CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211843Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA Number	211843
Submission Date	08/31/2018
Submission Type	505(b)(2)
Generic name	Tiopronin
Brand name	THIOLA EC
Applicant	Mission Pharmacal Company
Dosage form	Enteric coated tablets
Strength	100 mg and 300 mg
Mechanism of action	An active reducing agent which undergoes thiol-disulfide exchange with cystine to form water soluble mixed disulfide of tiopronin-cystine
Indications	Prevention of cystine (kidney) stone formation in patients with severe homozygous cystinuria (b) (4) high fluid intake, alkali and diet modification, (b) (4)
Associated IND	135653
OCP Division	Office of Clinical Pharmacology DCP-1
OND Division	Division of Cardiovascular and Renal Products (DCRP)
Primary Reviewer	Anusha Ande, PhD
Secondary Reviewer	Sudharshan Hariharan, PhD

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1. EXECUTIVE SUMMARY

Mission Pharmacal Company is seeking approval for 100 and 300 mg strengths of THIOLA enteric coated (EC) tablets via a 505(b)(2) New Drug Application (NDA). Tiopronin is currently approved as an immediate release (IR) tablet at a single strength of 100 mg under the brand name THIOLA ®, also marketed by Mission. THIOLA ® is indicated for the prevention of cystine (kidney) stone formation in patients with severe homozygous cystinuria with urinary cystine greater than 500 mg/day, who are resistant to treatment with conservative measures of high fluid intake, alkali and diet modification, or who have adverse reactions to d-penicillamine in adults and children above 9 years of age. (b) (4) No additional clinical efficacy data in adults is presented in this application and no new claims are being sought with this application.

The applicant has conducted two clinical pharmacology studies: 1) A relative bioavailability (BA) study to bridge THIOLA EC to THIOLA IR tablets: open label, randomized, 4-way crossover study to evaluate the pharmacokinetic (PK) profile of a single oral dose of THIOLA 300 mg EC (fasted) compared to a single oral dose of three tablets of THIOLA ® 100 mg IR in fasted healthy subjects (replicate design); 2) A food effect study for THIOLA EC tablets: open label randomized, 2-way crossover PK study to evaluate the bioavailability of a single oral dose of THIOLA 300 mg EC (fasted) compared to a single oral dose THIOLA 300 mg EC (fed) in healthy subjects. The applicant is relying on the relative BA study for bridging to the efficacy and safety of the listed drug (NDA 019569). In addition to the two PK studies, the applicant has submitted dissolution data and dose linearity information in support of a biowaiver request for the 100 mg strength of THIOLA EC tablets.

1.1 Recommendations

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 1(OCP/DCP1) has reviewed the NDA submission. The results of the relative BA study and food effect study support approval of THIOLA EC tablets for the proposed indication at the doses (b) (4). The food effect study results support administration of THIOLA EC tablets with or without food provided the drug is administered the same way every time. The clinical pharmacology section of the proposed label was updated to reflect the current Guidance on Clinical Pharmacology Section of Labeling for Human Prescription Drug and Biological Products.

1.2 Post-Marketing Requirements and Commitments

(b) (4) A pediatric age appropriate formulation is not currently available. The adult dosage form can be used in pediatric patients weighing greater than 20 kg who can swallow the tablet whol (b) (4) Therefore, OCP/DCP1 recommends conducting a randomized, 2-way crossover single oral dose study to evaluate the relative bioavailability of 300 mg THIOLA EC (crushed) mixed in apple sauce compared to 300 mg THIOLA EC intact tablet in healthy subjects as a post-marketing commitment (PMC) to support providing dosing instructions to pediatric patients weighing less than 20 kg or who cannot swallow the tablet whole.

1.3 Summary of important clinical pharmacology and biopharmaceutics findings

- The results of the relative BA study show that peak exposure (C_{max}) of THIOLA EC tablet decreased by 22% compared to THIOLA IR tablet, but the total exposure, AUC_{0-t} and AUC_{0-inf} remained similar between the two formulations. The results of the relative BA study are summarized in **Table 3**.

- The results from food effect study demonstrate that fed state administration of the THIOLA EC tablet decreased the plasma exposure of tiopronin compared to the fasted state as measured by C_{max} by 13%, while AUC_{0-t} and $AUC_{0-\infty}$ were lowered by 25% and 22%, respectively. The results of the food effect study are summarized in **Table 4**.

- The bioanalytical method used to measure tiopronin is validated and the performance of the method in the clinical studies is acceptable as per the specifications outlined in the *Bioanalytical Method Validation Guidance* (See **Table 2**).

2. QUESTION BASED REVIEW

This is an abridged version of the question-based review. For a detailed review of the clinical pharmacology of THIOLA®, refer to the original NDA 19,569.

