 to 0.8%
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in polypropylene tubes at ambient temperature under white light (QCs stored at -20°C and -80°C) Cumulative Short-Term Stability: 62 hours in polypropylene tubes at ambient temperature under white light (total of all thaw cycles) (QCs stored at -20°C and -80°C)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 228 days at approximately 5000 µg/mL in methanol in a polypropylene container at -20°C
Processed Stability (Hrs)	Post-Preparative Stability: 105 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 151 hours in a polypropylene 96 well plate at 5°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C an d-80°C)-thaw (ambient temperature) cycles in polypropylene tubes under white light
Long-Term Storage Stability (Days)	Long-Term Stability: 155 days in polypropylene tubes at -20°C and -80°C
Dilution Integrity	Up to 100 µg/mL, diluted 5-fold

Selectivity	No significant interference at the retention time and mass transition of SYM-1219 was observed from endogenous components in any of the 10 human plasma (EDTA) lots screened or of metronidazole (IS) in any of the 10 human plasma (EDTA) lots screened

Table 4.1-2 Validation Summary of Analytical Methods for Quantification of secnidazole in Human Urine (Adapted from (b) (4) 10889-02 Study Report)

Validation Summary	(b) (4) Validation Study (b) (4) 10889-02
Analyte	SYM-1219
Internal Standard (IS)	(b) (4)
Method Description	Dilution procedure with analysis/detection by LC-MS/MS
Limit of Quantitation (µg/mL)	0.0500 µg/mL
Average Recovery of Drug (% Mean)	102% at 0.150 µg/mL 96% at 0.750 µg/mL 100% at 7.50 µg/mL
Average Recovery of IS (% Mean)	99%
Standard Curve Concentrations (µg/mL)	0.0500, 0.100, 0.200, 0.400, 0.800, 1.50, 3.00, 8.00 and 10.0 µg/mL
QC Concentrations (µg/mL)	LLOQ QC, 0.150, 0.750, and 7.50 µg/mL
QC Intra-Batch Precision Range (% CV)	1.1 to 6.7%
QC Intra-Batch Accuracy Range (% Bias)	-10.0 to 4.0%
QC Inter-Batch Precision Range (% CV)	2.5 to 7.7%
QC Inter-Batch Accuracy Range (% Bias)	-7.3 to -5.4%
Bench-Top Stability (Hrs)	Short-Term Stability: 24 hours in polypropylene tubes at ambient temperature under white light Cumulative Short-Term Stability: 61 hours in polypropylene tubes at ambient temperature under white light (total of all thaw cycles)
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 228 days at approximately 5000 µg/mL in methanol in a polypropylene container at -20°C
Processed Stability (Hrs)	Post-Preparative Stability: 156 hours in a polypropylene 96 well plate at 5°C Processed Sample Integrity: 211 hours in a polypropylene 96 well plate at 5°C
Freeze-Thaw Stability (Cycles)	6 freeze (-20°C)-thaw (ambient temperature) cycles in polypropylene tubes under white light
Long-Term Storage Stability (Days)	Long-Term Stability: 116 days in polypropylene tubes at -20°C
Dilution Integrity	Up to 500 µg/mL, diluted 100-fold
Selectivity	No significant interference at the retention time and mass transition of SYM-1219 was observed from endogenous components in any of the 10 human urine lots screened or of metronidazole (IS) in any of the 10 human urine lots screened

Quality control samples concentration range is acceptable for both plasma and urine.
Accuracy and precision of the calibration curve samples and quality control samples were within 15% (20% at the lower limit of quantitation) for both plasma and urine.

Stability demonstrated in samples under varying conditions of storage for both plasma and urine.

For the secnidazole and oral contraceptive DDI study, multiple validated assays were used for quantification of ethinyl estradiol, norethindrone and secnidazole in human plasma and urine. See Appendix 4.2.2 of the individual study report for this DDI study for additional details of the validation and bioanalytical assay performance for EE, norethindrone, and secnidazole.

In general the validation and performance of the bioanalytical assays for secnidazole and all other relevant analytes were acceptable.

4.2 Individual Clinical Pharmacology Report Reviews

	Study No.	Study information
4.2.1	SYM-1219-101	A Phase 1, Open-label, 2-Part Study to Assess the Pharmacokinetics and Safety of a 1 gram or 2 gram Single Dose of SYM-1219 in Healthy Female Volunteers (Part A) and to Determine the Effect of SYM-1219 on the Pharmacokinetics of Ethinyl Estradiol (EE2) and Norethindrone (NET) in Healthy Female Volunteers (Part B)
4.2.2	SYM-1219-102	A Phase 1, Open-label, Single-dose, Randomized, 3-way Crossover Study to Assess the Pharmacokinetics and Safety of 2 Grams of SYM-1219 Administered Orally as Granules in Applesauce Under Fed and Fasted Conditions in Healthy Female Volunteers
4.2.3	SYM-1219-103	A Phase 1, Open-Label, Single-Dose, Randomized, 3-Way Crossover Study to Assess the Pharmacokinetics and Safety of SYM-1219 Containing 2 Grams of Secnidazole Administered Orally as Granules in Pudding, Yogurt, or Applesauce under Fasting Conditions in Healthy Female Volunteers
4.2.4		In Vitro Study Reviews

4.2.1 Study # SYM-1219-101
TITLE OF STUDY:

A Phase 1, Open-label, 2-Part Study to Assess the Pharmacokinetics and Safety of a 1 gram or 2 gram Single Dose of SYM-1219 in Healthy Female Volunteers (Part A) and to Determine the Effect of SYM-1219 on the Pharmacokinetics of Ethinyl Estradiol (EE) and Norethindrone (NET) in Healthy Female Volunteers (Part B)

STUDY OBJECTIVES:

- Determine the plasma and urine PK of SYM-1219 granules sprinkled over 4 ounces of applesauce.
- Determine the safety of SYM-1219 granules sprinkled over 4 ounces of applesauce.
- Evaluate the effect of a single dose of SYM-1219 on the PK of ethinyl estradiol (EE) and norethindrone (NET).

SELECTION OF DOSES IN THE STUDY:

The doses of SYM-1219 oral granules in this clinical trial were single doses of either 1 g or 2 g. The choice of these doses was based on the large number of previous patient exposures for secnidazole worldwide and the results of the 7-day rat and 7-day dog toxicology studies. In addition, results from the 28-day rat and dog studies also support the single dose administration of 1 g or 2 g to healthy volunteers as toxicities were not observed following a single dose. In Part B of the study, a commercially available oral contraceptive, Ortho Novum® 1/35, was chosen to study the potential interaction of a common oral contraceptive combination product (EE/NET) and SYM-1219.

STUDY DESIGN:
This was two-part, Phase 1, open-label study.

Duration of treatment:
Part A: Single 1 g or 2 g dose of SYM-1219 granules
Part B: Two doses of EE/NET; Single 2 g dose of SYM-1219 granules

Part A

In Part A of the study, subjects received a single dose of SYM-1219 granules (either 1 g for Group A1 or 2 g for Group A2) and remained in the in-patient unit under supervision until the 168 hours post-dose PK blood draw and the final study assessments were completed (Day 8). Blood was obtained for determination of SYM-1219 plasma concentrations at the following times: pre-dose (within 30 minutes of dosing), and then at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 18, 24, 36, 48, 72, 96, 120, 144, and 168 hours post-dose. Urine was also collected at pre-specified times for determination of SYM-1219 concentrations.

Part B

In Part B of the study, all subjects received EE/NET on Day 1 of **Period 1** and blood was obtained at predetermined times over a 24-hour period to assess EE/NET PK. Subjects were discharged from the clinic following the completion of the assessments on Day 2 and returned 7-10 days later for **Period 2**. In **Period 2**, subjects were randomized to either Group B1 or Group B2. Group B1 subjects received 2 g of SYM-1219 and EE/NET on the same Day (**Day 1 of Period 2**), and Group B2 subjects received 2 g of SYM-1219 and EE/NET on separate days (2 g of SYM-1219 on Day 1 of **Period 2**, then EE/NET on Day 2 of **Period 2**).

Bioanalytical Method Description:

Plasma and urine secnidazole (SYM-1219) and plasma EE/NET concentrations were determined by (b) (4) using validated bioanalytical procedures. The lower limits of quantification (LLOQ) were 0.200 µg/mL for secnidazole in plasma, 0.0500 µg/mL for secnidazole in urine, 2.00 pg/mL for EE, and 0.0500 ng/mL for NET.

Ethinyl estradiol standard curve concentrations (pg/mL) were 2.00, 5.00, 10.0, 20.0, 50.0, 100, 200, 400, and 500 pg/mL and QC Concentrations (pg/mL) were 6.00, 75.0, 225, and 375 pg/mL.

Norethindrone standard curve concentrations were (ng/mL) 0.0500, 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 8.00, and 10.0 ng/mL and QC Concentrations (ng/mL) were 0.150, 0.750, 7.50 ng/mL. The inter-batch and intra-batch accuracy and precision results for QC were within ±15.0%. Ethinyl estradiol and norethindrone plasma samples demonstrated freeze-thaw and long- term storage stability. No interference at the retention time and mass transition for ethinyl estradiol and norethindrone was observed in human plasma samples.

A set of 9 non-zero calibration standards, ranging from 0.200 µg/mL to 50.0 µg/mL was prepared for SYM-1219 in plasma. QC concentrations for SYM-1219 were: 0.2 µg/mL, 0.600 µg/mL, 3.00 µg/mL, 37.5 µg/mL. The standard curve range of secnidazole in urine was from 0.500 µg/mL to 10.0 µg/mL. No interference at the retention time and mass transition for secnidazole was observed from any endogenous components in human urine.

Linearity was indicated by a correlation coefficient of ≥0.990 from the standard curve. The LC-MS/MS method for the determination of SYM-1219 in human plasma (EDTA) and urine met the requirements as specified in the validation protocol (Appendix 4.1). Stability was demonstrated for SYM-1219 in human plasma (EDTA) and urine samples and solutions under varying conditions of storage.

For precision and accuracy (PA) batches, the inter-batch and intra-batch precision and accuracy results for QC samples prepared at LLOQ, low, medium, and high concentrations met acceptance criteria for both plasma and urine. Accuracy (% Theoretical) for LLOQ QC was within 80.0 – 120.0%, expressed as % Bias within ± 20.0%. Accuracy (% Theoretical) for low, medium, and

high QCs was within 85.0 – 115.0%, expressed as % Bias within ± 15.0%. The difference between the lowest and highest means recovery values was ≤ 20.0% which met the acceptance criteria for both plasma and urine.

Precision (% CV) for the LLOQ QC was ≤ 20.0%. Precision (% CV) for low, medium, and high QCs was ≤ 15.0%.

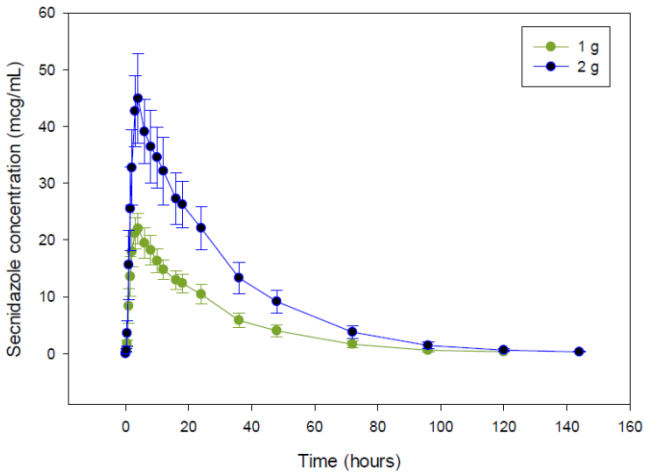
Reviewer Comment: In general, the bioanalytical assay validation and QC performance for secnidazole, ethinyl estradiol (EE), and norethindrone (NET) were acceptable.

SUMMARY OF PHARMACOKINETIC RESULTS:

Part A:
Pharmacokinetics:
Twenty-eight (28) subjects were randomized into and completed Part A. Mean (±SD) plasma concentrations of secnidazole by dose and time point are displayed graphically below (Figure 1) and a summary of the pharmacokinetic parameters is presented in Table 1.

The mean plasma concentration-time plot shows secnidazole plasma levels for both the 1 g and 2 g doses increased and reached the highest concentration at approximately 4 hours after administration of SYM-1219 (Figure 1). After 4 hours the mean concentrations declined and were, on average, no longer measurable (<0.200 µg/mL) at 120 hours for the 1 g dose and 144 hours for the 2 g dose.

