Building and evaluation of a PBPK model for erythromycin in healthy adults

Version	**0.1**
OSP Version	8.0
Author	André Dallmann (AndreDlm)

Table of Contents

- 1 Introduction
- 2 Methods
  - 2.1 Modeling Strategy
  - 2.2 Data
  - 2.3 Model Parameters and Assumptions
- 3 Results and Discussion
  - 3.1 Final input parameters
  - 3.2 Diagnostics Plots
  - 3.3: Concentration-Time Profiles
    - 3.3.1 Model Building
    - 3.3.2 Model Verification
- 4 Conclusion
- 5 References

1 Introduction

The presented model building and evaluation report evaluates the performance of a PBPK model for erythromycin in healthy adults.

Erythromycin a macrolide antibiotic used for the treatment of a number of bacterial infections, including respiratory tract infections, skin infections, chlamydia infections, and others. Erythromycin is available in the form of various salts and formulations, for example as:

- erythromycin lactobionate for injection
- erythromycin base in enteric-coated capsules or tablets for oral administration
- erythromycin stearate in filmcoated tablets for oral administration
- erythromycin ethylsuccinate in suspension or in filmcoated tablets for oral administration

In its free form as base, erythromycin is easily hydrolyzed in acidic aqueous solution (Mordi 2000). Therefore, orally administered erythromcyin is given in the form of enteric-coated formulations or as more acid-stable salts or esters of erythromycin (e.g. erythromycin ethylsuccinate). Once in the small intestine, erythromycin is rapidly absorbed displaying a highly variable bioavailability (Chun 1977, Mather 1981). Erythromycin diffuses in most tissues and accumulates in leukocytes and phagocytes (Miller 1984, Carlier 1987). About 70% of erythromycin is bound to plasma proteins (Barre 1987). Erythromycin has been shown to be a substrate for various transporters including P-gp and OATP1B1. The latter has been shown to critically affect erythromycin disposition (Lancaster 2012). Erythromycin is extensively metabolized through N-demethylation catalyzed by CYP3A. Metabolism via CYP4F11 has also been suggested (Kalsotra 2004). Biliary excretion also appears to play an important role in erythromycin clearance (Acocella 1968, Chelvan 1979), but tits contribution to total elimination remains unknown. The dose fraction excreted unchanged in urine is minimal and highly variable; reported fractions range from 0.018 ± 0.005 to 0.171 ± 0.11 (mean ± SD) (Pasic 1987, Austin 1980). Erythromycin is a moderate inhibitor of CYP3A4 and listed by the FDA as clinical index inhibitor for CYP3A.

The presented erythromycin PBPK model as well as the respective evaluation plan and evaluation report are provided open-source (https://github.com/Open-Systems-Pharmacology/Erythromycin-Model).

2 Methods

2.1 Modeling Strategy

The general workflow for building an adult PBPK model has been described by Kuepfer et al. (Kuepfer 2016). Relevant information on the anthropometry (height, weight) was gathered from the respective clinical study, if reported. Information on physiological parameters (e.g. blood flows, organ volumes, hematocrit) in adults was gathered from the literature and has been incorporated in PK-Sim® as described previously (Willmann 2007). The applied activity and variability of plasma proteins and active processes that are integrated into PK-Sim® are described in the publicly available 'PK-Sim® Ontogeny Database Version 7.3' (PK-Sim Ontogeny Database Version 7.3).

The PBPK model was developed based on clinical data of healthy adult subjects obtained from the literature, covering different formulation types and erythromycn salts. Multiple doses and dosing schedules following intravenous (IV) and oral (PO) administration were included in model building. Mass balance information on urinary excretion of unchanged erythromycin after IV administration was also accounted for during the model building process.

Unknown parameters were simultaneously optimized using all available PK data, in particular:

- 4 data sets following single IV administration of 4 different doses of erythromycin (125 mg, 250 mg, 300 mg, 500 mg) as erythromycin lactobionate
- 6 data sets following single and multiple PO administration of 3 different doses of erythromycin (250 mg, 500 mg, 1000 mg) as film-coated tablets containing erythromycin stearate
- 2 data sets following single PO administration of 500 mg erythromycin as enteric-coated tablets containing erythromycin as base
- 2 data sets following single and multiple PO administration of 2 different doses of erythromycin (250 mg, 500 mg) as enteric-coated capsules containing pellets of erythromycin as base

Structural model selection was mainly guided by visual inspection of the resulting description of data and biological plausibility. The following parameters were identified using the Parameter Identification module provided in PK-Sim® and MoBi® (Open Systems Pharmacology Documentation):

- `Dissolution shape` (for the film-coated tablet containing erythromycin stearate)
- `Dissolution time (50% dissolved)` (for the film-coated tablet containing erythromycin stearate)
- `Dissolution lag time` (for the enteric-coated tablet containing erythromycin as base)
- `Dissolution shape` (for the enteric-coated tablet containing erythromycin as base)
- `Dissolution time (50% dissolved)` (for the enteric-coated tablet containing erythromycin as base)
- `Solubility at reference pH` (for the enteric-coated tablet containing erythromycin as base)
- `Dissolution lag time` (for the enteric-coated capsule containing pellets of erythromycin as base)
- `Dissolution shape` (for the enteric-coated capsule containing pellets of erythromycin as base)
- `Dissolution time (50% dissolved)` (for the enteric-coated capsule containing pellets of erythromycin as base)
- `Specific intestinal permeability (transcellular)`
- `Transport Protein - OATP1B1 - kact`
- `Metabolizing Enzyme - CYP3A4 - kact`
- `Total Hepatic Clearance - Specific clearance`
- `GFR fraction`

Details about input data (physicochemical, *in vitro* and clinical) can be found in Section 2.2.

Details about the structural model and its parameters can be found in Section 2.3.

2.2 Data

2.2.1 In vitro / physicochemical data

A literature search was carried out to collect available information on physicochemical properties of eryhtromycin. The obtained information from the literature is summarized in the table below and is used for model building.

Parameter	Unit	Literature	Description
Molecular weight	g/mol	733.9 (drugbank.ca)	Molecular weight
pK_a (basic)		8.8 (Lien 1974); 8.88 (McFarland 1997)	Acid dissociation constant
logP		2.48 (Lien 1974); 2.92 (Capobianco 1994); 3.06 (McFarland 1997)	Partition coefficient between octanol and water
f_u		0.27 ± 0.05[a] (Sun 2010); 0.28 ± 0.04[a] (Iliopoulou 1982); 0.305 ± 0.028[a] (Barre 1987); 0.326 (Xu 2009)	Fraction unbound in human plasma
Water solubility (erythromycin lactobionate)	mg/L	200000 (U.S. Patent 2,761,859)	Solubility of erythromycin lactobionate in water
Water solubility (erythromycin stearate)	mg/L	182 (Jones 1969)	Solubility of erythromycin stearate in water
Water solubility (erythromycin base)	mg/L	2100 (Manna 1998)	Solubility of free erythromycin as base in water

[a] mean ± SD

2.2.2 In vitro data on mechanism-based inhibition of CYP3A

A literature search was carried out to collect quantitative information the meachnism-based inhibition of CYP3A by erythromycin, in particular measured values for K_I and k_{inact}. The gathered information is summarized in the table below and is used for model building.

Victim compound	In vitro system	Enzyme	K_I [µM]	k_{inact} [min^{-1}]	Concentration range of erythromycin [µM]	Concentration of victim compound [µM]	Reference
testosterone	rec cDNA CYP3A4	CYP3A4	1.04	0.0293	0 - 20	100	Akiyoshi 2013
testosterone	rec cDNA CYP3A4	CYP3A4	1.21	0.0164	0 - 20	100	Akiyoshi 2013
testosterone	rec cDNA CYP3A4	CYP3A4	0.415	0.0159	0 - 20	100	Akiyoshi 2013
testosterone	rec cDNA CYP3A4	CYP3A4	2.24	0.0293	0 - 20	100	Akiyoshi 2013
testosterone	rec cDNA CYP3A4	CYP3A4	0.753	0.0248	0 - 20	100	Akiyoshi 2013
midazolam	rec cDNA CYP3A4	CYP3A	9.5	0.16	50-fold range	10	Atkinson 2005
midazolam	rec cDNA CYP3A4	CYP3A	8.82	0.12	50-fold range	10	Atkinson 2005
midazolam	rec cDNA CYP3A4	CYP3A	8.3	0.09	50-fold range	10	Atkinson 2005
testosterone	rec cDNA CYP3A4	CYP3A	9.5	0.06	4 - 200	16.7	Atkinson 2005
triazolam	HLM	CYP3A	5.4	0.069		400	Aueviriyavit 2010
testosterone	rec cDNA CYP3A4	CYP3A4	5.3	0.12	5 - 20	200	Chan 2000
midazolam	cryopreserved HEP	CYP3A	60	0.081	3 - 300	30	Chen 2011
midazolam	cryopreserved HEP	CYP3A	67.9	0.079	3 - 300	30	Chen 2011
midazolam	rec cDNA CYP3A4	CYP3A4	0.762	0.0648	0.1 - 30	4	Ishikawa 2017
testosterone	rec cDNA CYP3A4	CYP3A4	1.00	0.0604	0.1 - 30	150	Ishikawa 2017
nifedipine	rec cDNA CYP3A4	CYP3A4	0.794	0.0766	0.1 - 30	6	Ishikawa 2017
triazolam	HLM	CYP3A4	15.9	0.062	3 - 100	300	Kanamitsu 2000
triazolam	HLM	CYP3A4	17.4	0.055	3 - 100	300	Kanamitsu 2000
triazolam	rec cDNA CYP3A4	CYP3A4	19.1	0.173	3 - 100	300	Kanamitsu 2000
triazolam	rec cDNA CYP3A4	CYP3A4	18.9	0.097	3 - 100	300	Kanamitsu 2000
testosterone	HLM	CYP3A4	29.4	0.0271		250	Kosaka 2017
testosterone	HLM	CYP3A4	30	0.040	0.3 - 300		Kozakai 2013
midazolam	HLM	CYP3A4	12	0.035	0.3 - 300		Kozakai 2013
midazolam	HLM	CYP3A4	20	0.033	0.3 - 300		Kozakai 2013
midazolam	cryopreserved HEP	CYP3A	25.15	0.08	0.13 - 100	30	Mao 2011