2.1 General Attributes of the Drug Product

THIOLA EC tablets are formulated as enteric-coated, delayed release, white, round tablets in two dose strengths of 100 mg and 300 mg. 100 mg tablets are approximately 8.0 mm in diameter, 3.5 mm thick, weighing approximately 185.92 mg with "T1" imprinted on one side with red ink. 300 mg tablets are approximately 11.1 mm in diameter, 5.0 mm thick, weighing approximately 536.83 mg with "T3" imprinted on one side with red ink.

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

THIOLA EC tablets contain the following inactive ingredients: lactose (monohydrate), hydroxypropyl cellulose, hydroxypropyl cellulose (low substitute), magnesium stearate, hydroxypropyl methylcellulose E5, Eudragit® L 100-55, talc, and triethyl citrate. (b) (4)

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

Tiopronin is an active reducing agent which undergoes thiol-disulfide exchange with cystine to form a mixed disulfide of Tiopronin-cysteine:

$2R\text{-}SH + R'\text{-}S\text{-}S\text{-}R' \quad 2\ R\text{-}S\text{-}S\text{-}R' + 2H^+$

Tiopronin Cystine $\rightleftharpoons$ Tiopronin-cysteine

From this reaction, a water-soluble mixed disulfide is formed and the amount of sparingly soluble cystine is reduced.

THIOLA EC is indicated for the prevention of cystine (kidney) stone formation in patients with severe homozygous cystinuria (b) (4) high fluid intake, alkali and diet modification, who are not responsive to these measures alone, (b) (4).

2.1.4 What are the proposed dose(s)?

The proposed doses are the same as those for the listed drug THIOLA IR.

The recommended initial dosage in adult patients is 800 mg/day. In clinical studies, the average dose of THIOLA IR was about 1000 mg/day. The recommended initial dosage in pediatric patients is 15 mg/kg/day.

Administer THIOLA EC in 3 divided doses with or without food at the same times each day.

In patients who had shown severe toxicity to d-penicillamine, THIOLA EC might be begun at a lower dosage.

Measure urinary cystine 1 month after starting THIOLA EC, and every 3 months thereafter. Adjust THIOLA EC dosage to maintain urinary cystine concentration less than 250 mg/liter.

2.2 Specific Review Questions

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

The applicant submitted two clinical pharmacology studies (**Table 1**): 1) The pivotal relative bioavailability study (MPC-Tiopronin-002) compared the bioavailability of tiopronin from 1 tablet of 300 mg THIOLA EC and 3 tablets of 100 mg THIOLA IR following single oral dose administration; 2)The food effect study (MPC-Tiopronin-001) evaluated the effect of high-fat meal on the bioavailability of single dose of 300 mg THIOLA EC tablets.

Table 1. Summary of clinical pharmacology studies

Study	Type	Design	Study participants
MPC-Tiopronin-002	Relative Bioavailability study	Open label, randomized, two-treatment, four-period, two-sequence, full replicate crossover, single oral dose comparative bioavailability study in healthy adults under fasted state administration	Enrolled: 40 (25 men and 15 women) Completed: 33 (22 men and 11 women) Age Range: 21-53 years

MPC-Tiopronin-001	Food effect study	Open-label, randomized, two-way crossover study to evaluate the bioavailability of a single oral dose of THIOLA 300 mg EC in fed compared to fasted state in healthy subjects	Enrolled: 12 Completed: 12 (8 men and 4 women) Age Range: 24-43 years

Source: Clinical Study Reports of Study No. MPC-Tiopronin-002 and MPC-Tiopronin-001

2.2.2 Are the active moieties in the plasma appropriately identified and measured to assess pharmacokinetic parameters?

A validated LC-MS/MS analytical method for determining total tiopronin in human plasma treated with K_2EDTA was used for the bioanalysis of this study. Tiopronin-d3 was used as the internal standard (IS) for tiopronin. Sample preparation was done by protein precipitation technique. The chromatography was performed using a Gemini C6- Phenyl C18, 3 µm, 4.6×50 mm column and analyzed by electrospray mass spectrometry in the negative ion mode. LCMS/ MS system consists of an Agilent UPLC 1260 Infinity and a SCIEX API4000 MS. A gradient program was used to elute the analytes using 0.1% formic acid in water as mobile phase A (MPA) and 0.1% formic acid in MeCN:MeOH (25:75 v/v) as mobile phase B (MPB). With a flow rate of 1.0 mL/min, tiopronin eluted at approximately 1.08 minutes and its disulfide eluted at 1.39 minutes respectively. The internal standard d3-tiopronin co-eluted with tiopronin. Calibration curve was found to be linear from 50 ng/mL to 5000 ng/mL. Accuracy and precision of QC samples were ≤15% (and ≤20% at LLOQ), and calibration curves for the LC-MS/MS bioanalytical assay were within acceptable limits. Greater than two-thirds (75.2%) of the incurred samples concentration results were within 20% of the original concentration of the respective samples, thus meeting the acceptance criteria for incurred samples reanalysis.