Figure 1 Mean (±SD) Secnidazole Plasma Levels by SYM-1219 Treatment Group (Part A)



Source: Applicant's Summary of Clinical Pharmacology Studies, Page 46, Figure 1

Table 1 Plasma Pharmacokinetics of Secnidazole After a Single Oral Dose of SYM-1219 Administered to Fasted Healthy Female Subjects (Part A)

Parameter	SYM-1219 1 gram (N=14)	SYM-1219 2 grams (N=14)
C_{max} (µg/mL)		
n	14	14
Mean (SD)	22.62 (2.871)	45.43 (7.642)
%CV	12.69	16.82
Geometric Mean (SD)	22.45 (2.938)	44.84 (7.467)
Median	22.75	45.05
Min, Max	17.4, 26.5	34.5, 58.3
T_{max} (h)		
n	14	14
Median	3.060	4.000
Min, Max	2.00, 6.00	3.00, 4.05
AUC_{0-t} (h*µg/mL)		
n	14	14
Mean (SD)	609.66 (96.685)	1322.40 (230.256)
%CV	15.86	17.41
Geometric Mean (SD)	602.94 (92.067)	1305.35 (214.383)
Median	587.42	1290.41
Min, Max	487.6, 832.5	1048.5, 1899.5
$AUC_{0-\infty}$ (h*µg/mL)		
n	14	14
Mean (SD)	618.89 (98.093)	1331.63 (230.159)
%CV	15.85	17.28
Geometric Mean (SD)	612.09 (93.248)	1314.74 (214.081)
Median	595.25	1299.10
Min, Max	498.5, 847.0	1055.1, 1911.9
$t_{1/2}$ (h)		
n	14	14
Mean (SD)	17.05 (1.611)	16.86 (2.649)
Median	16.79	17.13
Min, Max	14.7, 20.4	11.3, 20.4
λz (1/h)		
n	14	14
Mean (SD)	0.04099 (0.003757)	0.04220 (0.007544)
Median	0.04129	0.04047
Min, Max	0.0339, 0.0471	0.0340, 0.0613

Source: Table 14.1.2.4, Listing 16.2.1.11.2 in SYM-1219-101 Clinical Study Report (CSR)

Descriptive statistics of secnidazole urine pharmacokinetic parameters by dose are provided in Table 2 below. The dose related parameter (Ae_{0-168}) appeared to exhibit dose proportionality between the two doses. Doubling the dose from 1 g to 2 g resulted in a 2.25-fold increase in mean Ae_{0-168} (0.136 and 0.306 g for the 1 g and 2 g dose, respectively). CLr was similar between the two doses suggesting renal clearance was dose independent over the 1-2 g dose range. The mean CLr values (3.742 and 3.935 mL/min) are low relative to the glomerular filtration rate (GFR) which is >90 mL/min in healthy subjects with normal renal function. Over the 168-hour

collection period, the %FE values were similar between doses with 13.6% and 15.3% of secnidazole excreted unchanged into the urine for the 1 g and 2 g doses, respectively.

Table 2 Urine Pharmacokinetics of Secnidazole After a Single Oral Dose of SYM-1219 Administered to Fasted Healthy Female Subjects (Part A)

Parameter	SYM-1219 1 gram (N=14)	SYM-1219 2 grams (N=14)
$Ae_{0\text{-}168}$ (g)		
n	14	14
Mean (SD)	0.136 (0.0238)	0.306 (0.0711)
%CV	17.478	23.234
Geometric Mean (SD)	0.134 (0.0241)	0.300 (0.0602)
Median	0.140	0.299
Min, Max	0.10, 0.18	0.22, 0.52
CLr (mL/min)		
n	14	14
Mean (SD)	3.742 (0.8255)	3.935 (1.0568)
%CV	22.060	26.859
Geometric Mean (SD)	3.650 (0.8701)	3.801 (1.0532)
Median	3.965	3.962
Min, Max	2.37, 4.89	2.23, 6.19
%FE		
n	14	14
Mean (SD)	13.602 (2.3773)	15.300 (3.5549)
%CV	17.478	23.234
Geometric Mean (SD)	13.403 (2.4100)	14.991 (3.0081)
Median	13.981	14.943
Min, Max	10.19, 17.56	11.03, 26.20

Source: Table 14.1.2.5, Listing 16.2.1.12.1 in SYM-1219-101 CSR

- The pharmacokinetics of secnidazole following administration of oral granules was dose proportional when 1 g and 2 g doses were compared.
- The PK parameters for secnidazole exhibited low inter-subject variability demonstrating the reproducible performance of the SYM-1219 granules formulation.
- The T_{max} of secnidazole was approximately 3-4 hours following oral granules administration.
- The half-life of secnidazole following oral granules administration was approximately 17 hours and was independent of the dose administered.
- There was low excretion of unchanged secnidazole into the urine, representing approximately 13-15% of the administered dose.

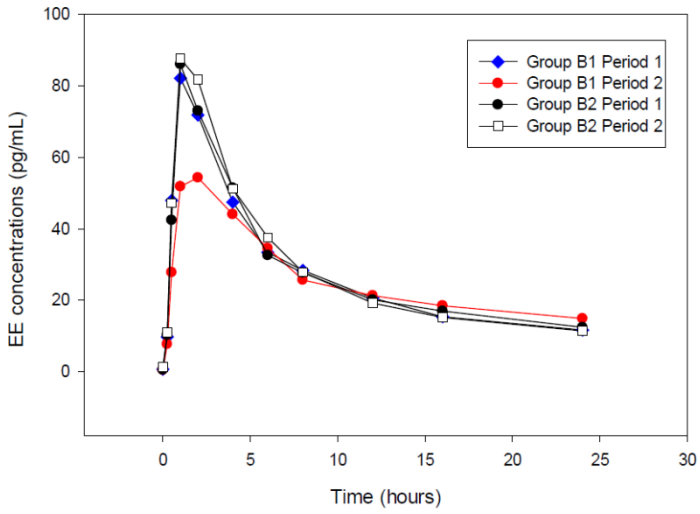
Part B:
Pharmacokinetics:

EE
Mean plasma concentration-time profiles of EE by period and treatments are shown in Figure 2, when EE/NET was administered alone (**Period 1, Groups B1 & B2**), and when EE/NET was co-administered with SYM-1219 on Day 1 (**Period 2, Group B1**), or when SYM-1219 oral granules were given on Day 1 and EE/NET was administered alone on Day 2 (**Period 2, Group B2**).

In Period 1, Group B1, EE C_{max} was 80.63 pg/mL when EE/NET was administered alone, decreased by 29% when EE/NET was co-administered with SYM-1219 (59.88 pg/mL). Both AUC_{0-t} and $AUC_{0-\infty}$ values were similar when comparing EE/NET co-administered with secnidazole oral granules and EE/NET administered alone.

NET
Mean plasma concentration-time profiles of NET are shown in Figure 3. NET plasma levels when EE/NET was administered alone and when EE/NET was administered co-administered with SYM-1219 appeared to be nearly superimposable for both treatment groups. In Period 1, Group B1 mean C_{max} was 9.18 ng/mL and Period 2, Group B1 C_{max} was 10.07 ng/mL. NET plasma concentrations over the 24 hour period were similar between all treatment groups.

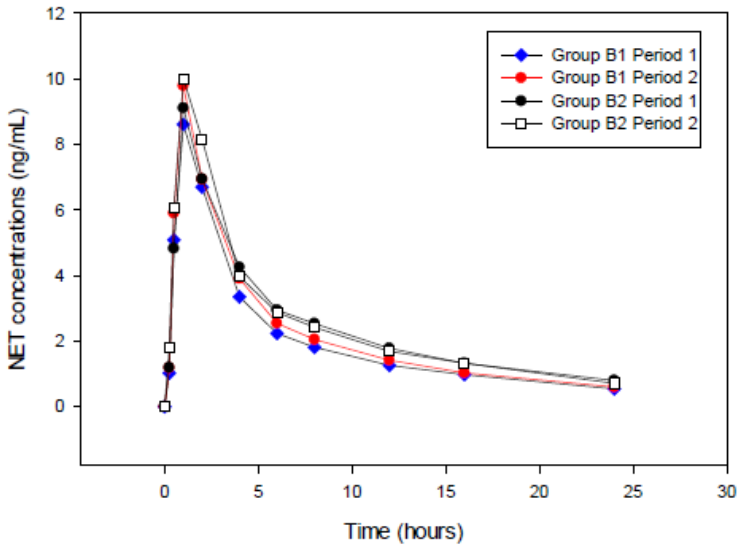
Figure 2 Mean EE Plasma Levels by Period and Treatment*



Source: Applicant's Summary of Clinical Pharmacology Studies, Page 60, Figure 3

***Period 1:** all subjects in **Groups B1 and B2** received a single dose of ethinyl estradiol/norethindrone (EE/NET) alone on Day 1.
Period 2: subjects in **Group B1** received EE/NET concomitantly with 2 g SYM-1219 on Day 1; subjects in **Group B2** received 2 g SYM-1219 on Day 1 followed by EE/NET on Day 2.

Figure 3 Mean NET Plasma Levels by Period and Treatment*



Source: Applicant's Summary of Clinical Pharmacology Studies, Page 61, Figure 4

***Period 1:** all subjects in **Groups B1 and B2** received a single dose of ethinyl estradiol/norethindrone (EE/NET) alone on Day 1.
Period 2: subjects in **Group B1** received EE/NET concomitantly with 2 g SYM-1219 on Day 1; subjects in **Group B2** received 2 g SYM-1219 on Day 1 followed by EE/NET on Day 2.

The pharmacokinetic parameters and 90% confidence intervals (CIs) for EE and NET are presented in Table 3. The EE C_{max} geometric mean ratio (GMR) for **Period 2, Group B1** was 71 % and the 90% CI was 63%-80% indicating co-administration of EE/NET with secnidazole oral granules resulted in a reduction in EE peak plasma concentrations of approximately 30%.

Table 3 EE and NET Plasma Pharmacokinetic Parameters Following Administration of EE/NET and SYM-1219

Parameter	Period 1 (EE/NET) Mean (%CV)	Period 2 (SYM-1219 + EE/NET) Mean (%CV)	GMR (90% CI)
EE			
Group B1* (N=26)			
C_{max}, pg/mL	81 (27)	60 (42)	71 (63–80)
AUC_{0-t}, h•pg/mL	644 (24)	616 (31)	94 (90–99)
AUC, h•pg/mL	911 (29)	954 (29)	105 (98–114)
Group B2* (N=25)			
C_{max}, pg/mL	89 (36)	93 (35)	105 (98–112)
AUC_{0-t}, h•pg/mL	681 (29)	667 (26)	99 (95–103)
AUC, h•pg/mL	995 (29)	937 (34)	93 (87–100)
NET			
Group B1* (N=26)			
C_{max}, ng/mL	9 (56)	10 (44)	113 (100–128)
AUC_{0-t}, h•ng/mL	50 (70)	54 (45)	116 (106–126)
AUC, h•ng/mL	58 (75)	62 (48)	116 (106–127)
Group B2* (N=25)			
C_{max}, ng/mL	10 (56)	11 (48)	117 (103–131)
AUC_{0-t}, h•ng/mL	61 (93)	61 (67)	111 (104–118)
AUC, h•ng/mL	75 (109)	72 (72)	109 (102–116)

*During period 1, all subjects in groups 1 and 2 received a single dose of EE/NET on day 1. Study drug administration during period 2 was as follows: subjects in group 1 received EE/NET followed immediately by 2 g SYM-1219 on day 1; subjects in group 2 received 2 g SYM-1219 on day 1 followed by EE/NET on day 2.

AUC=area under the curve; AUC_{0-t}=area under the curve from time zero to the last measurable concentration; CI=confidence interval; C_{max}=maximum plasma concentration; CV=coefficient of variation; EE=ethinyl estradiol; GMR=geometric mean ratio; NET=norethindrone.

Source: Applicant's Summary of Clinical Pharmacology Studies, Page 63, Table 20

Sponsor DDI Summary:

- When secnidazole 2g oral granules were co-administered with EE/NET there was a statistically significant but not clinically relevant decrease (29%) in the C_{max} for EE. There was no effect from secnidazole 2g oral granules on EE AUC_{0-t} and $AUC_{0-\infty}$ when taken immediately after administration of EE/NET.
- When SYM-1219 was administered 1 day before EE/NET administration there was no effect on EE C_{max} or AUC.

- When secnidazole 2g oral granules were co-administed with EE/NET there was a small increase (13-16%) in the rate and extent of absorption (C_{max} and AUC) of NET. This increase was not clinically relevant.
- There was no effect from secnidazole 2g oral granules on NET AUC_{0-t} and $AUC_{0-\infty}$ when EE/NET was taken one day after administration of secnidazole 2g oral granules. NET C_{max} showed a small increase (17%) when taken one day after administration of SYM 1219. This increase was not clinically relevant.