Victim compound	In vitro system	Enzyme	K_I [µM]	k_{inact} [min^{-1}]	Concentration range of erythromycin [µM]	Concentration of victim compound [µM]	Reference
midazolam	HLM	CYP3A	10.8	0.032			Mao 2016
midazolam	cryopreserved HEP	CYP3A	30.7	0.05	0 - 300	20	Mao 2016
midazolam	cryopreserved HEP	CYP3A	59.2	0.062	0 - 100	5	Mao 2016
midazolam	cryopreserved HEP	CYP3A	80.3	0.052	0 - 100	20	Mao 2016
midazolam	rec cDNA CYP3A4	CYP3A4	7.47	0.042	2 - 50	8	McConn 2004
midazolam	HLM	CYP3A4	10.9	0.046	2 - 100	8	McConn 2004
midazolam	primary HEP	CYP3A	11	0.07	0.1 - 10	3	McGinnity 2006
midazolam	HLM	CYP3A4	10	0.036	0 - 25		Obach 2007
testosterone	HLM	CYP3A4	9.8	0.039	0 - 25	500	Obach 2007
testosterone	rec cDNA CYP3A4	CYP3A4	0.92	0.058	5 - 100	250	Polasek 2006
testosterone	HLM	CYP3A4	12.8	0.037	5 - 100	250	Polasek 2006
midazolam	rec cDNA CYP3A4	CYP3A4	5.1	0.30	0.5 - 50	100	Ring 2005
testosterone	rec cDNA CYP3A4	CYP3A4	0.92	0.058			Teng 2010
testosterone	HLM	CYP3A4	4.579	0.0115			Teng 2010
domperidone	HLM	CYP3A4	18.4	0.022	2.5 - 200	500	Ung 2009
domperidone	rec cDNA CYP3A4	CYP3A4	4.1	0.026	2.5 - 200	500	Ung 2009
midazolam	HLM	CYP3A4	12.1	0.0215	0 - 100	25	Watanabe 2007
nifedipine	HLM	CYP3A4	11.3	0.0295	0 - 100	50	Watanabe 2007
testosterone	HLM	CYP3A4	10.9	0.0352	0 - 100	200	Watanabe 2007
midazolam	HLM	CYP3A	1.48	0.017	0.5 - 500	20	Xu 2009
midazolam	cryopreserved HEP	CYP3A	20.0	0.016	0.5 - 500	20	Xu 2009
midazolam	cryopreserved HEP	CYP3A	109	0.055	0.5 - 500	20	Xu 2009
midazolam	HLM	CYP3A	81.8	0.0665	20 - 400	10	Yamano 2001
midazolam	HLM	CYP3A	15.7	0.1			Zhang 2006
testosterone	rec cDNA CYP3A4	CYP3A4	5	0.34	1 - 50	200	Zhang 2009
testosterone	HLM	CYP3A4	15.7	0.09	1 - 50	200	Zhang 2009

Victim compound	In vitro system	Enzyme	K_I [µM]	k_{inact} [min^{-1}]	Concentration range of erythromycin [µM]	Concentration of victim compound [µM]	Reference
midazolam	HLM	CYP3A4	26.5	0.041	2.5 - 50	10	Zimmerlin 2011

Note: Abbreviations: HEP: human hepatocytes; HLM: human liver microsomes; rec cDNA CYP3A4: human recombinant c-DNA CYP3A4 enzymes (e.g. supersomes, baculovirus-insect cell system, E. coli transfected cells)

2.2.3 Clinical data

A literature search was carried out to collect available PK data on erythromycin in healthy adults.

The following publications were found and used for model building and evaluation:

Publication	Study description
Austin 1980	IV administration of 125 mg, 250 mg, 500 mg, and 900 mg as erythromycin lactobionate; single dose
Barre 1987	IV administration of 500 mg as erythromycin lactobionate; single dose
Huppertz 2011	IV administration of 1000 mg as erythromycin lactobionate; single dose
Pasic 1987	IV administration of 300 mg as erythromycin lactobionate; single dose
Sun 2010	IV administration of 125 mg and PO administration of 250 mg (salt and formulation type not specified); single dose
Iliopoulou 1982	PO administration of 500 mg as film-coated tablet containing erythromycin stearate; multiple dose
Josefsson 1982	PO administration of 500 mg as film-coated tablet containing erythromycin stearate and 250 mg, 500 mg, 1000 mg as enteric coated capsules containing pellets of erythromycin base; single dose
Kavi 1988	PO administration of 500 mg as film-coated tablet containing erythromycin stearate; single dose
Malmborg 1978	PO administration of 500 mg as film-coated tablet containing erythromycin stearate; multiple dose
Miglioli 1990	PO administration of 1000 mg erythromycin stearate; multiple dose
Posti 1983	PO administration of 500 mg as film-coated tablets containing erythromycin stearate, as enteric-coated tablets containing erythromycin base, and as enteric-coated tablets containing erythromycin stearate; single dose
Schreiner 1984	PO administration of 500 mg as film-coated tablets containing erythromycin stearate and as enteric-coated capsule containing pellets of erythromycin base; single dose
Yakatan 1980	PO administration of 250 mg as film-coated tablet containing erythromycin stearate, as enteric-coated tablet containing erythromycin base, and as enteric-coated capsules containing erythromycin estolate; single and multiple dose
Birkett 1990	PO administration of 250 mg as enteric-coated capsules containing pellets of erythromycin base; single dose
Kivistö 1997	PO administration of 500 mg as enteric-coated capsules containing erythromycin base; multiple dose
DiSanto 1981	PO administration of 500 mg as unprotected tablets containing erythromycin base, as film-coated tablets containing erythromycin base, as enteric-coated tablets containing erythromycin base and as film-coated tablets containing erythromycin stearate; single and multiple dose
Kroboth 1982	PO administration of 500 mg as enteric-coated tablets containing erythromycin base; single dose

2.3 Model Parameters and Assumptions

2.3.1 Dissolution and absorption

Development of an adequate absorption model for erythromycin was complicated by the large intersubject variability in the absorption kinetics of erythromycin (Chun 1977, Mather 1981). Additionally, multiple formulation types are available entailing different absorption kinetics (Chun 1977, Yakatan 1980, Mather 1981). The herein presented model was developed for the following oral dosage forms:

- film-coated tablets containing erythromycin stearate
- enteric-coated tablets containing erythromycin as free base
- enteric-coated capsules containing pellets of erythromycin as free base

It was assumed that the solubility of erythromycin as stearate in the film-coated tablets and as free base pellets in enteric-coated capsules is high and was therefore fixed to a value of 500 mg/L. Solubility of erythromycin base in enteric-coated tablets was optimized to capture the relatively lower C_{max} value of this formulation observed in the herein used *in vivo* PK studies. For all three formulations types, dissolution kinetics was described by a Weibull function with different parameter values for each formulation type. Optimized parameters were the `Dissolution shape` and `Dissolution time (50% dissolved)`. Additionally, for the enteric-coated formulations, a `lag time` was applied in the model to account for the passage through the stomach, while the film-coated tablet was assumed to dissolve immediately upon entering the stomach, i.e. no lag time was applied here. `Specific intestinal permeability (transcellular)` was the same for all formulations and simultaneously optimized with the other parameters.

2.3.2 Distribution

With an average fraction unbound in human plasma of approximately 0.30, erythromycin is moderately protein-bound. In the developed model, the `fraction unbound (plasma, reference value)` was set to 0.305 which is the value reported by Barre 1987. `Lipophilicity` was fixed to a value of 2.82 which is the average of three experimentally measured values reported in the literature (Lien 1974, Capobianco 1994, McFarland 1997). The observed PK data were found to be best described using the model for estimating intracellular-to-plasma partition coefficients according to the method by `Rodgers and Rowland` (Rodgers 2005, Rodgers 2006). Cellular permeabilities were automatically calculated using the method `Charge dependent Schmitt` (Open Systems Pharmacology Documentation). Active transfer of erythromycin by OATP1B1 was modeled as Michaelis-Menten kinetics using an `Influx` transporter type; the `Km` value of 13.2 µM was taken from the literature (Lancaster 2012) and `kcat` was optimized. The gene expression profile of OATP1B1 (default symbol for the gene: LST-3TM12) was loaded from the internal PK-Sim® database using the expression data quantified by RT-PCR (Open Systems Pharmacology Documentation).

2.3.3 Elimination

Erythromycin is extensively metabolized via N-demethylation catalyzed by CYP3A. Kinetics of this biotransformation was described by a Michaelis-Menten process with `kcat` being optimized and `Km` fixed to a value of 70 µM, the average of reported values in the literature (Riley 1997, Wang 1997). The gene expression profile of CYP3A4 was loaded from the internal PK-Sim® database using the expression data quantified by RT-PCR (Open Systems Pharmacology Documentation).

Although it has also been observed that erythromycin is metabolized via CYP4F11 (Kalsotra 2004), this elimination pathway was not accounted for in the model because its contribution to overall elimination was assumed to be low. In humans, CYP4F11 is mainly expressed in the liver and to a much lesser extent in the kidney (Cui 2000) and the CYP4F family makes up approximately 15% of all hepatic CYP enzymes (Michaels 2014). The K_m and V_{max} values for the CYP4F11-mediated biotransformation reported by Kalsotra 2004 are similar to those measured for CYP3A4 (Riley 1997, Wang 1997), suggesting that the relative mass balance of these two metabolism pathways mainly depends on the absolute amount of each enzyme in the liver. While no information on total CYP4F11 in the human liver could be found in the literature, CYP4F11 expression in the liver of cynomolgus monkeys was observed to be approximately 6-fold lower than that of CYP3A4 (Uehara 2015). Hence, it was assumed that CYP4F11-mediated metabolism of erythromycin can be neglected in humans.

Additional elimination pathways suggested for erythromycin are acid-catalyzed degradation (hydrolysis) the acidic milieu of the stomach (Mordi 2000) and biliary excretion (Acocella 1968, Chelvan 1979), but no quantitative information on the mass balance of these pathways could be found in the literature. Additionally, mechanism-based inhibition of CYP3A4 by erythromycin might constitute another clearance process which was neither considered in the model. However, a `total hepatic clearance` process was implemented in the model which could at least partly account for other elimination pathways not explicitly accounted for in the model.

The reported dose fractions of erythromycin undergoind unchanged renal excretion after IV administration range from 0.018 ± 0.005 to 0.171 ± 0.11 (mean ± SD) (Pasic 1987, Austin 1980). This information was accounted for in the model by implementing a glomerular filtration process and optimizing the `GFR fraction` to match the observed dose fractions excreted unchanged in urine.

2.3.2 Autoinhibition

There is abundant evidence from *in vitro* studies that erythromycin irreversibly inhibits CYP3A (e.g. Larrey 1983) and the FDA lists erythromycin as moderate index inhibitor for CYP3A. Ample data quantifying the mechanism-based inhibition of CYP3A4 by erythromycin was collected from the literature (see section 2.2.2). The data listed in the Table in section 2.2.2 can be statistically summarized as follows:

Parameter [unit]	Min	Q1	Geometric mean	Median	Arithmetic mean	Q3	Max
K_I [µM]	0.420	4.89	8.71	10.9	18.4	19.3	109
k_{inact} [min^{-1}]	0.0115	0.0314	0.0504	0.0535	0.0664	0.0772	0.340

In the PBPK model, `Ki` and `kinact` were fixed to the geometric mean values tabulated above.