Analytical methods were validated and performed within acceptable limits as shown in **Table 2**.

Table 2. Summary of bioanalytical sample analysis and method validation

Analyte	Tiopronin
Method	LC-MS/MS
Internal Standard	Tiopronin-d3
Matrix	Human K_2EDTA Plasma
Extraction	Protein Precipitation
LLOQ (ng/mL)	50
CCs (ng/mL)	50, 150, 350, 500, 1500, 2500, 3500, 5000
QCs (ng/mL)	LQC – 150 MQC – 500 HQC – 3500

Accuracy Intra-assay Accuracy (% Nominal) Inter- assay Accuracy (% Nominal)	 87.8 to 117 91 to 105
Precision Inter- assay Precision (%CV) Intra- assay precision (%CV)	 2.11 to 10.5 5.83 to 11.3
Recovery%	LQC- 104 MQC-93.2 HQC-98.9

CCs: Calibration Curve standards, QCs: Quality Control Samples, LLOQ: Lower Limit of Quantification

Source: Method validation and analytical reports (bioanalytical-report-17missp2glps462.pdf, method-validation-report-17missp2glps424.pdf)

2.2.3 What is the relative bioavailability of THIOLA EC tablets compared to THIOLA IR tablets? Does it support the proposed dosing?

The geometric mean ratios along with its 90% confidence interval (CI) of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for 300 mg THIOLA EC compared to THIOLA IR are summarized in **Table 3**.

Table 3. Results from relative bioavailability study

Parameter	**Geometric Least Squares Mean**		**T/R Ratio (%)**	**T/R 90% CI**	**Intrasubject variability %CV**	
	T (N=39)	**R (N=39)**			**T (N=39)**	**R (N=39)**
C_{max} (ng/mL)	6,216.2	7,958.1	78.1	73.2 - 83.3	29.5	11.4
AUC_{0-t} (ng.hr/mL)	31,662	33,935.2	93.3	89.2 - 97.6	20.9	7
$AUC_{0-\infty}$ (ng.hr/mL)	39,357.9	40,568.8	97	93.8 - 100.3	14.3	7.5

T: THIOLA EC tablet, R: THIOLA IR tablet; Geometric least squares mean of T and R represent the mean from four periods.

Source: Clinical Study Report of Study MPC-Tiopronin-002

- The time to reach peak tiopronin plasma concentration for THIOLA EC was prolonged compared to THIOLA IR. The mean T_{max} for tiopronin was 3 hours (range: 1 to 6 hours) and 1 hour (range: 0.5 to 2.1 hours) for THIOLA EC and THIOLA IR respectively. As designed, the EC dosage form showed a delayed absorption profile, which indicates transit out of the stomach into the intestine before dissolution and absorption of the dose.
- The plasma exposure for THIOLA EC 300 mg was decreased compared to 3 THIOLA IR 100 mg. Cmax was decreased by 22%, while AUC_{0-t} and $AUC_{0-\infty}$ decreased by only 7% and 3%, respectively.

- While the products are not equivalent based on C_{max}, the AUC of THIOLA EC tablet is equivalent to the IR tablets. The total systemic exposure (AUC) is more clinically relevant to efficacy i.e., reduction of cystinuria, because total systemic exposure over the interdosing interval is a close reflection of the amount of tiopronin excreted in urine where it reacts with cystine to form water-soluble mixed disulfide tiopronin-cystine. Moreover, tiopronin is dosed to effect; therefore, small changes in peak concentration or total systemic exposure do not warrant adjusting the dose of THIOLA EC tablets. Overall, the results of the relative BA study establish an adequate PK bridge between THIOLA EC and IR tablets.
- Intra-subject variability of tiopronin pharmacokinetics (PK) following administration of THIOLA EC tablets is slightly higher than that following THIOLA IR (C_{max}: 29.5% for EC tablets and 11.4% for IR tablets, AUC_{0-t}: 20.9% for EC tablets and 7% for IR tablets). However, the intra-subject variability is not greater than 30%, a threshold used for defining highly variable drugs. In addition, the time course of tiopronin plasma concentration following administration of THIOLA EC tablets are reasonably similar within the same subject between two different periods. Hence, the pharmacokinetic performance of THIOLA EC tablets is acceptable.

2.2.4 What is the effect of food on the bioavailability of the drug from the drug product?
There is a food effect when THIOLA EC is administered with a standard meal compared to the fasted state. The geometric mean ratios along with its 90% CI of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for administration of THIOLA EC with high-fat meal compared to fasted state administration is summarized in the **Table 4**.