Sponsor Safety Conclusions:

- Administration of SYM-1219 in conjunction with EE/NET was well tolerated. Overall, a higher incidence of AEs was observed when SYM-1219 was administered with EE/NET as compared to the administration of EE/NET alone. All observed adverse events were mild and generally resolved without treatment.
- There were no clinically relevant effects of administration of secnidazole 2g oral granules with EE/NET on laboratory parameters. No notable differences were observed between treatment groups in the mean, median, or min/max changes from a baseline in any laboratory parameter.
- None of the mean changes seen in heart rate, PR interval, QRS interval, QT interval, QTc interval, QTcB interval, QTcF interval or RR interval were clinically important.

SPONSOR'S CONCLUSIONS:
Based on these results, co-administration of SYM-1219 and EE/NET, either on the same day or 1 day apart, had no clinically relevant effects on the bioavailability of EE or NET. These results indicate that contraceptive efficacy should be maintained during co-administration of SYM-1219 and EE/NET.

Reviewer's Comments:
The Reviewer agrees with the Applicant's conclusions that the combination oral contraceptive, EE/NET, may be co-administered with secnidazole granules. However, the Reviewer notes that there was a 29% decrease in the C_{max} for EE which is deemed to not be clinically relevant, when secnidazole granules were co-administered with EE/NET.

4.2.2 Study # SYM-1219-102

TITLE OF STUDY:

A Phase 1, Open-label, Single-dose, Randomized, 3-way Crossover Study to Assess the Pharmacokinetics and Safety of 2 Grams of SYM-1219 Administered Orally as Granules in Applesauce Under Fed and Fasted Conditions in Healthy Female Volunteers

OBJECTIVES:

- Compare the pharmacokinetics of SYM-1219 granules containing 2 g of secnidazole manufactured by (b) (4) and manufactured by Catalent Pharma Solutions (Catalent) administered in applesauce under fasted conditions in healthy female volunteers
- Compare the pharmacokinetics of 2 g secnidazole granules manufactured by Catalent administered in applesauce under fed and fasted conditions in healthy female volunteers
- Evaluate the safety of single doses of 2 g secnidazole granules administered in applesauce in healthy female volunteers

STUDY DESIGN:

The study was conducted at a single study center in the United States. This was an open-label, randomized, 3-way crossover study of 2 g of secnidazole administered as granules in applesauce under fed and fasted conditions in healthy female volunteers. A total of 24 subjects were to be enrolled to ensure that 18 subjects completed the study.

Subjects underwent screening procedures up to 30 days before the initial dose of study drug to determine eligibility. Subjects determined to be eligible for the study were enrolled and randomly assigned to receive each of the following 3 treatments in a crossover fashion according to the randomization schedule:

Three doses of 2 g secnidazole granules were administered in applesauce as follows:

- Treatment A: One dose of SYM-1219 under fasted conditions (SYM-1219-(b) (4))
- Treatment B: One dose of SYM-1219 under fasted conditions (SYM-1219-Cat)
- Treatment C: One dose of SYM-1219 under fed conditions (SYM-1219-Cat)

There was a minimum of a 7-day washout period between each dose of SYM-1219. Subjects were admitted to the clinical research unit on Day –1 of Period 1 and remained confined in the clinical unit until all assessments were completed on Day 5 of Period 3.

On Day 1 of each period, subjects received 1 of 3 treatments according to their treatment assignment. The following instructions were followed for Treatments A, B, and C:

Fasted treatments: after an overnight fast of at least 10 hours, subjects received the study drug (SYM-1219-(b) (4) or SYM-1219-Cat) administered as granules containing 2 g of secnidazole in applesauce followed by 240 mL of water. No food was allowed for 4 hours after SYM-1219 administration.

Fed treatment: after an overnight fast of at least 10 hours, subjects were served a high-fat, high-calorie breakfast 30 minutes before the scheduled dose (breakfast consisted of 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 ounces of hash browns, 8 ounces of whole milk; this meal contained approximately 150 protein calories, 250 carbohydrate calories, and 500-600 fat calories). The study drug (SYM-1219-Cat) was administered within 5 minutes of the scheduled dosing time as granules containing 2 g of secnidazole administered in applesauce followed by 240 mL of water. No other food was allowed for 4 hours after SYM-1219 administration.

During each treatment period, pharmacokinetic (PK) blood samples were collected for the determination of secnidazole plasma concentrations following administration of SYM-1219 at the following time points: before dosing (within 30 minutes of dosing) and then at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 18, 24, 36, 48, 72, and 96 hours after dosing.

In each treatment period, an aliquot of urine was collected before dosing as baseline for potential metabolite urine concentration assessment. Thereafter, total urine was collected for the possible determination of concentrations of secnidazole and any metabolites of secnidazole at the following time intervals: 0 to 6, 6 to 12, 12 to 24 hours, and then continuing at 12-hour intervals up to 96 hours after dosing (24 to 36, 36 to 48, 48 to 60, 60 to 72, 72 to 84, and 84 to 96 hours).

Secnidazole and metabolite urine concentrations were not analyzed.

Safety assessments were performed at specified time points during each treatment period and at the conclusion of the study.

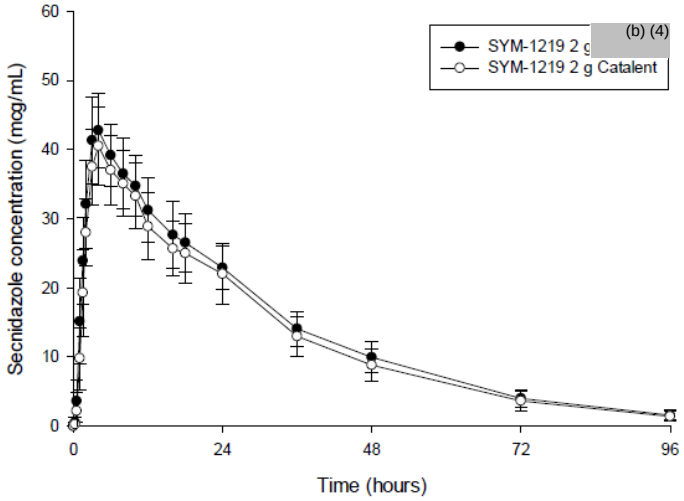
SUMMARY OF PHARMACOKINETICS:

The bioequivalence component of the SYM-1219-102 study compared the pharmacokinetics of secnidazole 2 g oral granules manufactured by (b) (4) and manufactured by Catalent Pharma Solutions (Catalent) administered in applesauce under fasted conditions in healthy female volunteers.

Mean (± SD) plasma concentrations of secnidazole by treatment and time point are shown in Figure 1. Descriptive statistics of secnidazole plasma pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, t½, and ƛz values by treatment are presented in Table 1. The median time to maximum plasma concentration, T_{max}, was 4.00 h for the SYM-1219-(b) (4) Fasted and SYM-1219-Cat Fasted treatments. The mean half-lives were similar for both treatment groups: 17.90 h for SYM-1219-(b) (4) Fasted and 17.53 h for SYM-1219-Cat Fasted.

The statistical evaluation of the pharmacokinetic parameters C_{max}, AUC_{0-t}, and AUC_{0-inf} is presented in Table 2. The 90% confidence intervals for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for the SYM-1219-(b) (4) Fasted and SYM-1219-Cat Fasted formulations demonstrated bioequivalence between the two treatment groups as all of the confidence interval comparisons were within the 80%-125% equivalence limits. The statistical evaluation for T_{max} is presented in Table 3. T_{max} for the SYM-1219-(b) (4) Fasted and SYM-1219-Cat Fasted formulations was comparable.

Figure 1 Mean (±SD) secnidazole concentration time profiles following single dose administration of SYM-1219 2 g manufactured at (b) (4) and Catalent



Source: Applicant's Summary of Biopharmaceutics Studies and Associated Analytical Methods, Page 26, Figure 4

Table 1 Summary of Secnidazole Plasma Pharmacokinetic Parameters by (b) (4) and Catalent Treatment

Parameter	SYM-1219-(b) (4) Fasted (A) (N=23)	SYM-1219-Cat Fasted (B) (N=23)
C_{max} (µg/mL)		
Mean (SD)	43.58 (5.701)	41.21 (5.489)
%CV	13.08	13.32
Median	45.20	39.90
Min, Max	30.8, 52.5	32.7, 56.2
T_{max} (h)		
Median	4.000	4.000
Min, Max	3.00, 4.00	3.00, 6.00
AUC_{0-t} (h*µg/mL)		
Mean (SD)	1321.62 (195.043)	1224.06 (217.138)
%CV	14.76	17.74
Median	1275.50	1169.20
Min, Max	983.8, 1714.4	868.7, 1621.7
AUC_{0-inf} (h*µg/mL)		
Mean (SD)	1364.74 (212.690)	1261.47 (236.505)
%CV	15.58	18.75
Median	1301.70	1203.70
Min, Max	997.9, 1817.4	874.3, 1750.4
$t_{1/2}$ (h)		
Mean (SD)	17.90 (2.792)	17.53 (2.785)
Median	17.70	17.10
Min, Max	11.7, 22.1	12.6, 24.6
λz (1/h)		
Mean (SD)	0.0397 (0.00675)	0.0405 (0.00627)
Median	0.0392	0.0406
Min, Max	0.031, 0.059	0.028, 0.055

Source: CSR SYM-1219-102, Table 14.2.6 and Listings 16.2.14.1 – 16.2.14.3

Table 2 Statistical Evaluation of C_{max}, AUC_{0-t}, and AUC_{0-inf} for the SYM-1219 2 g (b) (4) and Catalent Treatments

Parameter Treatment	Comparison	% Ratio: 100*Test/Reference	
		Geometric LSMean % Ratio (SE)[a]	90% C.I.[a]
C_{max} (µg/mL)			
SYM-1219-(b) (4) Fasted (A)			
SYM-1219-Cat Fasted (B)	Test (B) / Reference (A)	95.15 (1.862)	(92.07, 98.33)
AUC_{0-t} (h*µg/mL)			
SYM-1219-(b) (4) Fasted (A)			
SYM-1219-Cat Fasted (B)	Test (B) / Reference (A)	93.15 (2.485)	(89.06, 97.42)
AUC_{0-inf} (h*µg/mL)			
SYM-1219-(b) (4) Fasted (A)			
SYM-1219-Cat Fasted (B)	Test (B) / Reference (A)	92.94 (2.528)	(88.78, 97.29)

Source: CSR SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

Table 3 Statistical Evaluation of T_{max} for the SYM-1219 2 g (b) (4) and Catalent Treatments

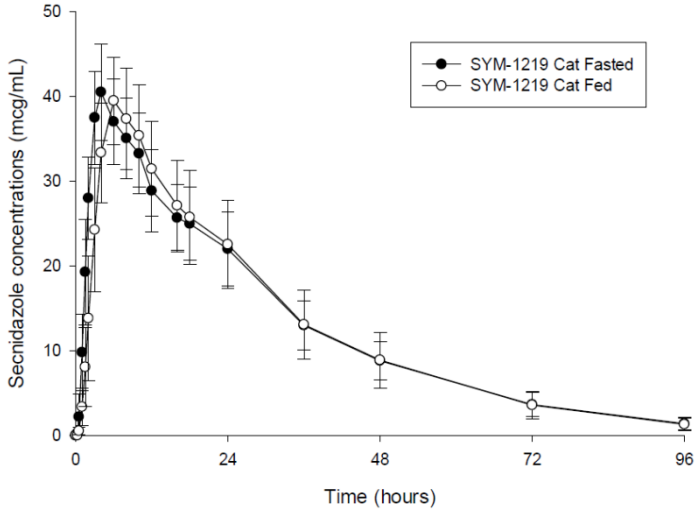
SYM-1219-Cat Fasted (B) - SYM-1219- (b) (4) Fasted (A)	Results (N=23)
Median	0.000
Min, Max	-1.00, 3.00
90% C.I.[a]	(0.000, 0.000)
P-value[b]	0.234

a: Symmetric distribution free 90% confidence interval for the median.
b: From the signed-rank test.

Source: CSR SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

Comparison of Pharmacokinetics under Fed vs. Fasted Conditions

Figure 2 Mean (±SD) Secnidazole Plasma Concentrations by following single dose administration of secnidazole 2 g oral granules (Cat) under fasted and fed conditions



Source: Applicant's Summary of Biopharmaceutic Studies and Associated Analytical Methods, Page 18, Figure 2

Mean (±SD) secnidazole plasma concentrations following single dose administration of 2 g oral granules (Cat) under fasted and fed conditions are shown in Figure 2. Descriptive statistics for Cmax, Tmax, AUC0-t, AUC0-inf, t½, and λz values are presented in Table 4. Cmax, AUC0-t, AUC0-∞ exhibited low intersubject variability with %CVs below 23.5% for all treatment groups. The median time to maximum plasma concentration, Tmax, was 4.00 h for the SYM-1219-Cat

Fasted treatments but was slightly longer (6.00 h vs 4.00 h) for the SYM-1219-Cat Fed group. The mean half-lives were similar for both treatment groups; 17.53 h for SYM-1219-Cat Fasted, and 16.92 h for SYM-1219-Cat Fed.