3 Results and Discussion

The PBPK model for erythromycin was developed and verified with clinical pharmacokinetic data.

The next sections show:

1. the final model parameters for the building blocks: Section 3.1.
2. the overall goodness of fit: Section 3.2.

3. simulated vs. observed concentration-time profiles for the clinical studies used for model building and for model verification: Section 3.3.

3.1 Final input parameters

The compound parameter values of the final PBPK model are illustrated below.

Compound: Erythromycin

Parameters

Name	Value	Value Origin	Alternative	Default	
Solubility at reference pH	500 mg/l	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	Erythromycin stearate film-coated tablet	True	
Reference pH	7	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	Erythromycin stearate film-coated tablet	True	
Solubility at reference pH	500 mg/l	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	Erythromycin base enteric coated pellets	False	
Reference pH	7	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	Erythromycin base enteric coated pellets	False	
Solubility at reference pH	200 mg/ml	Publication-In Vitro-Hoffhine, Jr Charles E. "Aqueous soluble salts of erythromycin." U.S. Patent 2,761,859, issued September 4, 1956.	Erythromycin lactobionate	False	

Name	Value	Value Origin	Alternative	Default	
Reference pH	7	Publication-In Vitro-Hoffhine, Jr Charles E. "Aqueous soluble salts of erythromycin." U.S. Patent 2,761,859, issued September 4, 1956.	Erythromycin lactobionate	False	
Solubility at reference pH	2.5002520571 mg/l	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	Erythromycin base enteric coated tablet	False	
Reference pH	7		Erythromycin base enteric coated tablet	False	
Lipophilicity	2.82 Log Units	Publication-In Vitro-Average of reported values in the literature (PMID: 9135031, PMID: 18611609, and: Lien, E. J., Kuwahara, J., & Koda, R. T. (1974). Diffusion of drugs into prostatic fluid and milk. Drug Intelligence & Clinical Pharmacy, 8(8), 470-475. http://journals.sagepub.com/doi/abs/10.1177/106002807400800803)	Literature (average value)	True	

Name	Value	Value Origin	Alternative	Default	
Fraction unbound (plasma, reference value)	0.305	Publication-In Vivo-PMID: 3606934	Barre 1987	True	
Specific intestinal permeability (transcellular)	0.00057882652836 cm/min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	Fitted	True	
Is small molecule	Yes				
Molecular weight	733.927 g/mol	Internet-drugbank.ca			
Plasma protein binding partner	Unknown				

Calculation methods

Name	Value	
Partition coefficients	Rodgers and Rowland	
Cellular permeabilities	Charge dependent Schmitt	

Processes

Metabolizing Enzyme: CYP3A4-Biotransformation_fitted

Molecule: CYP3A4

Parameters

Name	Value	Value Origin	
In vitro Vmax for liver microsomes	345 pmol/min/mg mic. protein	Publication-In Vitro-PMID: 9566442	
Km	70 µM	Publication-In Vitro-Average of reported values in the literature (PMID: 9107550 and PMID: 9566442)	
kcat	6.477728756 1/min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	

Systemic Process: Glomerular Filtration-fitted

Species: Human

Parameters

Name	Value	Value Origin	
GFR fraction	1.3151947529	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	

Inhibition: CYP3A4-MBI

Molecule: CYP3A4

Parameters

Name	Value	Value Origin	
kinact	0.05044312 1/min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	
K_kinact_half	8.705192 µmol/l	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	

Transport Protein: OATP1B1-fitted

Molecule: OATP1B1

Parameters

Name	Value	Value Origin	
Transporter concentration	1 µmol/l		
Vmax	11.66 pmol/ml/min	Publication-In Vitro-PMID: 22990751	
Km	13.2 µmol/l	Publication-In Vitro-PMID: 22990751	
kcat	18.3753550107 1/min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	

Systemic Process: Total Hepatic Clearance-fitted

Species: Human

Parameters

Name	Value	Value Origin	
Fraction unbound (experiment)	0.305		
Lipophilicity (experiment)	2.48 Log Units		
Plasma clearance	0 ml/min/kg		
Specific clearance	3.1542129297 1/min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:46	

Formulation: Erythromycin_Weibull_enteric-coated-pellets

Type: Weibull

Parameters

Name	Value	Value Origin	
Dissolution time (50% dissolved)	3.4504159922 min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Lag time	107.7187470594 min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Dissolution shape	1.1678641251	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Use as suspension	Yes		

Formulation: Erythromycin_Weibull_enteric-coated-tablet

Type: Weibull

Parameters

Name	Value	Value Origin	
Dissolution time (50% dissolved)	121.1893502595 min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Lag time	66.1803696499 min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Dissolution shape	1.5167999313	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Use as suspension	Yes		

Formulation: Erythromycin_Weibull_filmtablet

Type: Weibull

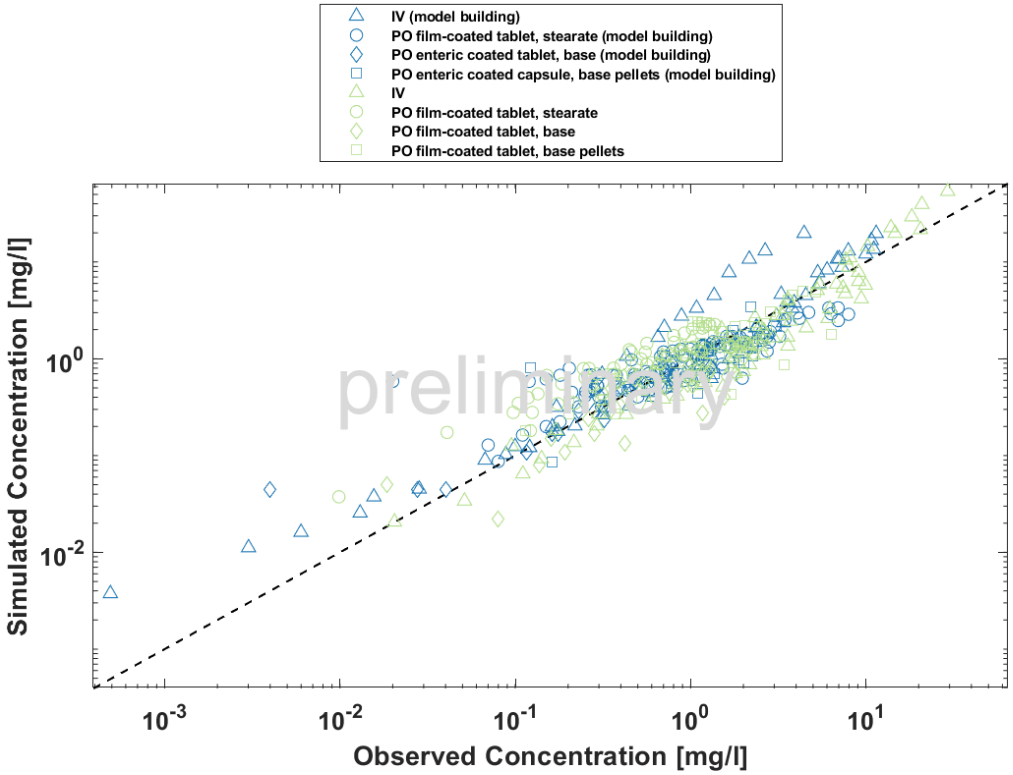
Parameters

Name	Value	Value Origin	
Dissolution time (50% dissolved)	27.6449832276 min	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Lag time	0 min	Other-Assumption	
Dissolution shape	0.3779018493	Parameter Identification-Parameter Identification-Value updated from '001' on 2019-10-01 13:29	
Use as suspension	Yes		

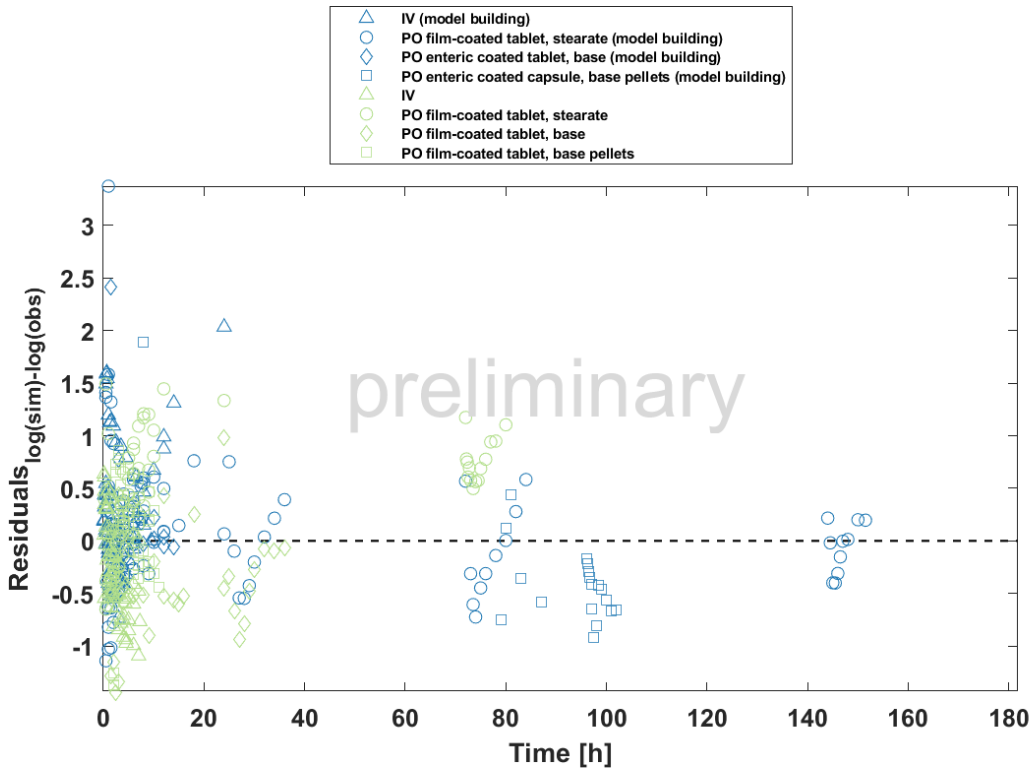
3.2 Diagnostics Plots

Below you find the goodness-of-fit visual diagnostic plots for the PBPK model performance of all data used presented in Section 2.2.2.

The first plot shows observed versus simulated plasma concentration, the second weighted residuals versus time.