Table 4. Geometric mean ratio (GMR) and 90% confidence interval for Fed/Fasted state administration of THIOLA EC tablets

Parameter	Geometric Least Squares Mean		Fed/Fasted Ratio (%)	Fed/Fasted 90% CI
	Fed	Fasted		
C_{max} (ng/mL)	5396.80	6200.5	87	77.5 - 97.7
AUC_{0-t} (ng.hr/mL)	24051.4	31939.3	75.3	68.3 - 83
$AUC_{0-\infty}$ (ng.hr/mL)	29504.1	37989.6	77.7	69 - 87.4

Source: Clinical Study Report of Study MPC-Tiopronin-001

The standardized breakfast (high fat diet) decreased the plasma exposure of the drug compared to the fasted state as measured by C_{max} by approximately 13%, while AUC_{0-t} and $AUC_{0-\infty}$ were lowered by 25% and 22%, respectively.

Since the drug is dosed to effect, the study results support administration of THIOLA EC tablet with or without food; however, we recommend that Thiola EC should be taken at the same time each day with a routine pattern with regard to meals.

2.2.5 Is the PK linear across the two dose strengths of 100 mg and 300 mg, which is required to support the biowaiver request for the lower dose strength of 100 mg?

The applicant submitted the PK data for tiopronin from 8 clinical and 1 non-clinical studies obtained from published literature. The clinical studies included the data for 3 tiopronin doses of 200, 300, and 500 mg. The data from MPC-Tiopronin-002 was also used for this assessment. These doses are in the proposed dose range of interest except for the 100 mg dose. To support linearity down to 100 mg dose, data from a dog study provided an overlapping dose range from 100 to 375 mg.

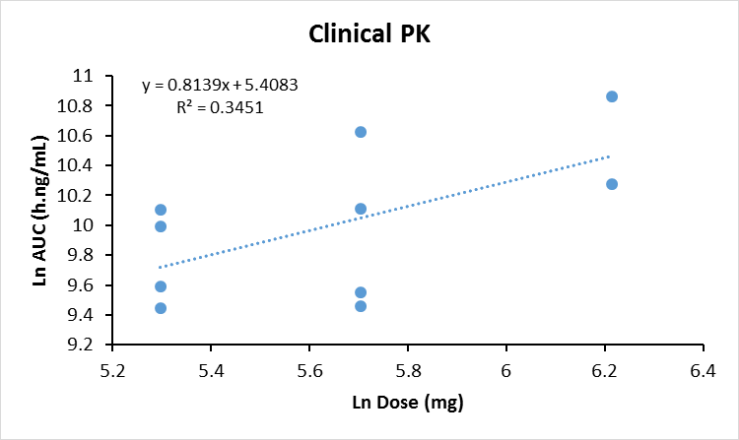


Figure 1: Plot of Ln $AUC_{0-\infty}$ versus Ln dose (Each *datapoint refers to mean AUC of all subjects from the individual study)*

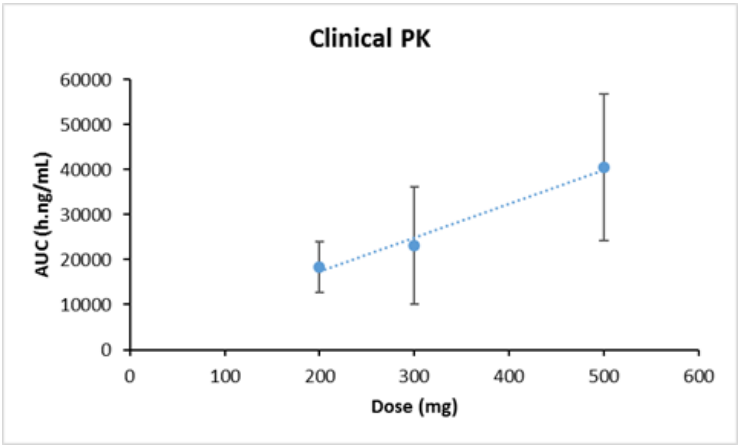


Figure 2: Plot of mean $AUC_{0-\infty}$ versus dose *[Datapoint at 200 mg refers to mean of AUC from four studies, datapoint at 300 mg refers to mean of AUC from three studies (one study contains two AUCs from two different formulations), datapoint at 500 mg refers to mean of AUC from two studies]*

Data from clinical studies exhibit close to dose proportional PK across the dose range of 200 to 500 mg (**Figure 1**). Since non-linearity typically tends to exhibit with increasing doses, it can be reasonably assumed that the 100 mg dose will fall mostly within the linear range in humans. Therefore, based on the available information, the reviewer agrees a linear PK for tiopronin is demonstrated between the two dose strengths of 100 and 300 mg.