The statistical evaluation of the pharmacokinetic parameters C_{max}, AUC_{0-t}, and AUC_{0-inf} is presented in Table 5. The 90% confidence intervals for C_{max}, AUC_{0-t}, and AUC_{0-inf} for the SYM-1219-Cat Fasted and SYM-1219-Cat Fed comparisons for C_{max}, AUC_{0-t}, and AUC_{0-inf} demonstrated similar exposures between the two treatment groups.

The statistical evaluation for T_{max} is presented in Table 6. Statistical differences were evident for T_{max} when the SYM-1219-Cat Fasted and SYM-1219-Cat Fed treatment groups were compared ($p<0.001$).

Table 4 Summary of Secnidazole Plasma Pharmacokinetic Parameters by Treatment

Parameter	SYM-1219-Cat Fasted (N=23)	SYM-1219-Cat Fed (N=23)
C_{max} (µg/mL)		
Mean (SD)	41.21 (5.489)	40.13 (4.871)
%CV	13.32	12.14
Median	39.90	40.90
Min, Max	32.7, 56.2	31.0, 47.7
T_{max} (h)		
Median	4.000	6.000
Min, Max	3.00, 6.00	4.00, 8.00
AUC_{0-t} (h*µg/mL)		
Mean (SD)	1224.06 (217.138)	1214.36 (272.581)
%CV	17.74	22.45
Median	1169.20	1197.00
Min, Max	868.7, 1621.7	754.5, 1673.3
AUC_{0-inf} (h*µg/mL)		
Mean (SD)	1261.47 (236.505)	1248.15 (291.629)
%CV	18.75	23.36
Median	1203.70	1237.30
Min, Max	874.3, 1750.4	762.0, 1769.4
$t_{1/2}$ (h)		
Mean (SD)	17.53 (2.785)	16.92 (2.520)
Median	17.10	16.70
Min, Max	12.6, 24.6	12.6, 21.6

Parameter	SYM-1219-Cat Fasted (N=23)	SYM-1219-Cat Fed (N=23)
λz (1/h)		
Mean (SD)	0.0405 (0.00627)	0.0418 (0.00619)
Median	0.0406	0.0416
Min, Max	0.028, 0.055	0.032, 0.055

Source: CSR SYM-1219-102, Table 14.2.6 and Listings 16.2.14.1 – 16.2.14.3

Table 5 Statistical evaluation of the effect of food on the C_{max}, AUC_{0-t}, and AUC_{0-inf} of secnidazole

Parameter Treatment	Comparison	% Ratio: 100*Test/Reference Geometric LSMean % Ratio (SE)	 90% C.I.
C_{max} (µg/mL)			
SYM-1219-Cat Fasted (B) SYM-1219-Cat Fed (C)	Test (C) / Reference (B)	97.36 (1.905)	(94.21, 100.62)
AUC_{0-t} (h*µg/mL)			
SYM-1219-Cat Fasted (B) SYM-1219-Cat Fed (C)	Test (C) / Reference (B)	98.10 (2.617)	(93.79, 102.60)
$AUC_{0-\infty}$ (h*µg/mL)			
SYM-1219-Cat Fasted (B) SYM-1219-Cat Fed (C)	Test (C) / Reference (B)	97.77 (2.659)	(93.40, 102.35)

Source: CSR SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

Table 6 Statistical evaluation of secnidazole T_{max} following administration of SYM-1219 under fasted and fed conditions

SYM-1219-Cat Fed (C) - SYM-1219-Cat Fasted (B)	Overall (N=23)
Median	2.000
Min, Max	-2.00, 4.00
90% C.I.[a]	(2.000, 3.000)
P-value[b]	<0.001
a: Symmetric distribution free 90% confidence interval for the median. b: From the signed-rank test.	

Source: CSR SYM-1219-102, Listings 16.2.14.1 – 16.2.14.3

The SYM-1219-Cat Fasted and SYM-1219-Cat Fed comparisons for Cmax, AUC0-t, and AUC0-inf demonstrated that the administration of SYM-1219 with a high fat meal did not have an impact on the pharmacokinetics of secnidazole and indicated that SYM-1219 can be administered without regard to meals.

Bioanalytical Method Description:

The analytical method was developed at [redacted] (b) (4), and validated according to the standard operating procedures (SOPs) in effect during the conduct of the validation. The LC-MS/MS method for the determination of SYM-1219 in human plasma (EDTA) met the requirements as specified in the validation protocol (Appendix 4.1). The SOPs were written

based on the good laboratory practices (GLP) principles described in 21 CFR Part 58 and the Guidance for Industry – Bioanalytical Method Validation (CDER, May 2001).

A set of 9 non-zero calibration standards, ranging from 0.200 µg/mL to 50.0 µg/mL was prepared for SYM-1219. QC concentrations for SYM-1219 were: 0.200 µg/mL, 0.600 µg/mL, 3.00 µg/mL, 37.5 µg/mL. Linearity was indicated by a correlation coefficient of ≥0.990 from the standard curve. The LC-MS/MS method for the determination of SYM-1219 in human plasma (EDTA) met the requirements as specified in the validation protocol. Stability was demonstrated for SYM-1219 in human plasma (EDTA) samples and solutions under varying conditions of storage. The inter-batch and intra-batch precision and accuracy results for QC samples prepared at LLOQ, low, medium, and high concentrations met acceptance criteria.

SYM-1219 Plasma Bioanalytical Method	
Linearity	linear (correlation coefficient ≥ 0.990)
Accuracy	85-115%
Precision	≤ 15%
Selectivity	no interference
Stability	Freeze-thaw; Long term stability, stock stability was demonstrated for SYM-1219

Stability was demonstrated for SYM-1219 in human plasma samples and solutions under several different conditions of storage.

Reviewer Comment: In general, the bioanalytical assay validation and QC performance for secnidazole were acceptable.

ECG Results: Pre-dose the mean heart rates with all 3 treatments were comparable at Day 1. Pre-dose mean PR, PRS, QT, QT_CB intervals were comparable with all treatments at Day 1.The statistical analysis of the frequent ECG sampling and triplicate ECG recording did not suggest that secnidazole was associated with any worrisome effect on ECG intervals. Mean changes in

QTcF were negative (ΔQTcF<0) at most post-dose time points. The categorical analysis of QTcF similarly led to the conclusion that secnidazole likely had no pharmacologic effect on QTcF.

SPONSOR'S CONCLUSIONS:

- The 90% confidence interval statistical evaluation demonstrated bioequivalence between the SYM-1219-(b)(4) Fasted and SYM-1219-Cat Fasted treatment groups and indicated the comparability of SYM-1219 manufactured at (b)(4) and Catalent.
- The SYM-1219-Cat Fasted and SYM-1219-Cat Fed comparisons for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ demonstrated that the administration of SYM-1219 with a high fat meal did not have an impact on the pharmacokinetics of secnidazole and indicated that SYM-1219 can be administered without regard to meals.

Safety Conclusions:

- Overall, SYM-1219 was well tolerated. Most adverse events were considered mild and no subjects reported SAEs or discontinued prematurely due to adverse events. No clinically significant changes were observed in vital signs or laboratory parameters. A higher incidence of headaches was observed in the two fasted groups (10 of 24 subjects; 41.7%) compared to the fed group (0%).
- None of the mean changes in heart rate, PR interval, QRS interval, QT interval, QTc interval, QTcB interval, QTcF interval or RR interval were clinically important.

Reviewer's Comments:

The Reviewer agrees with the Applicant's conclusions that there is no effect of food (high-fat meal) on the pharmacokinetics of secnidazole when admixed with applesauce, and that SYM-1219 (secnidazole granules) can be given without regard to food intake.

In addition, the Reviewer also agrees that the PK of secnidazole from either manufacturer (i.e., Cat or (b)(4)) was similar when SYM-1219 granules were given under fasted conditions.

4.2.3 Study # SYM-1219-103

TITLE OF STUDY: A Phase 1, Open-Label, Single-Dose, Randomized, 3-Way Crossover Study to Assess the Pharmacokinetics and Safety of SYM-1219 Containing 2 Grams of Secnidazole Administered Orally as Granules in Pudding, Yogurt, or Applesauce under Fasting Conditions in Healthy Female Volunteers

STUDY OBJECTIVES:

- To compare the PK of secnidazole 2 g oral granules admixed with pudding (**Test: Treatment A**) versus applesauce (**Reference: Treatment C**) in healthy female volunteers under fasting conditions.
- To compare the PK of secnidazole 2 g oral granules admixed with yogurt (**Test: Treatment B**) versus applesauce (**Reference: Treatment C**) in healthy female volunteers under fasting conditions.
- To evaluate the safety of a single dose of secnidazole 2 g oral granules admixed with pudding, yogurt, or applesauce in healthy female volunteers under fasting conditions.

STUDY DESIGN:

This was an open-label, randomized, 3-way crossover study. A total of 24 female subjects were enrolled to ensure that 18 subjects completed the study. On Day 1 of each period, subjects received 1 of 3 treatments according to the randomization scheme. In each treatment period, SYM-1219 granules containing 2 g of secnidazole were administered in 1 of the 3 foods (pudding, yogurt, or applesauce). Pharmacokinetic (PK) blood samples were collected for the determination of secnidazole plasma concentrations at the following time points: predose (within 30 minutes prior to dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, and 96 hours postdose. There was a minimum of a 7-day washout period between each dose of SYM-1219 granules.

Test Product, Dose, Mode of Administration:

The test products were:

Secnidazole 2 g oral granules, added to a single serving of approximately 4 oz. of chocolate pudding, administered as a single oral dose.

Secnidazole 2 g oral granules, added to a single serving of approximately 6 oz. of low-fat, vanilla yogurt, administered as a single oral dose.

Reference Product, Dose, Mode of Administration:

The reference product was secnidazole 2 g oral granules added to a single serving of approximately 4 oz. of unsweetened applesauce, administered as a single oral dose. Subjects received the study drug following an overnight fast of a minimum 10 hours with 240 mL of water. SYM-1219 was consumed within 5 minutes of adding the SYM-1219 granules to the food and within 5 minutes of the scheduled dosing time.

Duration of Treatment:

There were 3 periods of approximately 5 days each; washout phase was a minimum of 7 days between each dose of SYM-1219 granules. Total study duration was 7 weeks.

Bioanalytical Method Description:

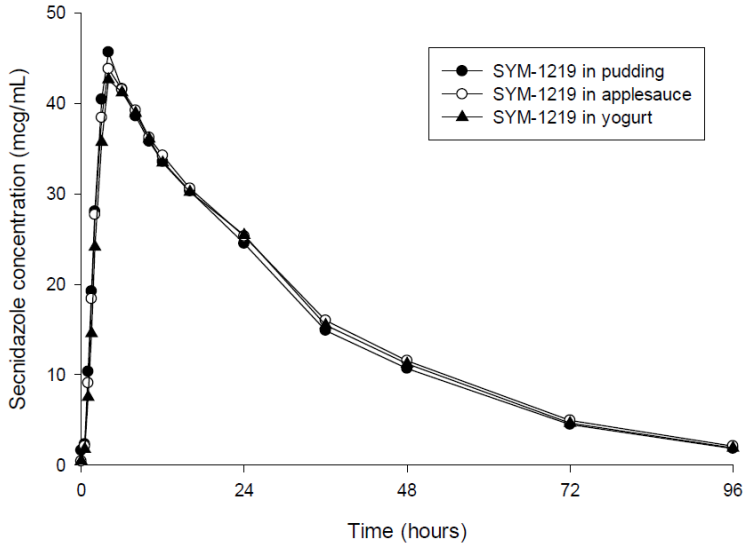
The LC-MS/MS method for the determination of SYM-1219 in human plasma (EDTA) met the requirements as specified in the validation protocol (Appendix 4.1).

The lower limit of quantitation for secnidazole was 0.200 µg/mL. The linear range of detection was from 0.200 µg/mL to 50.0 µg/mL. QC concentrations for SYM-1219 were: 0.200 µg/mL0.600 µg/mL, 3.00 µg/mL, 37.5 µg/mL. Linearity was indicated by a correlation coefficient of ≥0.990 from the standard curve. The reference standards materials for SYM-1219 and (b) (4) (internal standard) are light sensitive. All storage and handling of reference standard material for these analytes was conducted under UV-shielded light conditions (protected from white light). The validation method included testing for bench-top, stock, and processed stability as well as freeze-thaw and long-term storage stability. The inter-batch and intra-batch precision met the acceptance criteria.