Goodness of fit plot for concentration in plasma



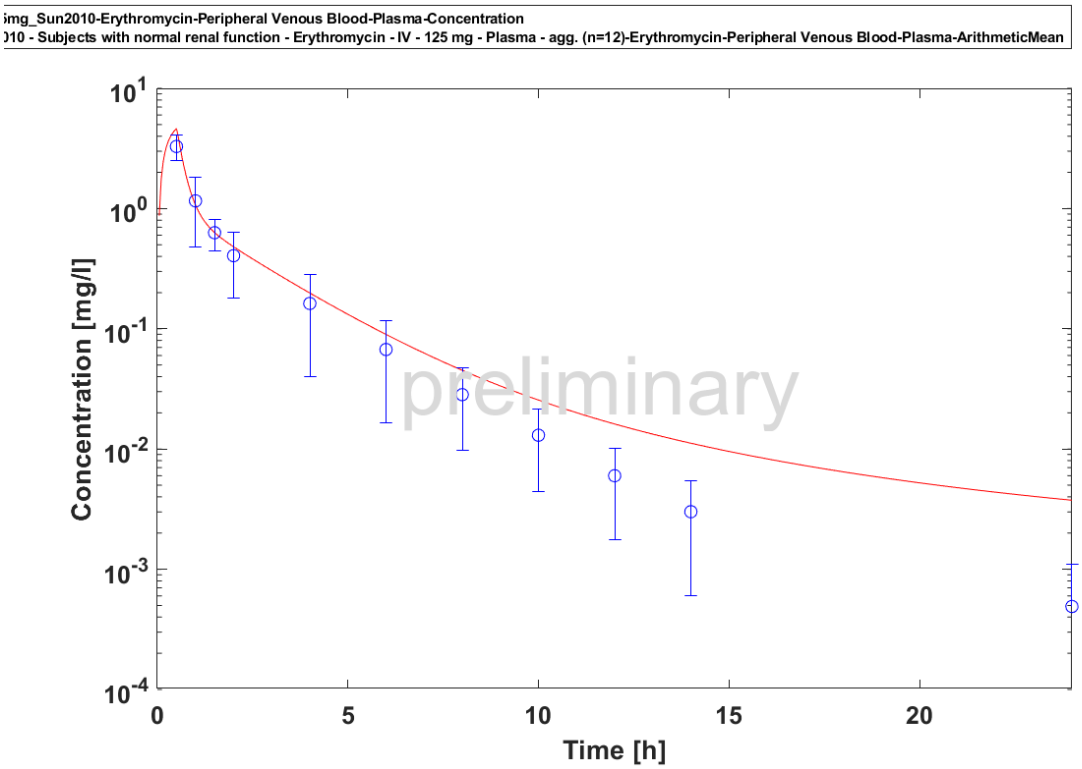
Goodness of fit plot for concentration in plasma

GMFE = 1.579291

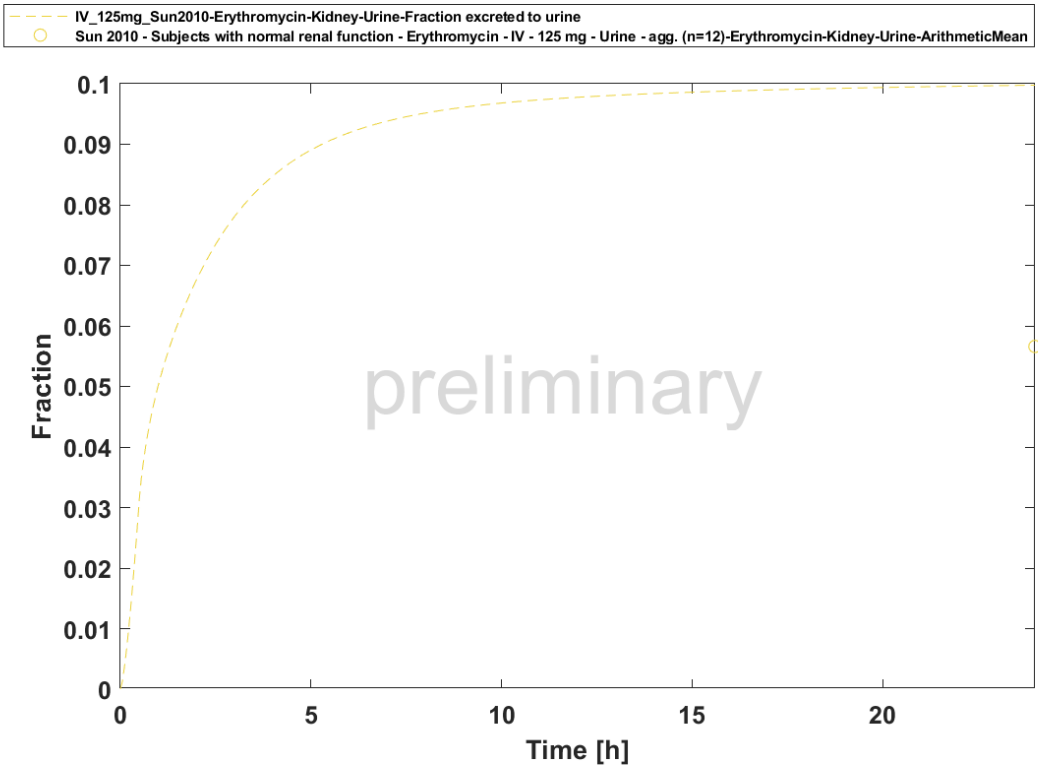
3.3: Concentration-Time Profiles

Simulated versus observed concentration-time profiles of all data listed in Section 2.2.2 are presented below.