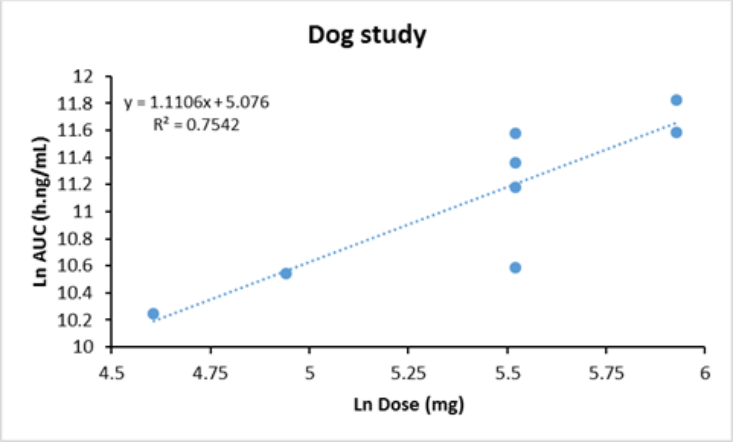


Figure 3: Plot of Ln $AUC_{0-\infty}$ versus Ln dose (Each *datapoint refers to individual dog in the study)*

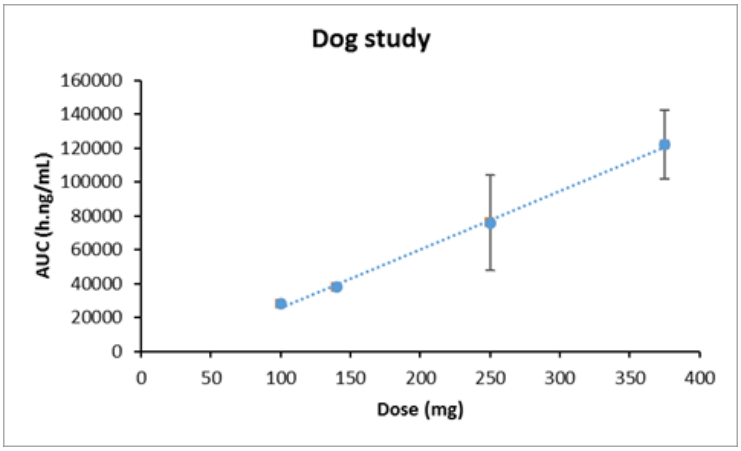


Figure 4: Plot of mean $AUC_{0-\infty}$ versus dose *(Datapoint at 250 mg refers to mean of AUC from 3 dogs, and datapoint at 375 mg refers to mean of AUC from 2 dogs)*

Based on the slope of the regression line on the natural log plot of dose versus $AUC_{0-\infty}$, linear PK is evident over a dose range of 100 to 375 mg in dogs (**Figure 3**), which is supportive of PK linearity down to 100 mg dose.

3. APPENDICES

3.1 Relative bioavailability study review

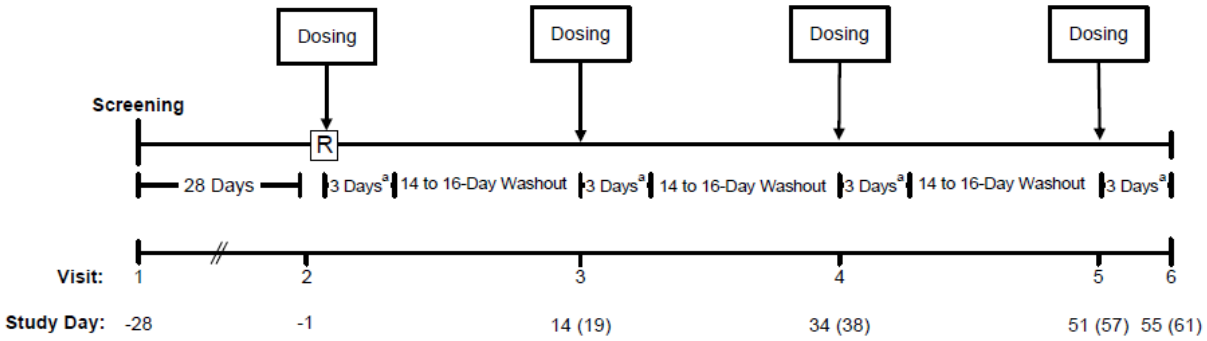
Study No: MPC-Tiopronin-002	**EDR:** \\cdsesub1\evsprod\nda211843\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5312-compar-ba-be-stud-rep\mpc-tiopronin-002\mpc-tiopronin-002-study-report-body.pdf
Study Date: September 18, 2017 to January 9, 2018	
Title of Study: Open Label, Randomized, 4-Way Crossover Study to Evaluate the Pharmacokinetic Profile of a Single Oral Dose of Tiopronin 300 mg EC (Fasted) Compared to a Single Oral Dose of Three Tablets of THIOLA ® 100 mg IR in Fasted Healthy Subjects (Replicate Design)	

Investigational Products
Test (T): THIOLA 300-mg EC Tablet (1 tablet)
Reference (R): THIOLA ® IR 100-mg Tablet (3 tablets)

Study:

- **Design:** Open label, randomized, two-treatment, two-sequence, four-period, single dose fully replicate crossover relative bioavailability study in fasted healthy adults. The study consisted of 6 visits: Visit 1, screening; Visit 2, randomization and first study treatment administration; Visits 3 to 5, study treatment administration; and Visit 6, study termination.