SYM-1219 Plasma Bioanalytical Method	
Linearity	linear (correlation coefficient ≥ 0.990)
Accuracy	85-115%
Precision	≤ 15%
Selectivity	no interference
Stability	Freeze-thaw; Long term stability, stock stability was demonstrated for SYM-1219

SUMMARY OF PHARMACOKINETICS:
Mean plasma secnidazole concentration-time profiles (linear scale) following single-dose administration of SYM-1219 granules in a 4 oz. of chocolate pudding, 6 oz. of low-fat, vanilla yogurt, and 4 oz. of unsweetened applesauce are presented in the Figure 1.

Figure 1 Mean Plasma Secnidazole Concentration-Time Profiles Following Pudding, Yogurt, and Applesauce (Treatment A – Pudding (Test), Treatment B – Yogurt (Test) and Treatment C – Applesauce (Reference))



Source: Applicant's Summary of Biopharmaceutics Studies and Associated Analytical Methods, Page 22, Figure 3

The summary of the plasma secnidazole PK parameters following single administration of SYM-1219 granules in a single serving of pudding, yogurt, and applesauce is presented in Table 1.

Table 1 Summary of Mean Plasma Secnidazole Pharmacokinetic Parameters Following Treatment A - Pudding (Test), Treatment B – Yogurt (Test), and Treatment C - Applesauce (Reference) (PK Population)

Pharmacokinetic Parameters	Treatment A (Pudding) (N=23)	Treatment B (Yogurt) (N=24)	Treatment C (Applesauce) (N=24)
AUC0-t (µg*hr/mL)[a]	1370 (19.6)	1390 (19.9)	1430 (20.8)
AUCinf (µg*hr/mL)[a]	1410 (21.9)	1440 (22.2)	1480 (23.6)
AUC%extrap (%)[b]	3.09 ± 2.46	3.47 ± 2.69	3.76 ± 2.99
Cmax (µg/mL)[a]	45.4 (10.8)	43.1 (11.7)	43.9 (10.3)
Tmax (hr)[c]	4.00 (3.99, 6.01)	4.00 (4.00, 8.00)	4.00 (3.02, 6.14)
t1/2 (hr)[b]	17.6 ± 4.41	18.1 ± 4.73	18.5 ± 4.85
lambda z (1/hr)[b]	0.0418 ± 0.0103	0.0408 ± 0.0101	0.0400 ± 0.0102

Treatment A = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of pudding (test)
Treatment B = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of yogurt (test)
Treatment C = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of applesauce (reference)

[a] Presented as geom. mean (geom. CV%)
[b] Presented as mean ± SD
[c] Presented as median (minimum, maximum)

Note: Subject 23 predose concentration value was more than 5% of Cmax (7.9%), therefore the concentrations of Subject 23, Period 3 (Treatment A) were not included in the statistical analysis.

Source: CSR SYM-1219-103 Tables 14.2.1.4, 14.2.1.5, 14.2.1.6

Overall, the PK profiles of secnidazole were well characterized with mean $AUC_{\%extrap}$ being less than 4%. The mean $t_{½}$ was less than 20% of the sampling interval following all treatments and values ranged from 17.6 to 18.5 hours across treatments.

Secnidazole extent of exposure (as measured by geometric mean AUC_{0-t} and AUC_{inf}) was similar following a single-dose administration of SYM-1219 granules in a single serving of pudding, yogurt, and applesauce. Rate of exposure (as measured by geometric mean C_{max}) was also similar following all treatments. The median time to reach peak secnidazole concentrations (T_{max}) was 4.00 hours in all treatments with low variability exhibited among subjects as individual values ranged between 3 and 8 hours.

The statistical comparisons of plasma secnidazole PK parameters following single-dose administration of SYM-1219 granules in a single serving of pudding (Test), yogurt (Test), and applesauce (Reference) are summarized in the Table 2 and Table 3 below.

Table 2 Summary of Statistical Comparisons of Plasma Secnidazole Pharmacokinetic Parameters Following SYM-1219 Administration in Pudding Relative to Applesauce (Treatment A Versus Treatment C, PK Population)

	Geometric LS Means			
Parameters	Treatment A (Pudding) (Test) (N=23)	Treatment C (Applesauce) (Reference) (N=24)	GMR(%)	90% Confidence Interval
AUC0-t (µg*hr/mL)	1381	1427	96.80	93.51 - 100.22
AUCinf (µg*hr/mL)	1428	1483	96.31	92.82 - 99.92
Cmax (µg/mL)	45.29	43.86	103.25	100.33 - 106.24

Treatment A = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of pudding (test)
Treatment C = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of applesauce (reference)
Parameters were ln-transformed prior to analysis.
Geometric least-squares (LS) means were calculated by exponentiating the LS means from the mixed-effects model
% Geometric mean ratio (GMR) = 100 × (test/reference)

Note: Subject 23 predose concentration value was more than 5% of Cmax (7.9%), therefore the concentrations of Subject 23, Period 3 (Treatment A) were not included in the statistical analysis.

Source: CSR SYM-1219-103, Table 14.2.1.14

Table 3 Summary of Statistical Comparisons of Plasma Secnidazole Pharmacokinetic Parameters Following SYM-1219 Administration in Yogurt Relative to Applesauce (Treatment B Versus Treatment C, PK Population)

	Geometric LS Means			
Parameters	Treatment B (Yogurt) (Test) (N=24)	Treatment C (Applesauce) (Reference) (N=24)	GMR(%)	90% Confidence Interval
AUC0-t (µg*hr/mL)	1393	1427	97.66	94.39 - 101.05
AUCinf (µg*hr/mL)	1444	1483	97.37	93.90 - 100.96
Cmax (µg/mL)	43.09	43.86	98.22	95.50 - 101.03

Treatment B = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of yogurt (test)
Treatment C = One dose of SYM-1219 granules containing 2 g of secnidazole in a single serving of applesauce (reference)
Parameters were ln-transformed prior to analysis.
Geometric least-squares (LS) means were calculated by exponentiating the LS means from the mixed-effects model
% Geometric mean ratio (GMR) = 100 × (test/reference)

Source: CSR SYM-1219-103, Table 14.2.1.15

Secnidazole extent of exposure (as measured by geometric LS means AUC_{0-t} and AUC_{0-inf}) and rate of exposure (as measured by geometric LS means C_{max}) were similar when secnidazole 2g

oral granules were administered in a single serving of pudding, yogurt or applesauce, with a maximum of 4% difference in geometric LS means. The 90% CIs around the GMRs derived from the analyses of the ln-transformed PK parameters AUC_{0-t}, AUC_{0-inf}, and C_{max} were within the 80.00 to 125.00% confidence interval, demonstrating bioequivalence between treatments (pudding versus applesauce and yogurt versus applesauce).

SPONSOR'S CONCLUSIONS:

- The pharmacokinetics of secnidazole following oral administration of SYM-1219 granules in pudding or yogurt has same relative bioavailability to that of SYM-1219 granules administered in applesauce.
- Based on the results of this study, secnidazole granules containing 2 g of secnidazole can be administered in pudding, yogurt, or applesauce.
- A single oral dose of secnidazole2 g granules admixed with pudding, yogurt, or applesauce was safe and generally well tolerated in healthy female volunteers.

Reviewer's Comments:

Administration of SYM-1219 granules with 4 oz. of chocolate pudding, 6 oz. of low-fat, vanilla yogurt, and 4 oz. of unsweetened applesauce, did not have an impact on the rate and extent of secnidazole absorption. Therefore, SYM-1219 granules can be admixed with any of these soft foods.

4.3.4 In Vitro Study Reviews

In vitro metabolic induction

Study 440004832: Evaluation of Induction Potential of Cytochrome P450 Isoforms by SYM-1219 in Cultured Human Hepatocytes

Objective: The purpose of this study was to determine the induction potential of cytochrome P450 isoforms by SYM-1219 in cultured human hepatocytes. Induction was measured by in situ catalytic activity and mRNA expression assays selective for cytochrome P450 (CYP) isoforms CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19.

Method: The study was designed to assess the potential of test article SYM-1219 to induce CYP isoforms CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19 mRNA and activity using primary cultured human hepatocytes. Prior to the induction assay, an assessment of the cytotoxicity potential of SYM-1219 to human hepatocytes was conducted using the MTT assay following a 2-day exposure period in preparations of human hepatocytes on collagen I-coated 24-well plates. Three separate donors of cryopreserved hepatocytes (lots 228, 307, and 321) were used. For the induction assessment, hepatocytes (same lots used for MTT) on collagen I-coated 24-well plates were exposed to SYM-1219 for a total of 2 days. Induction of CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19 was measured by mRNA expression assays selective for each CYP isoform by real time (RT)-PCR analysis. Induction of in situ catalytic activity was determined for CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19 using specific probe substrates. Positive control inducers and solvent vehicle controls were included in the assays.

Results: Summary tables (Table 1 and 2) are shown below.

Table 1 Summary of the Effects of SYM-1219 and Positive Controls (Omeprazole, Phenobarbital, Rifampicin) on CYP1A2, CYP2B6, and CYP3A4 mRNA Expression In Human Hepatocytes

Dose of SYM-1219	Mean ± SD CYP1A2 mRNA fold induction			Mean ± SD CYP2B6 mRNA fold induction			Mean ± SD CYP3A4 mRNA fold induction		
	Lot 228	Lot 307	Lot 321	Lot 228	Lot 307	Lot 321	Lot 228	Lot 307	Lot 321
100 µM	0.79 ± 0.16	0.85 ± 0.21	0.92 ± 0.096	1.1 ± 0.28	1.1 ± 0.21	0.91 ± 0.044	0.65 ± 0.093	0.93 ± 0.035	1.3 ± 0.20
300 µM	0.86 ± 0.21	0.57 ± 0.059	0.81 ± 0.14	1.3 ± 0.21	1.1 ± 0.13	1.3 ± 0.21	0.96 ± 0.12	1.0 ± 0.041	1.6 ± 0.23
1000 µM	0.56 ± 0.070	0.85 ± 0.19	0.89 ± 0.14	1.2 ± 0.28	3.3 ± 0.59	2.0 ± 0.48	0.96 ± 0.15	1.7 ± 0.11	2.4 ± 0.55
3000 µM	0.99 ± 0.15	1.4 ± 0.041	1.2 ± 0.27	3.7 ± 0.76	5.2 ± 1.3	3.9 ± 1.0	5.8 ± 0.83	18 ± 0.98	9.6 ± 3.1
5000 µM	1.0 ± 0.22	2.0 ± 0.48	1.6 ± 0.35	7.9 ± 1.5	13 ± 2.0	7.6 ± 1.5	22 ± 1.9	82 ± 24	33 ± 5.5
10000 µM	1.5 ± 0.15	3.9 ± 0.29	2.1 ± 0.22	23 ± 2.8	23 ± 3.4	15 ± 2.6	82 ± 14	242 ± 18	92 ± 13
Control[a]	67 ± 8.5	198 ± 5.2	51 ± 7.0	9.5 ± 0.72	9.4 ± 2.1	6.9 ± 1.8	101 ± 12	581 ± 91	57 ± 20

a= CYP1A2: Omeprazole; CYP2B6: Phenobarbital; CYP3A4: Rifampicin

Fold Induction: The mean fold change of treated samples compared to vehicle control samples

Table 2 Summary of the Effects of SYM-1219 and Positive Control (Rifampicin) on CYP2C8, CYP2C9, and CYP2C19 mRNA Expression in Human Hepatocytes

Dose of SYM-1219	Mean ± SD CYP2C8 mRNA fold induction			Mean ± SD CYP2C9 mRNA fold induction			Mean ± SD CYP2C19 mRNA fold induction		
	Lot 228	Lot 307	Lot 321	Lot 228	Lot 307	Lot 321	Lot 228	Lot 307	Lot 321
100 µM	1.1 ± 0.25	0.87 ± 0.37	1.6 ± 0.042	0.90 ± 0.13	0.87 ± 0.16	1.3 ± 0.32	1.5 ± 0.24	1.2 ± 0.22	1.3 ± 0.28
300 µM	0.93 ± 0.13	1.4 ± 0.13	1.3 ± 0.23	0.83 ± 0.036	1.1 ± 0.032	1.1 ± 0.22	1.5 ± 0.19	1.5 ± 0.20	1.1 ± 0.086
1000 µM	0.80 ± 0.13	1.3 ± 0.31	1.1 ± 0.17	0.85 ± 0.052	1.2 ± 0.094	1.0 ± 0.31	1.5 ± 0.021	1.5 ± 0.25	0.89 ± 0.062
3000 µM	2.2 ± 0.22	4.1 ± 0.63	2.9 ± 0.65	1.7 ± 0.10	2.3 ± 0.52	2.1 ± 0.25	1.6 ± 0.23	1.4 ± 0.41	1.2 ± 0.27
5000 µM	2.8 ± 0.283	4.5 ± 1.3	3.1 ± 1.0	2.9 ± 0.27	3.0 ± 0.39	2.6 ± 0.40	2.3 ± 0.19	1.4 ± 0.25	1.3 ± 0.33
10000 µM	7.2 ± 0.44	5.6 ± 0.16	5.0 ± 0.33	5.2 ± 0.26	4.9 ± 0.93	4.5 ± 0.14	3.6 ± 0.19	1.7 ± 0.27	2.6 ± 0.28
Control[a]	3.0 ± 0.024	4.5 ± 0.20	3.5 ± 0.37	3.7 ± 0.30	4.2 ± 0.64	3.1 ± 0.28	1.7 ± 0.10	1.1 ± 0.039	1.4 ± 0.18

a= Rifampicin

Fold Induction: The mean fold change of treated samples compared to vehicle control samples

Applicant's Conclusion: SYM-1219 was an in vitro inducer of CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19 in human hepatocytes under the conditions tested in this study. Treatment with SYM-1219 resulted in an induction response for CYP1A2 and CYP2C19

mRNA in two of the three hepatocyte lots, and for CYP2B6, CYP3A4, CYP2C8, and CYP2C9 mRNA in all three hepatocyte lots when tested up to a maximum concentration of 10000 µM. The concentration of 10000 µM is approximately 40-fold greater than the mean clinical C_{max} of approximately 245 µM SYM-1219.