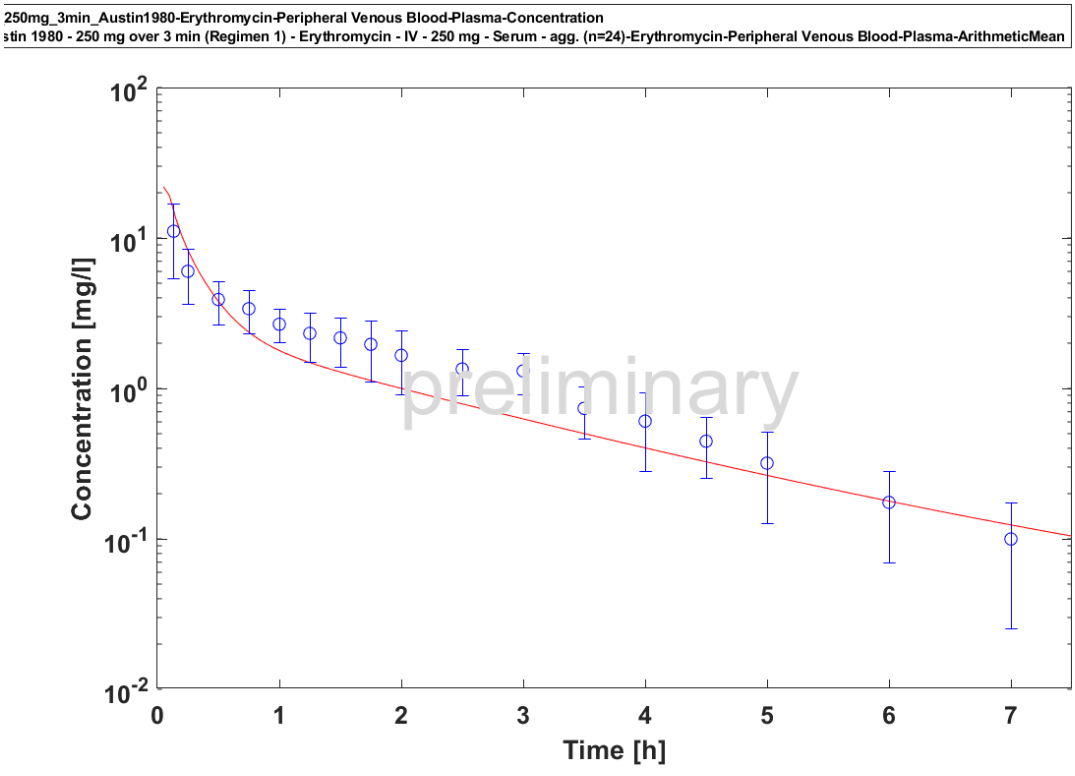
3.3.1 Model Building



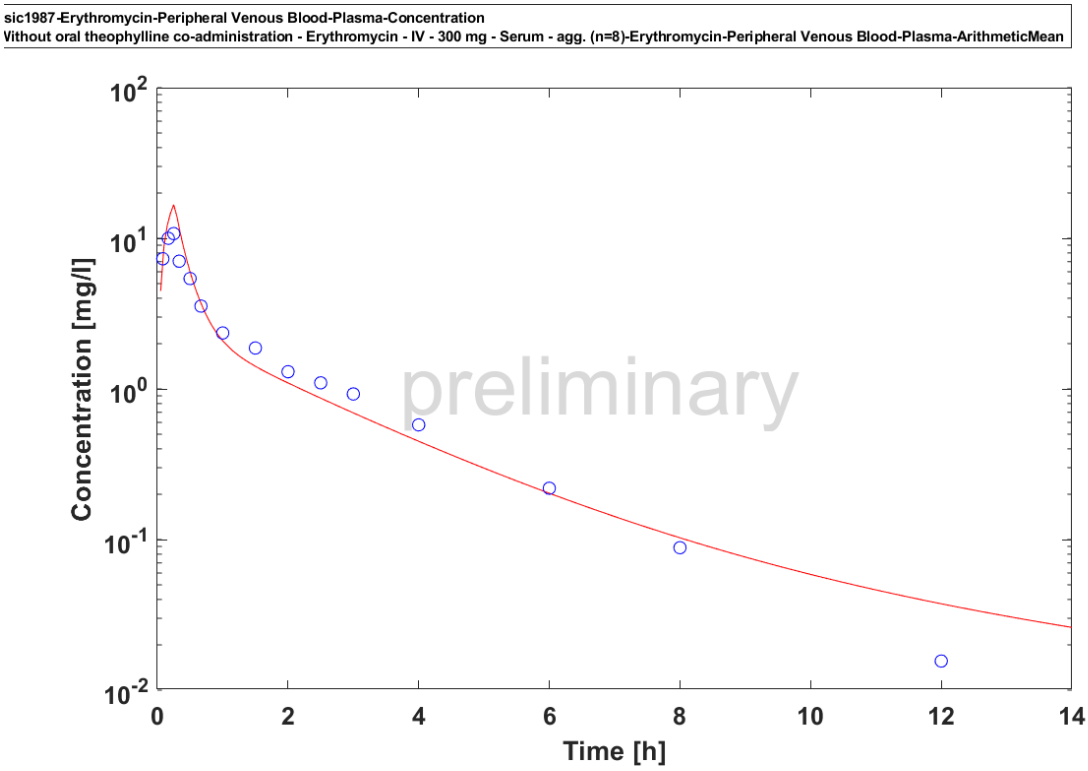
Time Profile Analysis



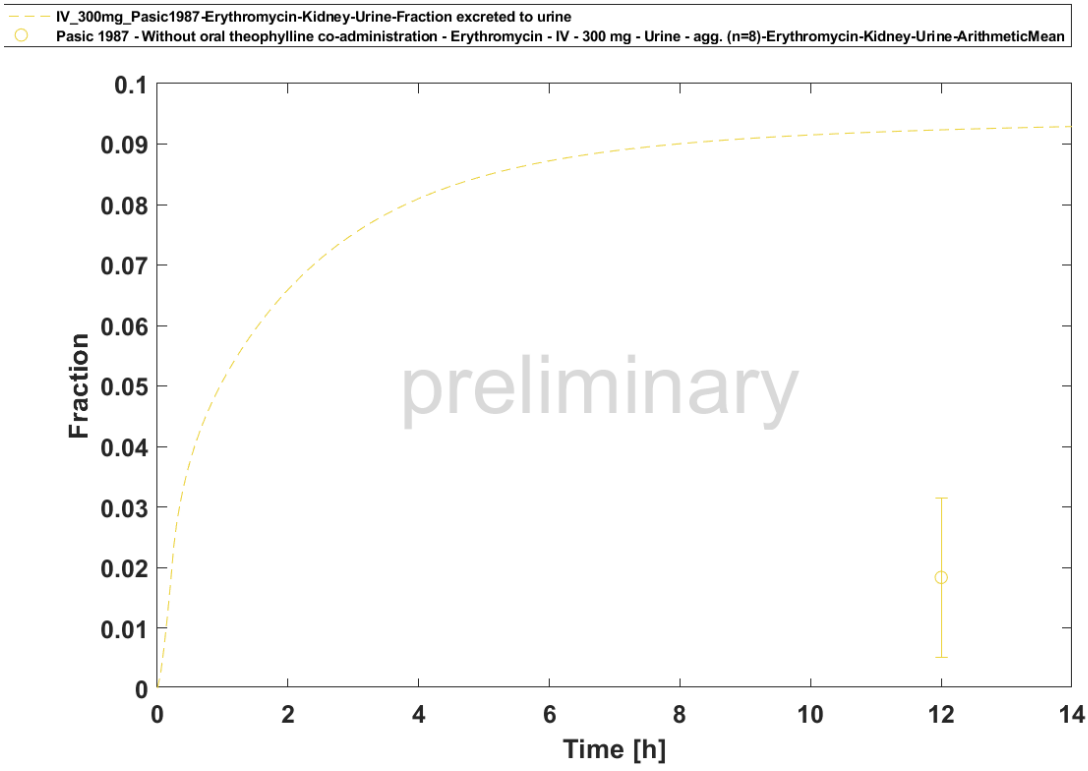
Time Profile Analysis 1



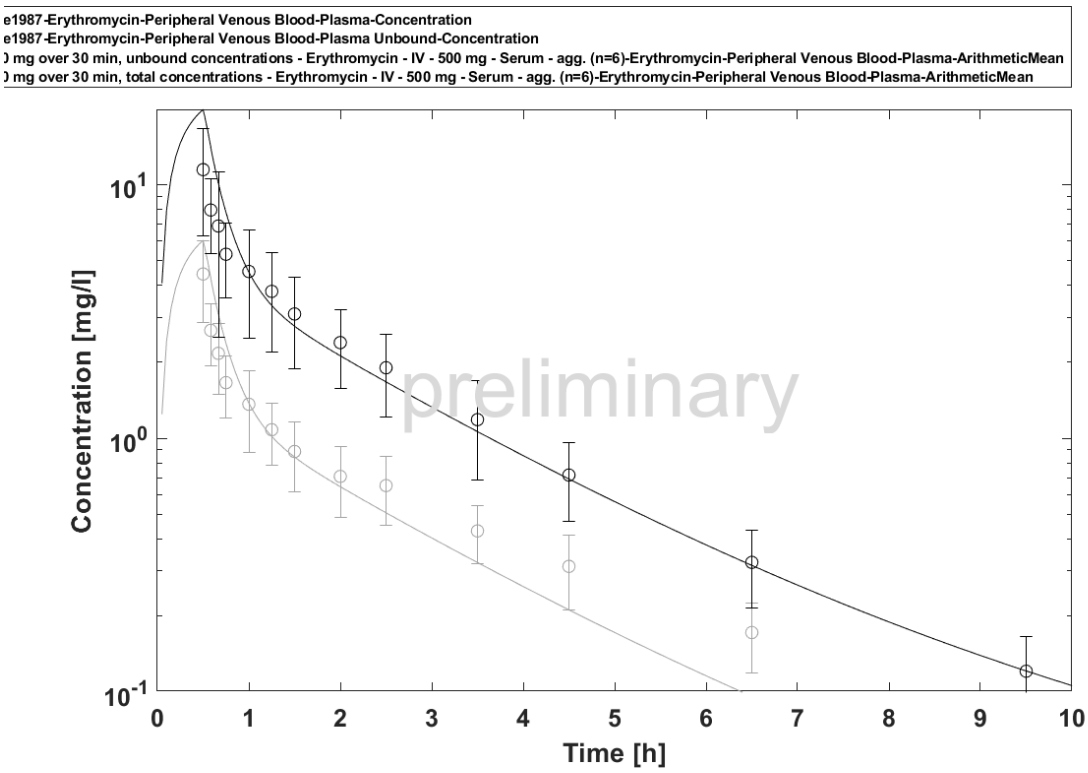
Time Profile Analysis



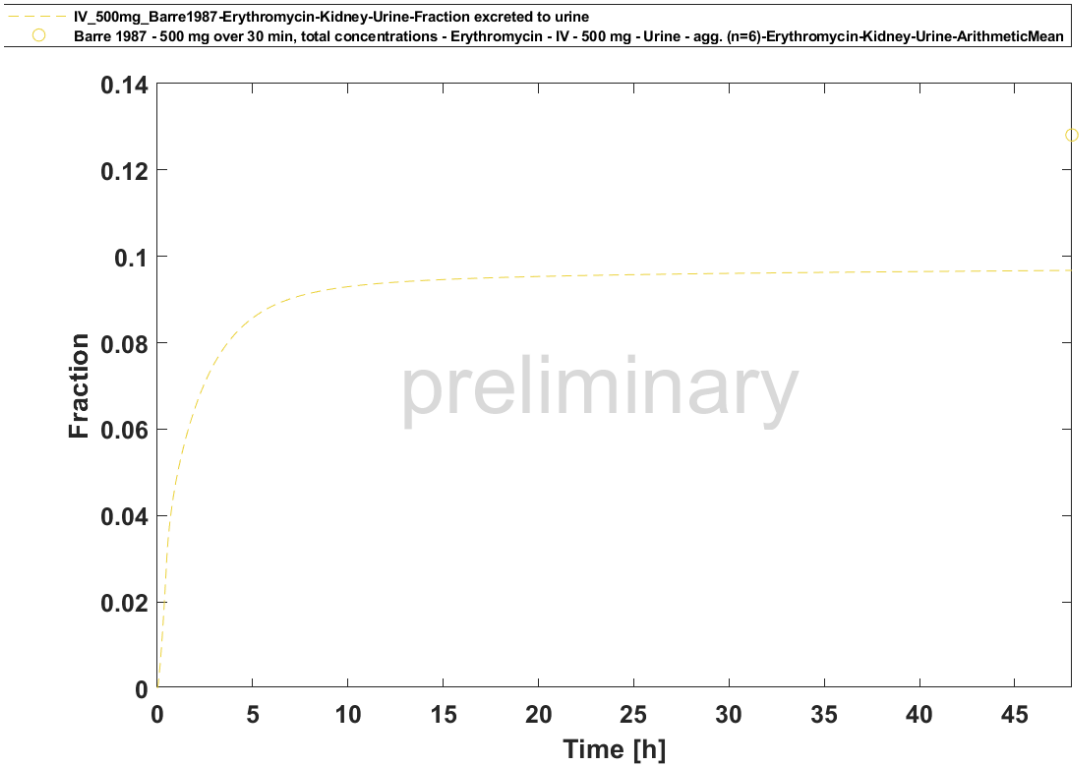