Figure 1: Study Schematic



All subjects were to be admitted to the study clinic 1 day prior to dose administration for each dosing period. Each dosing period consisted of admission, drug administration and pharmacokinetic sampling.
R = Randomization.
[a] Pharmacokinetic blood samples were to be drawn.

- **Washout:** There was a 2-week washout period (or 5½ half-lives) between each of the dosing periods. The washout period began 72 hours after study administration.
- **Study participants:** 33 healthy adults, 21-53 years of age.
- **Sampling times (h):** pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8,10,12, 24, 36, 48, 60, and 72 h post-dose.

Pharmacokinetic (PK) parameters calculated: AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{1/2}$, and K_{el}
Analytical Method:

- A validated LC-MS method, as described in section 2.2.2, was used for the estimation of total tiopronin in human K_2EDTA plasma using tiopronin d3 as Internal Standard.
- The analytical range for tiopronin in plasma was 50 ng/mL - 5000 ng/mL

Reviewer comment: The performance of the analytical method is acceptable per the specifications in Bioanalytical Method Validation Guidance.

Statistical Methods:
Each pharmacokinetic parameter was natural log transformed and analyzed using a linear mixed-effects model with fixed effects for treatment, period, and sequence and a random effect for subject nested in sequence.
For each pharmacokinetic parameter, the least squares mean was computed for each treatment

and the difference between treatments, along with its 90% confidence interval, was exponentiated. The geometric mean ratio (EC to IR) and its confidence interval were used to summarize the relative bioavailability of tiopronin formulations.

Results:
33 subjects completed the study. A total of 2,227 blood samples were collected for analysis of plasma tiopronin concentrations.

Table 1. Demographics

Age range	21-53 years
Males Females	22 (66.7%) 11 (33.3%)
Weight	52.2-98.9 kg

Table 2. Subject Disposition

	Treatment Sequence 1 N (%)	Treatment Sequence 2 N (%)	All subjects N (%)
Screened			175 (100)
Randomized	21 (52.5)	19 (47.5)	40 (22.5)
Completed	18 (85.7)	15 (78.9)	33 (82.5)
Not Completed	3 (14.3)	4 (21.1)	7 (17.5)
Protocol deviation	0	2 (50)	2 (28.6)
Withdrawal by subject	3 (100)	1 (25)	4 (57.1)
Other	0	1 (25)	1 (14.3)

- Seven subjects did not complete the study because of protocol deviations (2 subjects), withdrawal of consent (4 subjects), or other reasons (1 subject).
- Subject (b) (6) was excluded from the per-protocol population due to a protocol violation, the use of a concomitant medication. Thus, there is a total of 39 subjects included in the PK analyses dataset.

For statistical summary of relative BA data, refer to **Table 3** in section 2.2.3

Table 3. Summary of PK Parameters for tiopronin by treatment and period

Parameter	T1 Arithmetic Mean (SD) (N=37)	T2 Arithmetic Mean (SD) (N=33)	R1 Arithmetic Mean (SD) (N=36)	R2 Arithmetic Mean (SD) (N=33)
C_{max} (ng/mL)	6423.8 (2117.9)	6563.6 (1771.1)	8125.8 (1708.1)	8098.5 (1772.5)
AUC_{0-t} (ng.hr/mL)	32591.3 (8379.8)	32790.2 (4945.7)	34886.9 (5746.6)	33942.6 (5412.9)
$AUC_{0-\infty}$ (ng.hr/mL)	40221.9 (9433.7)	40058.5 (6904.1)	41328 (6734.9)	40904.7 (6792.6)
T_{max} (hr)*	3 (1 - 6)	3 (1.5 - 4.1)	1.1 (0.5 - 2.1)	1 (1 - 2)
$t_{1/2}$ (hr)	42.5 (11.3)	46.1 (15.4)	41.7 (9.3)	43.8 (6.9)

* Median (Range)