Reviewer's comment: The results from Study 440004832 show that secnidazole induces CYP2B6, CYP3A4, CYP2C8 at 3000 µM. Secnidazole showed greater induction of CYP1A2, CYP2B6, CYP3A4, CYP2C8, and CYP2C9 at 5000 µM and 10000 µM concentrations. Secnidazole is very low protein bound (<5%) and clinically relevant concentration is 245µM. Therefore, clinical DDI, in terms of induction potential, is not expected from the results of these in vitro studies.

Study 440006060: Determination of SYM-1219 CYP3A4 Relative Induction Score, R_3, EC_{50}, and E_{max} in Cultured Human Hepatocytes

Objective: The purpose of this study was to evaluate the CYP3A4 induction potential of SYM-1219 in cultured human hepatocytes using a Relative Induction Score (RIS) correlation method.

Method: Hepatocytes on collagen I-coated 96-well plates were incubated with the test article for a total of 2 days, with a medium change approximately every 24 hours. CYP3A4 induction was assessed by measuring mRNA expression by RT-PCR analysis. Hepatocytes from 1 pre-qualified donor lot were used. Positive controls, solvent vehicle controls, and triplicate samples/replicates were included in the assays. Induction parameters such as EC_{50}, E_{max}, and R_3 values as well as RIS were determined.

Results: The effects of prototypical CYP inducers rifampicin (RIF) at 10 µM and phenobarbital (PB) at 1000 µM, and test article SYM-1219 at 200, 500, 2000, 5000, 6500, 10000, 15000, and 20000 µM on CYP3A4 mRNA levels in human hepatocytes demonstrated that RIF and PB produced a mean of 11- and 11-fold increase, respectively, in CYP3A4 mRNA levels over the vehicle control for hepatocyte lot 312. Treatment of lot 312 hepatocyte culture with SYM-1219 (200 to 20000 µM) for two days resulted in a concentration-dependent induction in CYP3A4 mRNA. Maximal induction of 9.6-fold as compared to vehicle, occurred at 15000 µM SYM-1219, and represented 87% of the positive control inducer rifampicin response. The data were used to calculate induction parameters, which are presented in Table 3. The concentration of 20000 µM was excluded from curve fitting due to marked attenuation of the induction response. The estimated EC_{50} and E_{max} values were 7295 µM and 9.6-fold, respectively. The projected $C_{max,\ total}$ (R_3 equation, as described in the draft FDA guidance) that would be a likely inducer, was 85 µM. $C_{max,unbound}$ (RIS equation, as described in the EMA guideline) at which 20%

midazolam AUC change would be expected clinically was 14 µM (95% confidence interval 6.7-21).

A clinical total C_{max} of 245 µM was used to calculate an R_3 value for SYM-1219, as well as to estimate a RIS (the total C_{max} was used as a conservative estimate of unbound C_{max}). The obtained R_3 value of 0.76 (which is less than the R_3 cut-off value of 0.9) and RIS value of 0.31 (which is greater than the RIS calibration curve cut-off value of 0.018), respectively, each indicate a potential for drug-drug interaction. Based on the RIS value of 0.31, the predicted % decrease in midazolam AUC at a 245 µM plasma level of SYM-1219 is 77% (Table 3).

Table 3 Parameters for CYP3A4 mRNA induction by SYM-1219

Parameters	Values
RIS cut-off value[1]	0.018
R3 cut-off value[2]	0.9
Test article concentration range (µM)	200-20000
EC50 (µM)	7295
E_{max} (fold induction)	9.6
Clinical total C_{max} (µM)	245
Predicted unbound C_{max} (µM) that may cause 20% decrease in AUC[3]	14 (6.7—21)
Predicted total C_{max} (µM) that may cause R_3 <0.9[4]	85
Test article R_3[5]	0.76
Test article RIS[6]	0.31
RIS-based predicted % decrease in AUC[7]	77

[1]RIS cutoff value was derived from the pre-determined RIS calibration curve (unbound C_{max}-based) corresponding to a predicted 20% decrease in midazolam AUC in humans

[2]R_3cutoff value of 0.9 was obtained from FDA draft guidance (2012). A test article R_3 value of <0.9 indicates "likely inducer in vivo".

[3]Predicted unbound C_{max} is the predicted concentration that may cause 20% decrease in midazolam AUC in humans based on the RIS calibration curve (unbound C_{max}-based). Range was reported with 95% confidence interval (CI).

[4]Predicted total C_{max} is the predicted concentration that may cause $R_3 < 0.9$, indicating a "likely inducer in vivo".

[5]R_3 value (FDA Draft Guidance, 2012) based on a total C_{max} = 245 µM

[6]RIS (EMA Guideline, 2012). The clinical total C_{max} of 245 µM was used as a conservative estimate for clinical unbound C_{max}.

[7]Predicted % decrease in midazolam AUC based on test article RIS using the RIS calibration curve.

Applicant's Conclusions: The predictions suggest that SYM-1219 (tested up to 20000 µM) was an in vitro inducer of CYP3A4 mRNA expression and a likely clinical inducer. Although the in vitro data show some propensity towards induction with secnidazole, clinical induction studies are conducted with repeated administration of the inducing drug and this experimental design is not likely to be clinically relevant for SYM-1219 due the single dose regimen.

Reviewer's comment: Secnidazole induced CYP3A4 mRNA levels from 5000-15000 µM. The percentage of induction relative to positive control rifampicin was 15-87% for the 5000-15000 µM secnidazole concentration. Secnidazole's potential as an inducer for CYP3A4 at the concentration of 5000 µM is not expected to result in clinical DDI as CYP3A4 mRNA induction was demonstrated at a 20-fold higher concentration than clinically relevant concentration.

Inhibition of Drug-Metabolizing Enzymes

Study 440004831: Evaluation of Inhibition of Cytochromes P450 Catalytic Activities in Human Liver Microsomes by SYM 1219.

Objective: The purpose of this study was to determine whether selected test article(s) inhibit human cytochrome P450 (CYP) catalytic activity in vitro using model substrates and human liver microsomes.

Method: Reaction mixtures (400 µL) contained seven concentrations of test article (0, 10, 30, 100, 300, 1000, 3000 and 5000 µM, microsomal protein, an NADPH-regenerating system (1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride), and one concentration of probe substrate in 100 mM potassium phosphate buffer (pH 7.4). Reactions were stopped by addition of 100 µL stop solution (0.1% formic acid in acetonitrile containing a stable-isotope labeled internal standard) and placement on ice.

Pre-incubation reaction mixtures contained seven concentrations of test article (0, 10, 30, 100, 300, 1000, 3000 and 5000 µM, solubility permitting), microsomal protein, and an NADPH-regenerating system (1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium chloride) in 100 mM potassium phosphate buffer (pH 7.4).

After 30 min of preincubation time, a 40 µL (or 80 µL for CYP2C19 only) aliquot was transferred into a prewarmed secondary reaction mixture (400 µL final volume) containing the NADPH-regenerating system and one concentration of probe substrate in 100 mM potassium phosphate buffer (pH 7.4).

Results: Result summary is shown in Table 4 and 5.

Table 4 Summary of Results – SYM-1219 Direct Inhibition Evaluations

P450 Isoform	Test article - IC50 Value (µM)	Percent remaining at highest concentration	Positive Control	Acceptable Range - IC50 Value (µM)	Result - IC50 Value (µM)
CYP1A2	> 5000	80	7,8-Benzoflavone	0.0010-0.070	0.012
CYP2A6	> 5000	54	Tranylcypromine	0.040-0.60	0.21
CYP2B6	> 5000	68	Ketoconazole	0.45-15	3.0
CYP2C8	> 5000	84	Montelukast	0.0070-0.20	0.062
CYP2C9	> 5000	95	Sulfaphenazole	0.15-1.5	0.40
CYP2C19	3873	41	S-Benzylnirvanol	0.10-1.5	0.25
CYP2D6	> 5000	63	Quinidine	0.020-0.20	0.048
CYP2E1	> 5000	96	Chlormethiazole	13-275	56
CYP3A4/ Midazolam	3722	44	Ketoconazole	0.0030-0.15	0.019
CYP3A4/Testosterone	4306	46	Ketoconazole	0.0050-0.090	0.017

Table 5 Summary of Results – SYM 1219 Time-Dependent Inhibition (TDI) Evaluation

P450 Isoform	Plus NADPH		Minus NADPH		Positive Control	Acceptable range - IC50 value [a] (µM)	Result - IC50 value (µM) [a]
	Test article IC50 value [a]	Percent remaining at highest concentration	Test article IC50 value [a]	Percent remaining at highest concentration			
CYP1A2	> 500	92	> 500	89	Furafylline	0.0035-0.085	0.021
CYP2A6	> 500	90	> 500	85	8-Methoxypsoralen	0.0020-0.040	0.0059
CYP2B6	> 500	101	> 500	92	Ticlopidine	0.030-0.20	0.058
CYP2C8	> 500	95	> 500	86	Gemfibrozil glucuronide	0.080-9.5	0.48
CYP2C9	> 500	98	> 500	86	Tienilic acid	0.025-0.15	0.052
CYP2C19	> 1000	85	> 1000	67	S-Fluoxetine	0.60-15	4.7
CYP2D6	> 500	93	> 500	90	Paroxetine	0.015-0.20	0.038
CYP2E1	> 500	108	> 500	103	Diethyldithiocarbamate	0.10-0.85	0.30
CYP3A4/ Midazolam	> 500	88	>500	75	Azamulin	0.0010-0.015	0.0016
CYP3A4/ Testosterone	> 500	93	>500	84	Azamulin	0.0035-0.040	0.0046

[a] – IC50 value after a 30 min pre-incubation calculated based on inhibitor concentrations in the secondary incubation. The concentration of test article in the pre-incubation step was 5 to10-fold higher than the concentrations in the secondary incubation, which were used to calculate the IC50 value. Therefore, the test article was evaluated as a time-dependent inhibitor at concentrations up to 5000 µM.

Applicant's Conclusions: Secnidazole exhibited direct inhibition of CYP2C19 and CYP3A4, with IC_{50} values ranging from 3722 to 4306 µM. All other enzymes tested exhibited IC_{50} values > 5000 µM, although some clear and concentration dependent inhibition was observed at the higher concentration with some enzymes, notably CYP2A6, CYP2B6 and CYP2D6. No evidence of time-dependent inhibition was observed for any enzyme. In most cases, pre-incubation of SYM 1219 with NADPH and HLM causes a small reduction (rather than enhancement as would occur if TDI was present) in inhibition potential, suggesting evidence of inhibitor depletion.

Reviewer's comment: The Reviewer agrees with the Applicant's conclusions that inhibition potential of secnidazole towards CYP1A2, CYP1A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 is deemed to not be clinically relevant, based on the fact that IC_{50} values obtained in in vitro studies were much higher than the clinically relevant concentration of secnidazole.

Study 440004834: In vitro Reaction Phenotyping, Plasma Stability, and Human Liver Microsomal Stability Evaluation of SYM-1219

Objective: The purpose of this study was to evaluate metabolism of secnidazole by drug metabolizing enzymes.

Method: The test articles were assayed at a concentration of 1 µM. Incubations with cytochrome P450 were performed in 0.05 M potassium phosphate, pH 7.4 with an NADPH generating system (1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride) at 37ºC. At five incubation times (0, 5, 10, 20 and 30 min), aliquots were withdrawn from each incubation, combined with stop solution (acetonitrile containing an internal standard) and placed on ice.