Time Profile Analysis



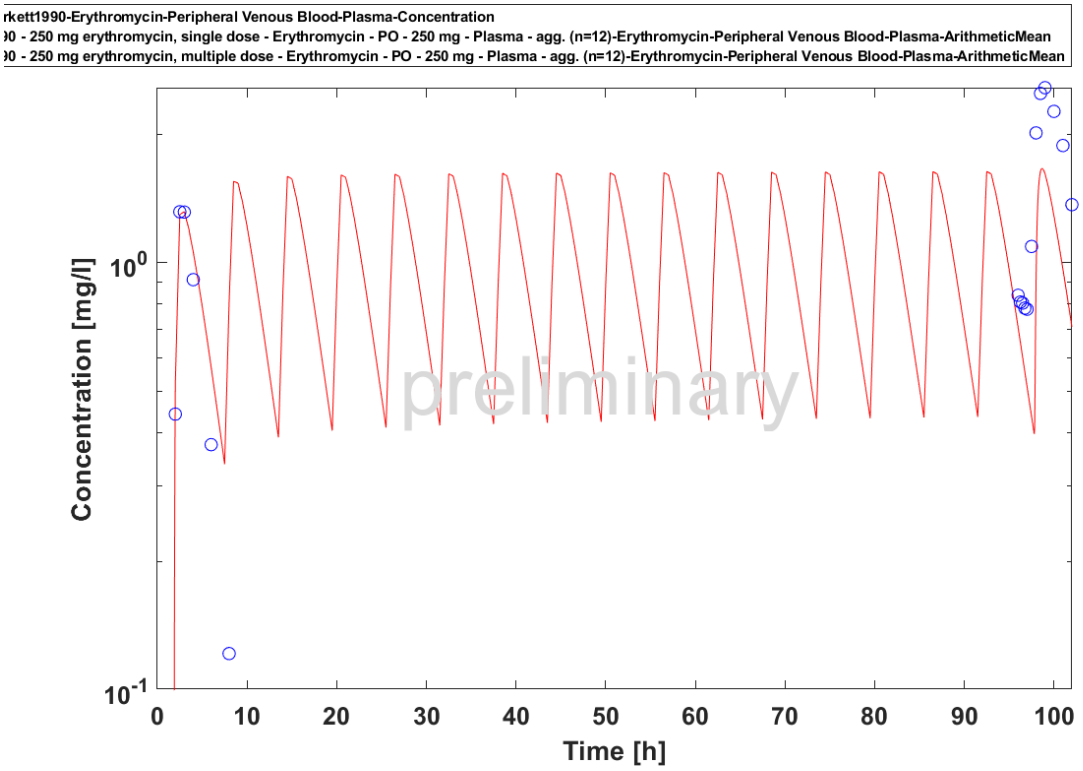
Time Profile Analysis 1



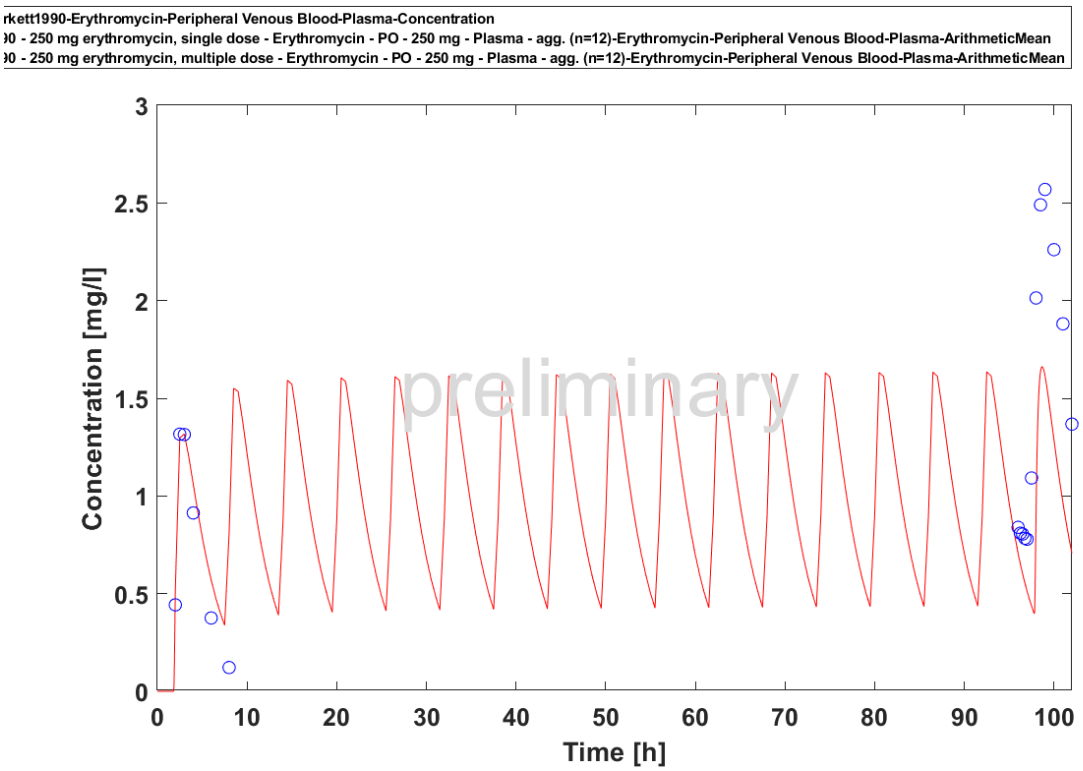
Time Profile Analysis



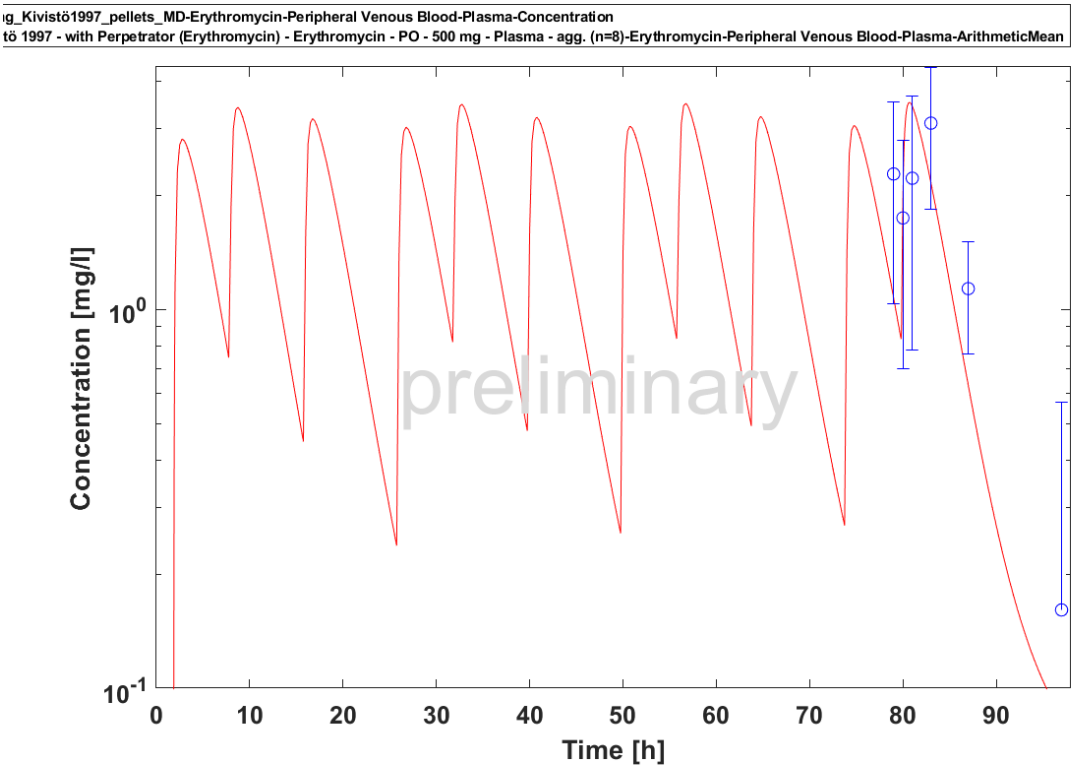
Time Profile Analysis 1



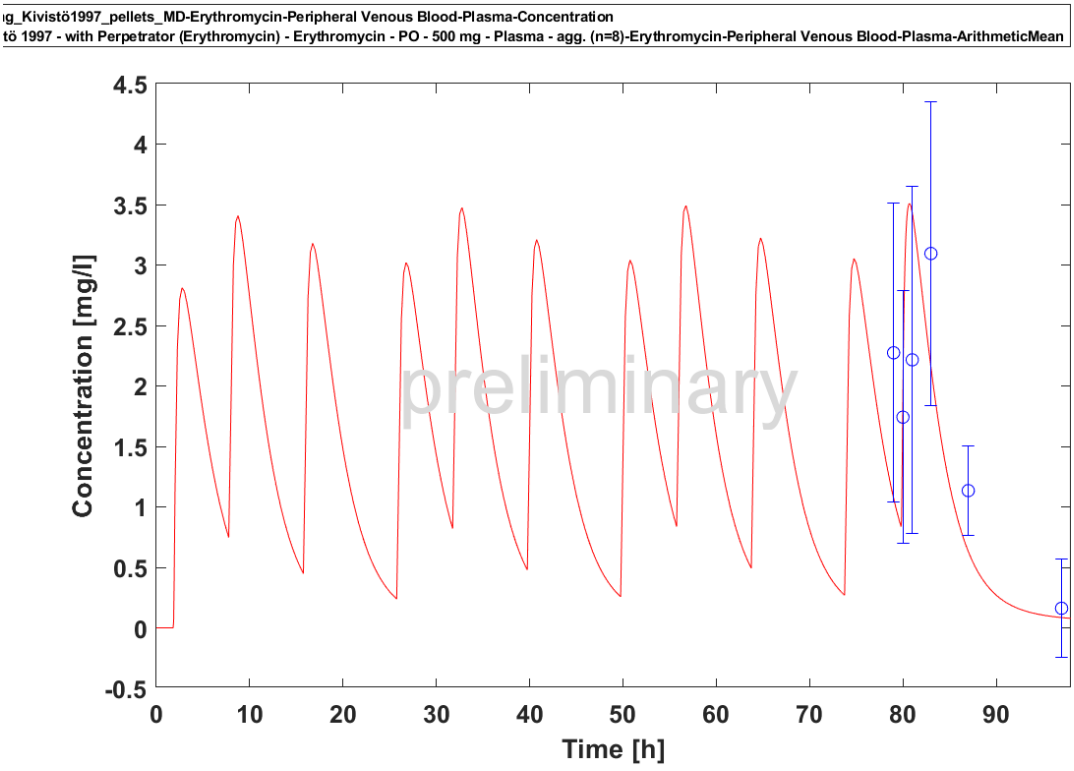
Time Profile Analysis