Source: Clinical Study Report Study MPC-Tiopronin-002

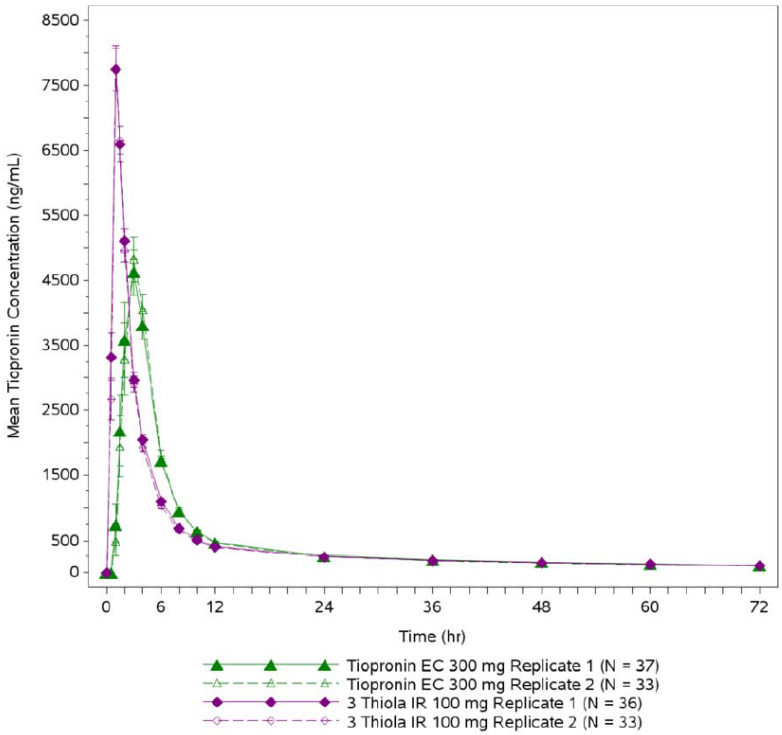


Figure 2: Plot of mean (SE) plasma tiopronin concentration vs time profiles by replicate periods and treatment

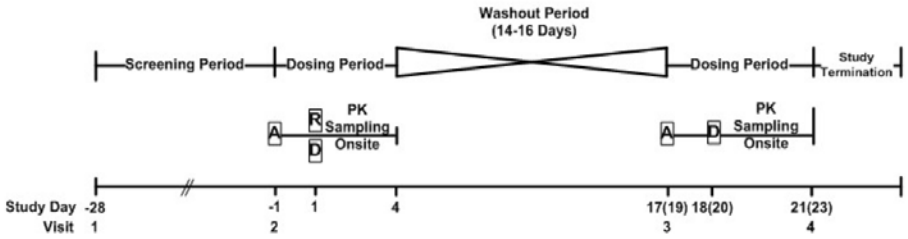
Source: Clinical Study Report Study MPC-Tiopronin-002

Conclusions:

When THIOLA IR and THIOLA EC single doses were given to fasted healthy subjects in a crossover study, the media time to peak plasma levels (T_{max}) was 1 (range: 0.5 to 2.1) and 3 (range: 1.0 to 6.0) hours, respectively. The peak exposure (C_{max}) and total exposure (AUC_{0-t}) of

tiopronin from THIOLA EC tablets were decreased by 22% and 7% respectively compared to THIOLA IR tablets.

3.2 Food effect study review

Study No: MPC-Tiopronin-001	**EDR:** \\cdsesub1\evsprod\nda211843\0001\m5\53-clin-stud-rep\533-rep-human-pk-stud\5334-extrin-factor-pk-stud-rep\mpc-tiopronin-001\mpc-tiopronin-001-study-report-body.pdf
Study Date: August 14, 2017 to September 12, 2017	
Title of Study: Open Label, Randomized, 2-Way Crossover Pharmacokinetic Study to Evaluate the Bioavailability of a Single Oral Dose of Tiopronin 300 mg EC (Fasted) Compared to a Single Oral Dose Tiopronin 300 mg EC (Fed) in Healthy Subjects.	
Investigational Product: THIOLA (tiopronin) 300 mg EC Tablet	
Study: • **Design:** Open label, randomized, 2-way, crossover study with 2 dosing periods: o A single dose of THIOLA EC 300 mg given orally in the fasted state with a 72-hour PK profile assessment, and o A single dose of THIOLA EC 300 mg given orally in the fed state with a 72-hour PK profile assessment. The study consisted of 4 visits: Visit 1, screening; Visit 2, randomization and first study drug dosing; Visit 3, second study drug dosing; and Visit 4, study termination (or early termination).  **Legend:** A = Admission D = Study drug dosing PK = Pharmacokinetic R = Randomization All subjects were admitted to the study clinic 1 day prior to study dose administration for each dosing period. Each dosing period consisted of admission, study drug dosing, and pharmacokinetic sampling over 72 hours. **Figure 1: Study Schematic** • **Washout:** There was a 2-week washout period (or 5½ half-lives) between each of the dosing periods. The washout period began 72 hours after study administration. • **Study participants:** 12 healthy adults (8 men and 4 women), 24- 43 years of age.	

- **Treatments Administered**
  Subjects received a single dose of THIOLA EC 300 mg on Study Day 1 and Study Day 18 to 20 in the fed or fasted state according to the randomization schedule.
  Fed State:
  A single dose of THIOLA EC 300 mg was administered with 240 mL of water, 30 minutes after the start of standardized high fat breakfast.
  Fasted State:
  A single dose of THIOLA EC 300 mg was administered with 240 mL of water after an overnight fasting for 12 hours.
- **Sampling times (h):** pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8,10,12, 24, 36, 48, 60, and 72 h post-dose.