Samples were analyzed on same day or stored at -20ºC for subsequent analysis. Prior to LC/MS/MS analysis, the samples were centrifuged to pellet the precipitated protein.

Incubations in plasma were initiated by the addition of the test compound to 1 mL of plasma (pre-warmed to 37°C) to yield a final concentration of 1 µM. At five incubation times (0, 15, 30, 60, 120 min) aliquots (50 µL) were taken and added to 200 µL of acetonitrile containing internal standard. The samples were subjected to vortex mixing for 1 min and then centrifugation at ~20°C for 10 min at 4,000 rpm. Aliquots of the clear supernatants were diluted with water (e.g. 7 parts water: 3 parts sample, v/v) and analyzed by LC/MS/MS.

Results: There was no conclusive evidence of metabolism by cDNA- expressed enzymes, however due to the nature of the assay, a role for P450 isoform involvement cannot be ruled out.

This is because the assay measures disappearance of parent compound, rather than formation of metabolite, and extent of loss was not substantial. Data suggest a possible role of CYP1A2, 2A6 and 3A4 in metabolism of SYM 1219. Supporting evidence of low or no role of P450 in the metabolism of SYM 1219 was provided by results with HLM (which contain all hepatic P450 isoforms) that showed no evidence of metabolic loss. Positive controls demonstrated a properly functioning model. Experiments that measure formation of metabolite would likely be needed to verify a role for these or other enzymes potentially involved in metabolic clearance.

Applicant's Conclusions: There was no conclusive evidence of metabolism of SYM 1219 by cDNA-expressed enzymes, human liver microsomes or human plasma. Based on extent and time-dependence of loss, there was suggestive evidence that CYP1A2, CYP2A6 and CYP3A4 can metabolize SYM-1219. It is likely that measuring formation of metabolite would be needed to verify a role for these or other enzymes potentially involved in metabolic clearance. There was no evidence of metabolism in human plasma. Positive controls demonstrated a properly functioning model.

Reviewer's comment: Reviewer notes that the Applicant has not provided the concentration of cDNA-expressed enzymes, human liver microsomes in the study. However, in the Study 1403044 (see below) the Applicant has conducted the detailed kinetic evaluation of cDNA-expressed human cytochrome P450 enzymes with secnidazole.

Study 1403044: Cytochrome P450 Reaction Phenotyping for Radiolabeled SYM 1219

Objective: The purpose of this study was to determine which human cytochrome P450 enzymes metabolize the test substance SYM 1219 at therapeutically relevant concentrations. Radiolabeled SYM-1219 was used.

Method: Two methods were used to determine which cytochrome P450 enzymes are principally responsible for the metabolism of SYM 1219.

These included:

- Analysis of enzyme kinetic parameters using individual, cDNA-expressed human cytochrome P450 enzymes and human liver microsomes.

- Inhibition of specific cytochrome P450 enzymes with selective chemical and immunochemical (antibody) inhibitors.

Incubations were performed in 0.1 M potassium phosphate buffer (pH 7.4) with and without an NADPH generating system (1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride). Two test substance concentrations, 100 µM and 5100 µM were incubated with HLM protein concentrations of 0.5, 1 and 1.5

mg/mL, in duplicate. An aliquot (0.2 mL) was removed at time points (0, 30, 60 and 90 minutes) and added to 0.1 mL acetonitrile. The samples were frozen at -20°C for subsequent analysis by HPLC with radiochemical detection.

The formation of metabolites by cDNA-expressed enzymes was determined. Two substrate concentrations were used, 200 μM and 6400 μM. Incubations were performed in 0.1 M potassium phosphate buffer (pH 7.4) with an NADPH generating system (1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride) and carried out for 0, 30, 60 and 90 min. Only the 90 min samples were ran initially. If significant metabolism was found, the 0, 30 and 60 min samples were analyzed. The samples were frozen at -20°C for subsequent analysis.

Results: Initial time dependence and protein dependence experiments were performed with 100 μM and 5100μM substrate with and without an NADPH regenerating system. A metabolite (M1) eluting at ~ 2.7 minutes was formed in a time and protein concentration dependent manner. A background peak was observed eluting at the same time as M1, suggesting that this was either a co-eluting contaminant or a contaminant with same structure as the metabolite. A second metabolite was also observed, M2, eluting at about 16.7 min, and this was also evaluated in the substrate concentration-dependence experiment. Results from the concentration-dependence experiment demonstrate that the Km of both M1and M2 formation is > 6400 μM. An Eadie-Hofstee plot of the data did not provide any particular insight into a potentially applicable enzyme kinetic model, possibly due to some variability in the data at very low substrate concentrations.

A panel of cDNA-expressed enzymes was examined for capacity to form M1 and M2 (Table 6). Results demonstrated that only CYP3A4 and CYP3A5 could generate a significant amount of M1 at 200μM substrate that was well above background peak area observed in buffer and insect control Supersomes. There was some evidence that CYP1A2, CYP2B6 and CYP2C8 could also catalyze M1 formation, but peak areas did not exceed 2.5-fold of background peak area, a level deemed to be the LLOQ. At 6400μM substrate concentration, results clearly demonstrated only CYP2B6, CYP3A4 and CYP3A5 could generate M1 formation. Only CYP3A4 and CYP3A5 were capable of generating M2 at both 200μM and 6400μM substrate. With cDNA-expressed enzymes, but not HLM, the metabolites M3, M4 and M5 were observed, and these were generated by CYP3A5 only and at 6400μM concentration only. In addition, some low level metabolism was apparently also generated by CYP3A5 only.

Applicant's Conclusions: SYM-1219 is slowly metabolized in human liver microsomes with generally ≤1% conversion to metabolites after 90 min incubations at 1.5mg/mL protein. Two metabolites were observed with HLM, M1 and M2. Substrate concentration dependence experiments demonstrate Km values for both metabolites were > 6400μM. Results from

independent experiments (cDNA-expressed enzymes and HLM ± selective inhibitors) confirmed a role for CYP3A4 and CYP3A5 in the metabolism of SYM-1219. A role for CYP2B6 was suggested from the cDNA expressed enzyme panel, however inhibitory monoclonal antibody to CYP2B6 failed to inhibit metabolite formation in HLM suggesting CYP2B6 has a minor or negligible role in SYM-1219 metabolism.

Table 6 M1-M5 Formation in 90 Min Incubations of SYM-1219 with cDNA-Expressed Enzymes and Estimated Rate of Metabolism in HLM

Incubation Condition	Replicate	Analyte	pmol/min/pmol (200 µM)	pmol/min/pmol (6400 µM)	Estimated rate of metabolism in HLM (200 µM substrate)	Estimated rate of metabolism in HLM (6400 µM substrate)
CYP2B6	A	M1	1	66	-	2756
CYP2B6	B	M1	-	59	-	2472
CYP3A4	A	M1	0.54	23	16	687
CYP3A4	B	M1	0.36	20	11	606
CYP3A5	A	M1	0.23	60	2	428
CYP3A5	B	M1	0.25	50	2	355
CYP3A4	A	M2	0.44	7	13	207
CYP3A4	B	M2	0.43	5	13	153
CYP3A5	A	M2	0.29	4	2	30
CYP3A5	B	M2	0.28	5	2	37
CYP3A5	A	M3	-	15	-	109
CYP3A5	B	M3	-	14	-	102
CYP3A5	A	M4	-	6	-	43
CYP3A5	B	M4	-	7	-	47
CYP3A5	A	M5	-	10	-	72
CYP3A5	B	M5	-	12	-	86

[1] - Dashes indicate BLOQ. For M1, which had a significant co-eluting background peak area, the quantitation limit was assigned as 2.5X background peak area. Of the enzymes evaluated, only those shown in the table exhibited significant peak areas.

Reviewer's comment: The reviewer agrees with the Applicant's conclusion regarding role of CYP3A4 and CYP3A5 in the metabolism of secnidazole.

Plasma Protein Binding

Study 14F128 SYMB: Binding to Human Plasma Proteins

Objectives: The objective of this study was to determine the percent bound of secnidazole in human plasma, using equilibrium dialysis.

Methods: The study was carried out in human plasma (Lot# [(b) (4)]H541560, [(b) (4)]H545409). All plasma was obtained from [(b) (4)] and collected on sodium heparin. A Pierce Rapid Equilibrium Dialysis Device (RED) was used for all experiments. Stock solutions of the test and control articles were first prepared in DMSO. An aliquot of the DMSO solution was dosed into 1.0 mL of plasma at a dosing concentration of 5µM for the test article and 10µM for the co-dosed control article warfarin. Plasma (300L), containing the test and control articles, was loaded into two wells of the 96-well dialysis plate. Blank PBS (500 µL) was added to each corresponding receiver chamber. The device was then placed into an enclosed heated rocker that was pre-warmed to 37°C, and allowed to incubate for four hours. After 4 hours of incubation, both sides were sampled. Aliquots (50µL for donor, 200µL for receiver) were removed from the chambers and placed into a 96-well plate. Plasma (50µL) was added to the wells containing the receiver samples, and 200L of PBS was added to the wells containing the donor samples. Two volumes of acetonitrile were added to each well, and the plate was mixed and then centrifuged at 3,000 rpm for 10 minutes. Aliquots of the supernatant were removed, diluted 1:1 into distilled water, and analyzed by LC-MS/MS.

Protein binding values were calculated as follows:
% Bound = [(PARR in Donor –PARR in Receiver) / (PARR in Donor)] 100%
PARR =peak area response ratio of test article to internal standard, including applicable dilution factors.

Results:

Test article	Species	% Bound Test article	% Bound Warfarin
SYM-1219 (secnidazole)	Human	< 5.0	99.3

Warfarin binding acceptance criteria: Human: ≥ 98.0 % bound

Applicant Conclusions: Secnidazole has low protein binding, <5.0% in human plasma.

Reviewer's comment: Although there were several typographical errors in the Applicant's method and results, i.e., 'L' was written instead of 'µL' at several places, the reviewer agrees with the Applicant's conclusions that secnidazole demonstrated low protein binding in human plasma.

Transporters

Study 13SYMBP1R1: Nonclinical Pharmacokinetic Drug Interactions in vitro: Evaluation of Secnidazole Substrate and Inhibition Potential for Efflux and Uptake Transporters

Objectives: The objective of the current study was to assess if secnidazole is a substrate and/or an inhibitor of the following transporters: P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1 and OATP1B3), organic anion transporters 1 and 3 (OAT1 and OAT3), and organic cation transporter 2 (OCT2).

Method:

P-gp and BCRP Substrate assessment:

Evaluation of secnidazole as a substrate of P-gp was carried out at concentrations of 2.3, 23, and 230, or 4300 µM in MDR1-MDCK and MDCK or Caco-2 cells, respectively. Permeability was evaluated in both the apical-to-basolateral (AP-to-BL) and basolateral-to-apical (BL-to-AP) directions by placing secnidazole (in HBSSg buffer, Hanks' Balanced Salt Solution containing 10 mM HEPES and 15 mM D-glucose) on the donor side (apical for AP-to-BL; basolateral for BL-to-AP) and HBSSg buffer on the receiver side.

Evaluation of secnidazole as a substrate of BCRP was carried out at concentrations of 2.3, 23, and 230, or 4300 µM in BCRP-MDCK and MDCK or Caco-2 cells, respectively.

P-gp and BCRP Inhibitor assessment:

Evaluation of secnidazole as an inhibitor of P-gp was carried out in Caco-2 and MDR1-MDCK cells with unidirectional approach (BL-to-AP). Inhibition of P-gp was assessed using 4300 µM secnidazole. Transport of the P-gp probe substrate digoxin (10 µM) across cell monolayers was used as an index of P-gp activity.

Evaluation of secnidazole as an inhibitor of BCRP was carried out in Caco-2 and BCRP-MDCK cells with unidirectional approach (BL-to-AP). Inhibition of BCRP was assessed at 4300 µM

secnidazole. Transport of the BCRP probe substrate cladribine (10 μM) across cell monolayers was used as an index of BCRP activity.

OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 Substrate assessment:

HEK cells transfected with human OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 were run in parallel with vector control cells, and these transfected HEK cells were used in the cellular uptake assay. For OATP1B1 and OATP1B3, the concentrations of secnidazole were 2.4, 24, and 240 μM; for OAT1, OAT3, and OCT2, the concentrations of secnidazole were 2.3, 23, and 230 μM. The incubation periods were 2, 5, and 10 minutes in transport buffer.

OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 inhibitor assessment:

HEK cell lines transfected with each uptake transporter of interest (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) were used to determine the inhibition potential of secnidazole. The uptake of a transporter-specific probe substrate was conducted with and without secnidazole in transfected and vector control cells.