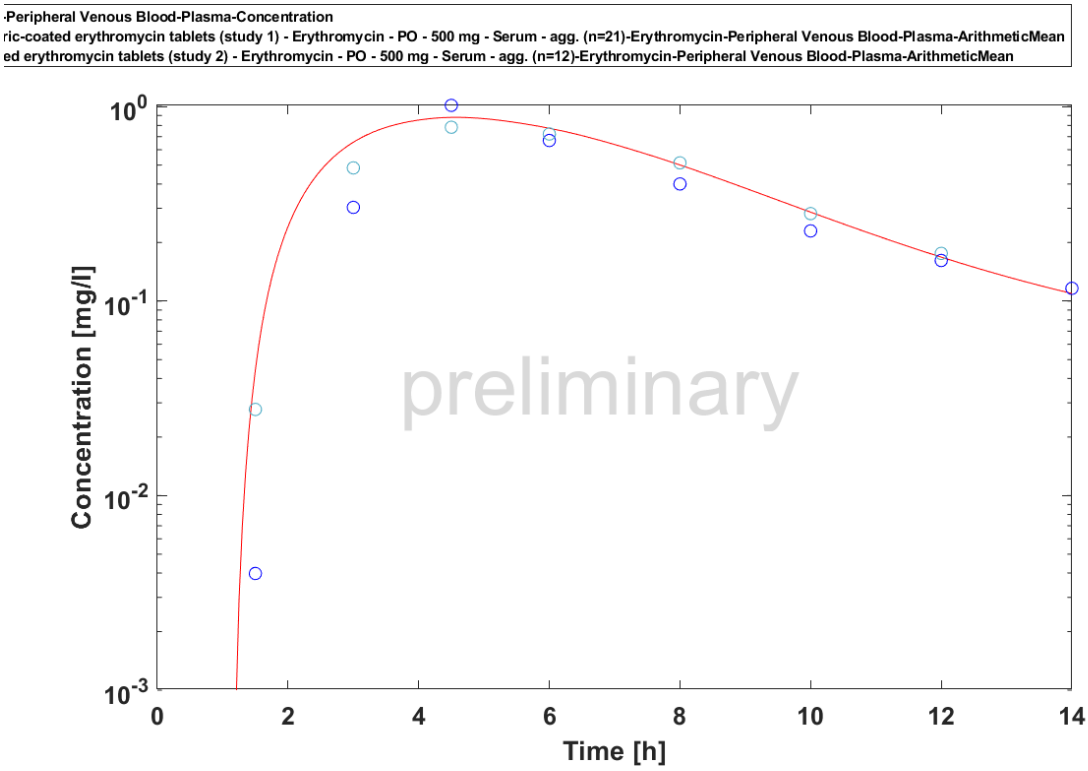
Time Profile Analysis 1



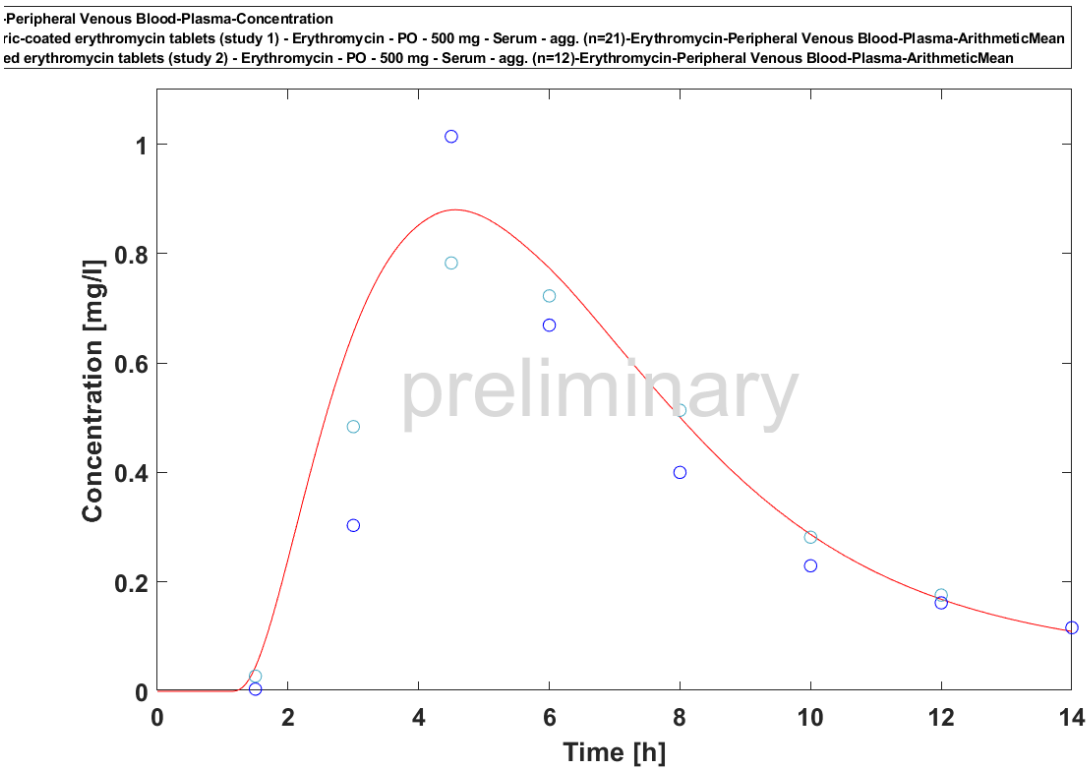
Time Profile Analysis



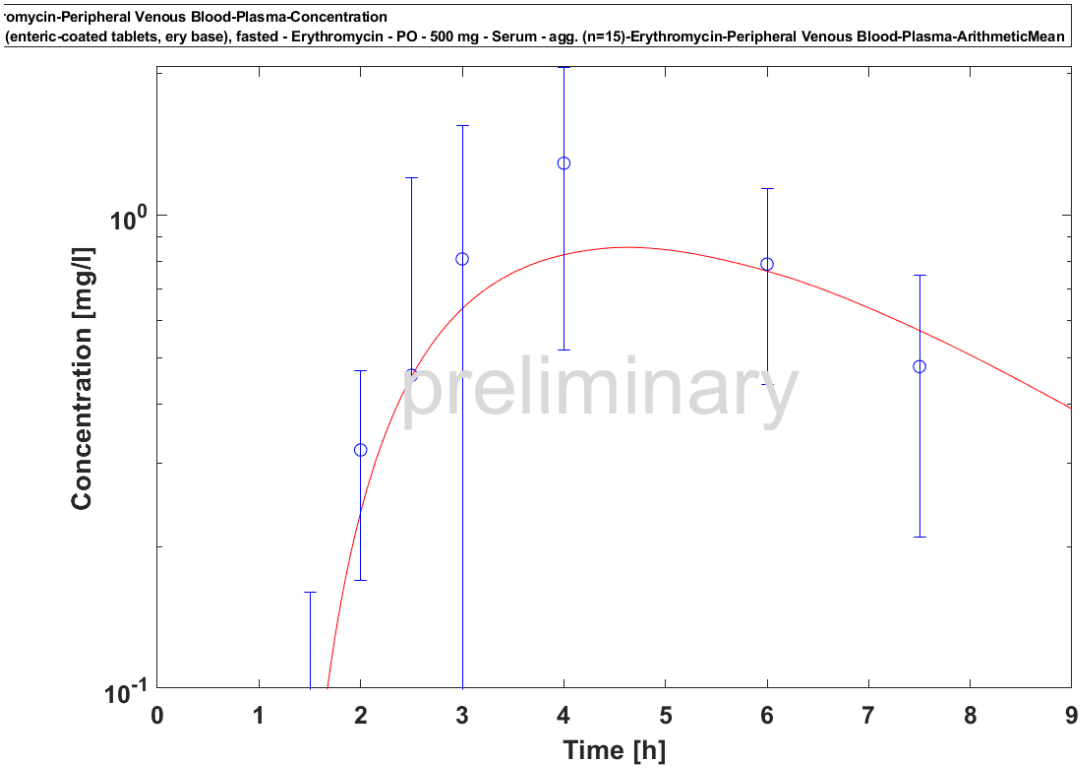
Time Profile Analysis 1



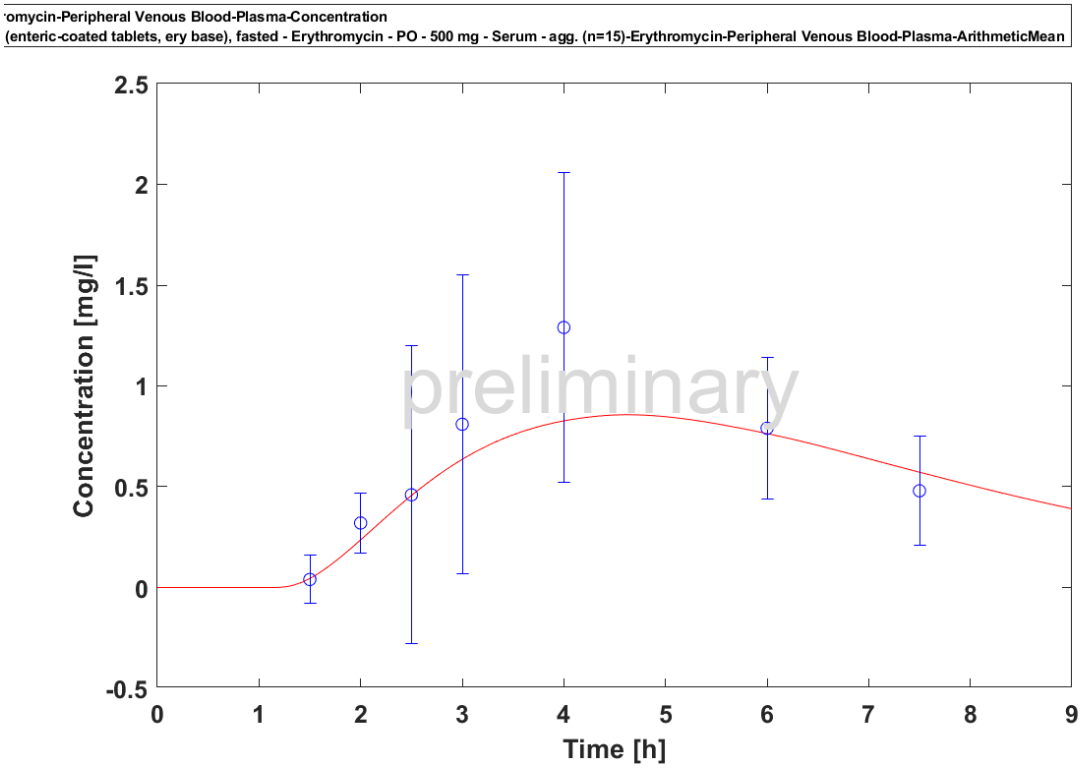
Time Profile Analysis



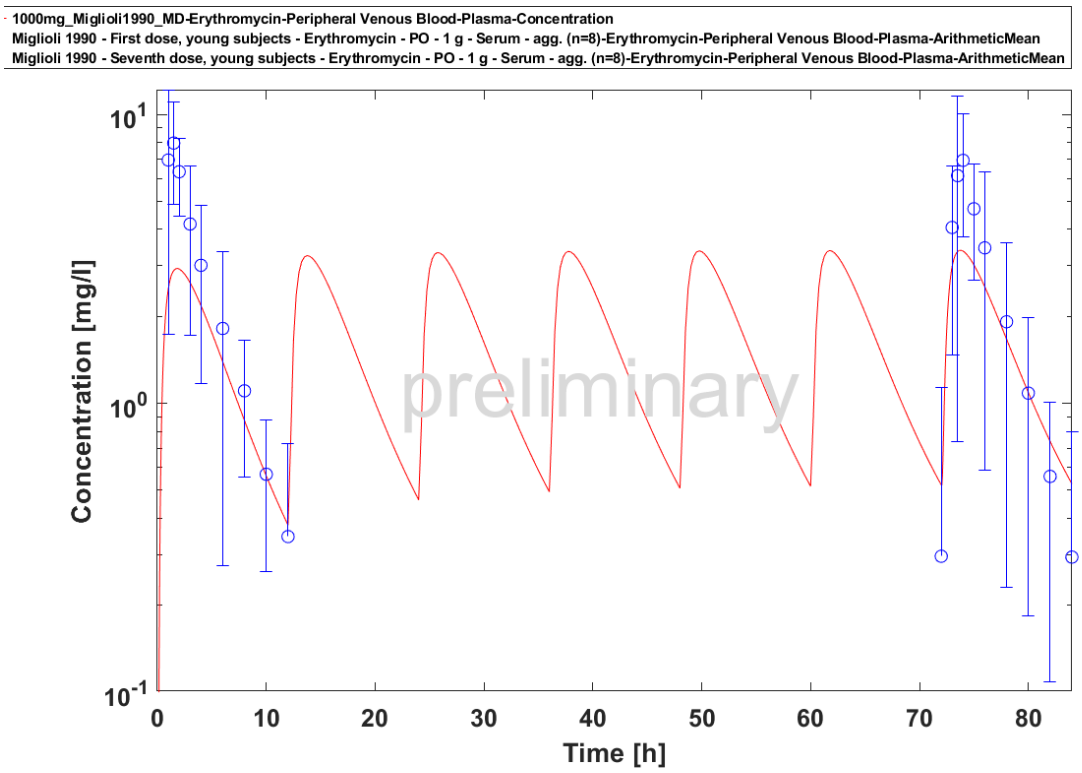