Pharmacokinetic (PK) parameters calculated: AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $t_{1/2}$, and K_{el}

Analytical Method:

- A validated LC-MS method, as described in section 2.2.2, was used for the estimation of total tiopronin in human K_2EDTA plasma using tiopronin d3 as Internal Standard.
- The analytical range for tiopronin in plasma was 50 ng/mL - 5000 ng/mL

Reviewer comment: The performance of the analytical method is acceptable per the specifications in Bioanalytical Method Validation Guidance.

Statistical Methods:

Natural log transformed $AUC_{0-\infty}$, AUC_{0-t}, and C_{max} were each analyzed using a general linear model with fixed effects for fed or fasted state, period, and sequence and a random effect for subject nested in sequence.

The least squares mean was computed for each fed and fasted state and the difference between treatments, along with its 90% confidence interval, was exponentiated. The geometric mean ratios and their confidence intervals were used to summarize the relative bioavailability of tiopronin between the fed and fasted states.

Results:

12 subjects completed the study. A total of 384 blood samples were collected for analysis of plasma tiopronin concentrations.

Table 1. Demographics

Age range	24 - 43 years
Males Females	8 (66.7%) 4 (33.3%)
Weight	60.4 - 89 kg

Source: Clinical Study Report Study MPC-Tiopronin-001

Table 2. Subject Disposition

	Fed/Fasted N (%)	Fasted/Fed N (%)	All subjects N
Screened			44
Randomized	6 (100)	6 (100)	12
Completed	6 (100)	6 (100)	12

Source: Clinical Study Report Study MPC-Tiopronin-001

For Statistical Summary of comparison of fed and fasted state, refer to **Table 4** in section 2.2.4

Table 3. Summary of PK Parameters

Parameter	Fed Arithmetic Mean (SD) (N=12)	Fasted Arithmetic Mean (SD) (N=12)
C_{max} (ng/mL)	5641.7 (1776.7)	6563.6 (1771.1)
AUC_{0-t} (ng.hr/mL)	24751.6 (6213.2)	32681.2 (7393.9)
$AUC_{0-\infty}$ (ng.hr/mL)	30566.5 (8569.2)	38901.6 (8938.3)
T_{max} (hr)*	3.5 (3 - 6)	3 (1.5 - 4.0)
$t_{1/2}$ (hr)	46.3 (10.6)	40.9 (3.9)

* Median (Range)
Source: Clinical Study Report Study MPC-Tiopronin-001

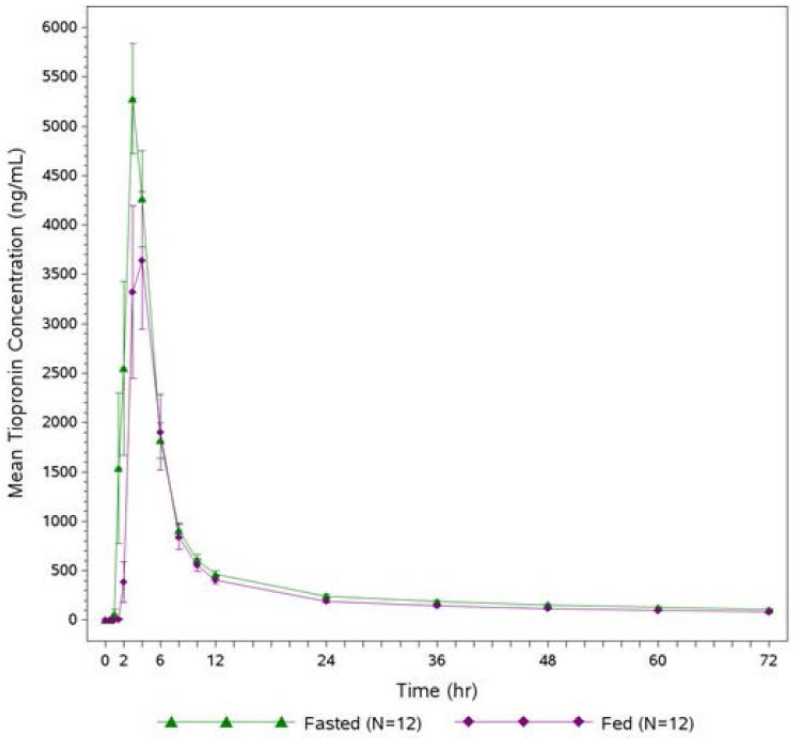


Figure 2: Plot of mean (SE) plasma tiopronin concentration vs time profiles
Source: Clinical Study Report Study MPC-Tiopronin-001

Conclusions:
When THIOLA EC single dose was given to fasted and fed healthy subjects in a crossover study, the peak exposure (C_{max}) and total exposure (AUC_{0-t}) of tiopronin in fed state were decreased by 13% and 25%, respectively, compared to fasted state. Since the drug is dosed to effect, the study results support administration of THIOLA EC tablet with or without food; however, at the same time each day with a routine pattern with regard to meals.

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