Results:
P-gp and BCRP Substrate assessment:

The net flux ratio of secnidazole was 0.802, 0.908, and 1.36 in MDR1-MDCK over MDCK cell systems at concentrations of 2.3, 23, and 230 μM; in the presence of valspodar (an inhibitor of P-gp), the efflux ratio of secnidazole was 1.15. In Caco-2 cells, the efflux ratios of secnidazole were 1.06, 1.34, and 1.10 in Caco-2 cells at the concentrations of 2.3, 23, and 230 μM, and in the presence of valspodar decreased efflux ratio of secnidazole was 1.11(Table 7). These results indicate that secnidazole was not a substrate of P-gp.

Evaluation of secnidazole as a substrate of BCRP was carried out at concentrations of 2.3, 23, and 230, or 4300 μM in BCRP-MDCK and MDCK or Caco-2 cells, respectively.

Table 7: Evaluation of Secnidazole as P-gp substrate

P-gp substrate assessment

Treatment	Net Flux Ratio (MDR1-MDCK/MDCK)
2.3 µM secnidazole	0.802
23 µM secnidazole	0.908
230 µM secnidazole	1.36

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio
		P_{app} ($\times 10^{-6}$cm/s)	Recovery (%)	P_{app} ($\times 10^{-6}$cm/s)	Recovery (%)	
2.30 µM secnidazole	1	18.4	86.2	20.4	79.4	1.06
	2	16.9	84.7	18.1	92.8	
	3	21.3	91.2	21.7	88.3	
	Average	18.9	87.3	20.1	86.8	
	SD	2.3	3.4	1.8	6.8	
23.0 µM secnidazole	1	19.1	87.0	23.1	89.7	1.34
	2	17.1	94.2	24.4	85.6	
	3	16.2	93.6	22.8	92.9	
	Average	17.5	91.6	23.4	89.4	
	SD	1.5	4.0	0.87	3.7	
4300 µM secnidazole	1	11.7	88.1	23.7	95.7	1.10
	2	35.9[a]	86.0	21.5	101	
	3	14.0	98.4	22.2	98.7	
	Average	20.6	90.8	22.6	98.4	
	SD	13	6.6	N.D.	2.6	
2.30 µM secnidazole + 1 µM valspodar	1	22.9	93.1	25.7	83.8	1.11
	2	19.9	95.8	22.9	94.6	
	3	19.8	96.9	20.7	93.2	
	Average	20.8	95.3	23.1	90.5	

The net flux ratio of secnidazole was 0.742, 0.913, and 1.14 in BCRP-MDCK over MDCK cell systems at the corresponding concentrations (Table 8); in the presence of Ko143 (an inhibitor of BCRP) the efflux ratio of secnidazole was 1.39. In Caco-2 cells and in the presence of Ko143, the efflux ratio of secnidazole was 1.38. These results indicate that secnidazole was not a substrate of BCRP.

Table 8: Evaluation of Secnidazole as BCRP substrate

BCRP substrate assessment

Treatment	Net Flux Ratio (BCRP-MDCK/MDCK)
2.3 µM secnidazole	0.742
23 µM secnidazole	0.913
230 µM secnidazole	1.14

P-gp and BCRP Inhibitor assessment:

Evaluation of secnidazole as an inhibitor of P-gp was carried out in Caco-2 and MDR1-MDCK cells with unidirectional approach (BL-to-AP). Inhibition of P-gp was assessed using 4300 µM secnidazole. Transport of the P-gp probe substrate digoxin (10 µM) across cell monolayers was used as an index of P-gp activity. The presence of 4300 µM secnidazole caused 13.3% inhibition and no inhibition towards P-gp in MDR1-MDCK and Caco-2 cells, respectively (Table 9). Since the percentage inhibition was less than 50%, the results indicated that secnidazole was not a significant inhibitor of P-gp at the tested concentration.

Table 9: Evaluation of Secnidazole as P-gp inhibitor

P-gp inhibitor assessment

Treatments	Corrected P_{app} ($\times 10^{-6}$cm/s)	Percentage inhibition
10 µM digoxin	14.6	N.A.
10 µM digoxin + 4300 µM secnidazole	12.6	13.3
10 µM digoxin + 1 µM valspodar	0	100

Evaluation of secnidazole as an inhibitor of BCRP was carried out in Caco-2 and BCRP-MDCK cells with unidirectional approach (BL-to-AP). Inhibition of BCRP was assessed at 4300 µM secnidazole.

Transport of the BCRP probe substrate cladribine (10 µM) across cell monolayers was used as an index of BCRP activity. The presence of 4300 µM secnidazole (Table 10) caused -0.310% (no inhibition) and 22.4% inhibition towards BCRP in BCRP-MDCK and Caco-2 cells, respectively. Since the percentage inhibition was less than 50%, the results indicated that secnidazole was not a significant inhibitor of BCRP at the tested concentration.

Table 10: Evaluation of Secnidazole as BCRP inhibitor

BCRP inhibitor assessment

Treatments	Corrected P_{app}	Percentage inhibition (%)
10 µM cladribine	9.28	N.A.
10 µM cladribine + 4300 µM secnidazole	9.31	-0.310
10 µM cladribine + 2 µM Ko143	0	100

Reviewer's comment: Secnidazole showed less than 50% inhibition of P-gp and BCRP at a concentration of 4300 µM. The Reviewer agrees with the Applicant's conclusion that secnidazole did not show significant inhibition of P-gp and BCRP.

OATP1B1, OATP1B3, OAT1, OAT3 and OCT2 uptake transporter substrate assessment: HEK cells transfected with human OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 were run in parallel with vector control cells, and these transfected HEK cells were used in the cellular uptake assay. For OATP1B1 and OATP1B3, the concentrations of secnidazole were 2.4, 24, and 240 µM; for OAT1, OAT3, and OCT2, the concentrations of secnidazole were 2.3, 23, and 230 µM. The incubation periods were 2, 5, and 10 minutes in transport buffer. In these uptake transporter-transfected HEK cells, secnidazole uptake was nearly identical to that in vector control cells under the tested conditions. Therefore, secnidazole was not a substrate of any of these transporters.

OATP1B1, OATP1B3, OAT1, OAT3 and OCT2 uptake transporter inhibitor assessment: HEK cell lines transfected with each of the uptake transporter of interest (OATP1B1, OATP1B3, OAT1, OAT3, or OCT2) were used to determine the inhibition potential of secnidazole. The uptake of a transporter-specific probe substrate was conducted with and without secnidazole in transfected and vector control cells. For OATP1B1 and OATP1B3, the initial test concentration of secnidazole was 2400 µM; for OAT1, OAT3, and OCT2, the initial test concentration of secnidazole was 2300 µM. Secnidazole caused 2.27% and -14.8% (no) inhibition of atorvastatin

uptake in OATP1B1-HEK cells and OATP1B3-HEK cells, respectively. Secnidazole caused 17.2% inhibition of p-aminohippurate uptake in OAT1-HEK cells, 16.4% inhibition of furosemide uptake in OAT3-HEK cells, and -5.86% (no) inhibition of methylphenylpyridinium iodide uptake in OCT2-HEK cells.

Applicant's Conclusions: Secnidazole is not a substrate of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2. Secnidazole is not a significant inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, or OCT2 at the tested concentration.

Reviewer's comment: The Reviewer agrees with the Applicant's conclusion that secnidazole is not a substrate/inhibitor of P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1, and OATP1B3 at the concentrations tested. Therefore, no clinical studies related to transporter DDI would be needed for secnidazole.

Ethanol Interaction

Study 150126: An investigation of SYM-1219 reversible and time-dependent inhibition of human aldehyde dehydrogenase 2 (ALDH2)

Objective: Purpose of this study was to evaluate reversible and time-dependent inhibition of human recombinant aldehyde dehydrogenase 2 by secnidazole.

Method: For the reversible inhibition assay, two mixes were prepared. Mix 1 contained 2X NAD+ (100 µM) in 0.1 M BES buffer (pH 7.4) and mix 2 contained 2X enzyme/substrate in 0.1 M BES buffer. The final concentrations of enzyme and substrate were 1.25 µg/mL (estimated to be 23 nM) and 0.1 µM propionaldehyde, respectively. To prepare plate containing test compound, to the first column of the 96-well plate, 72 µL of mix 1 was added, followed by 50 µL of mix 1 supplemented with 4% DMSO to columns 2 through 10. Then 3 µL of 50X upper concentration of the test or control compound dissolved in DMSO, was added to column 1. The test compound was diluted serially 1:3 through column 8 with the final 25 µL discarded. Column 10 served as the solvent only control. The reaction was initiated by adding 50 µL of mix 2 to each well. The fluorescence was measured with excitation/emission wavelength settings of 350/465 nm, respectively. Readings were taken at 4 minute intervals out to 24 min.

For the time-dependent inhibition assays, two mixes were prepared. Mix 1 contained 2X NAD+ (3000 μM) in 0.1 M BES buffer (pH 7.4) and Mix 2 contained 2X enzyme in 0.1 M BES buffer. Incubations for the pre-incubation were prepared by first adding 50 μL Mix 1 to each well followed by 2 μL of solvent vehicle (DMSO), DEAB or SYM 1219. The pre-incubation reaction was initiated by the addition of 50 μL of Mix 2 (2.5 μg/mL, estimated to be 46 nM) and carried out for 0.5, 1, 2, 3, 4, 8, 16 and 24 min, timed so that propionaldehyde could be added rapidly to each well using a multi-channel pipette. Immediately after the pre-incubation period, the residual ALDH2 catalytic activity was measured by adding saturating concentrations of propionaldehyde in a volume of 10 μL. The fluorescence was measured with excitation/emission wavelength settings of 350/465 nm, respectively. Readings were taken at 1 minute intervals out to 10 min. Assays were conducted in duplicate.

Results: In the reversible inhibition assay, the IC_{50} value for SYM-1219 was determined to be 503 μM (Table 11). It is likely that the true IC_{50} value for SYM-1219 is higher than the value obtained here. This is because in earlier experiments, the Sponsor found signal quenching of NADH fluorescence which tracked apparent inhibition response found in the enzyme assay, indicating the enzyme inhibition response found with SYM-1219 could be an artifact of fluorescence signal quenching. The IC_{50} for the positive control, daidzin, was found to be 4.7 μM, which was comparable to a literature value of 8.7 μM. The response with daidzin demonstrated a properly functioning test system.

In the time-dependent inhibition assay, SYM-1219 was pre-incubated with ALDH2 enzyme and NAD^+ for various periods up to 24 min (Table 12). At the end of the various pre-incubation periods, saturating concentrations of propionaledyde were added and the catalytic activity of ALDH2 was assessed. Whereas pre-incubation exhibited little or no time-dependent inhibitory effect with SYM-1219 at the 10 or 100 μM concentration examined, the positive control DEAB gave substantial time and concentration-dependent inhibition. Concentrations above 100 μM were not evaluated as SYM-1219 begins to exhibit quenching of fluorescence signal above 100 μM.

Table 11 Reversible Inhibition of ALDH2 by SYM-1219

SYM 1219 (µM)	nmol NADH/ min/mg	% Remaining	Xlfit data
1000	39	32	IC50 = 503 µM
333.3	79	63	SEE: 103 µM
111.1	99	79	
37.0	120	96	
12.3	137	110	
4.1	125	100	
1.4	112	90	
0.5	112	90	
0.2	117	94	
0	125	100	

SEE: standard error of the estimate

Table 12 Time Dependent Inhibition of ALDH2

	Velocity (nmol/min/mg)				
Preincubation Time (min)	Solvent Vehicle	0.2 µM DEAB	2 µM DEAB	10 µM SYM-1219	100 µM SYM-1219
24	136	33	15	131	104
16	121	29	16	113	97
8	112	39	23	110	102
4	108	60	27	101	92
3	114	63	29	104	92
2	102	76	33	101	92
1	101	87	45	103	89
0.5	98	96	67	104	91

Applicant's Conclusion: SYM-1219 exhibited an apparent IC_{50} of 503 µM in a reversible inhibition assay; inhibition of fluorescence signal was a result of quenching, rather than direct inhibition by the test article. The positive controls, diadzin for reversible inhibition, and DEAB for time-dependent inhibition, demonstrated a properly functioning test system. SYM-1219 at concentrations up to 100 µM, the highest concentration that could be tested, exhibited no substantial evidence of time or concentration-dependent inhibition.

Reviewer's comment: Secnidazole at concentrations up to 100 µM was tested in a time dependent inhibition study of the ALDH2 enzyme. However, due to assay method limitations, concentrations above 100 µM could not be evaluated. The IC_{50} of secnidazole for ALDH2 (503

μM) was higher than the clinically relevant concentration of 245 μM, which indicates that the likelihood of an interaction with concomitant ingestion of ethanol is low.

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/s/

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