Time Profile Analysis 1



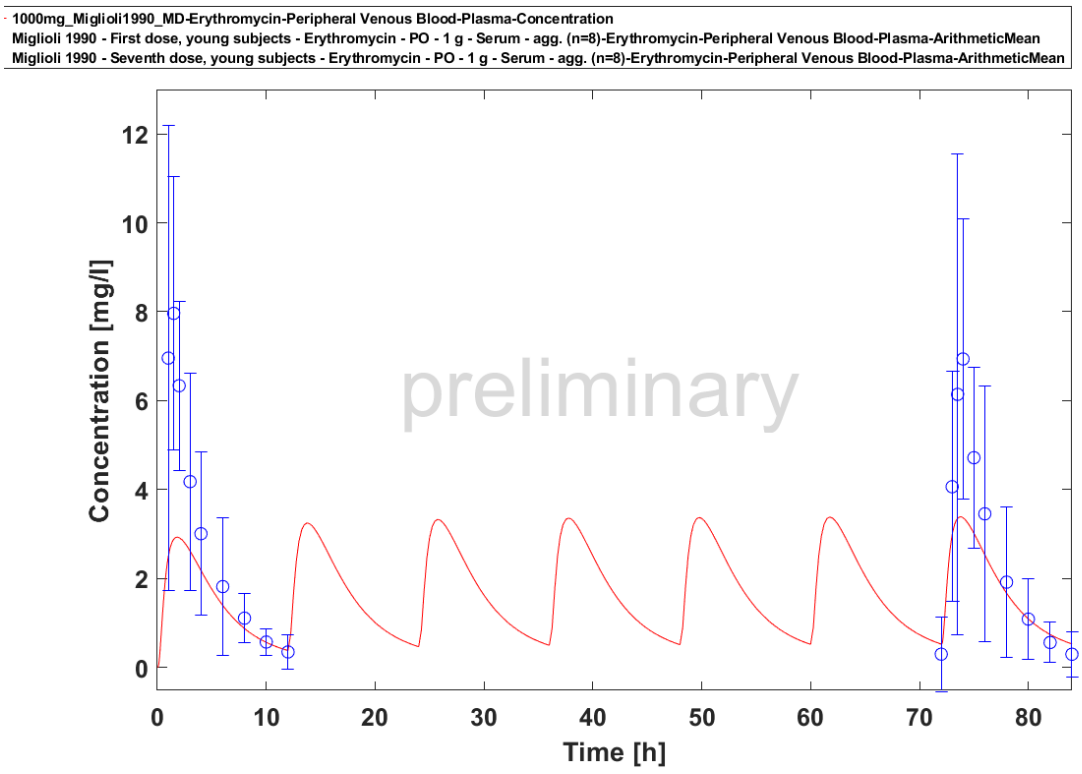
Time Profile Analysis



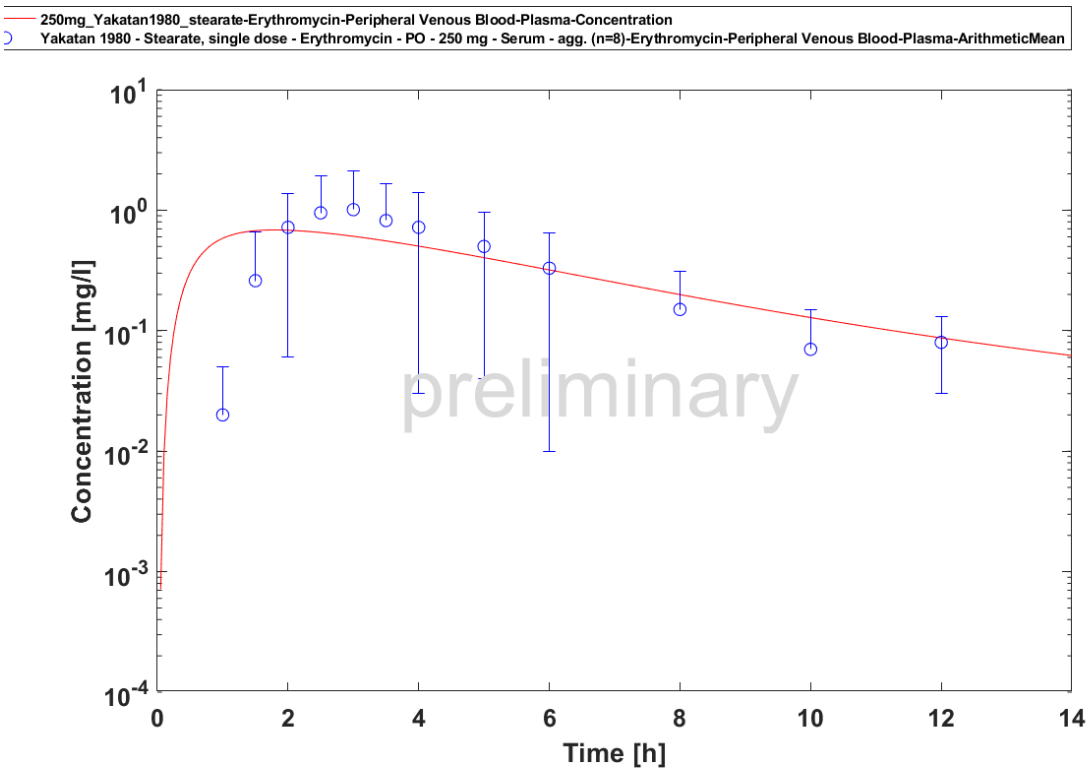
Time Profile Analysis 1



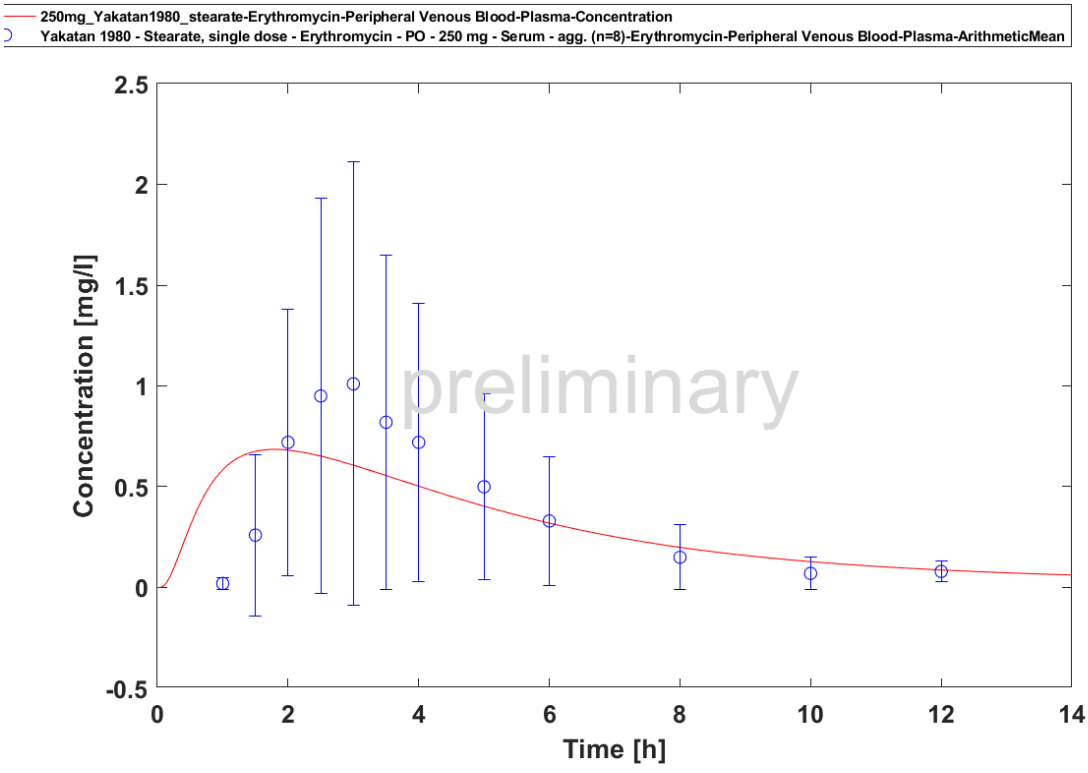
Time Profile Analysis



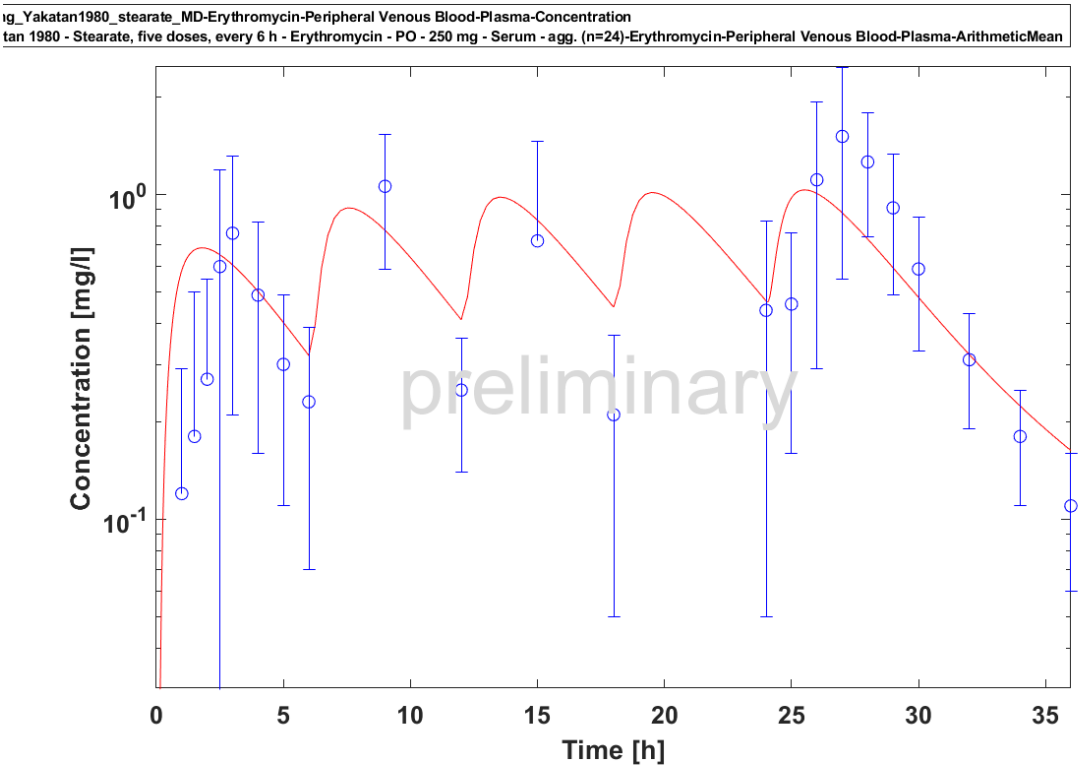
Time Profile Analysis 1



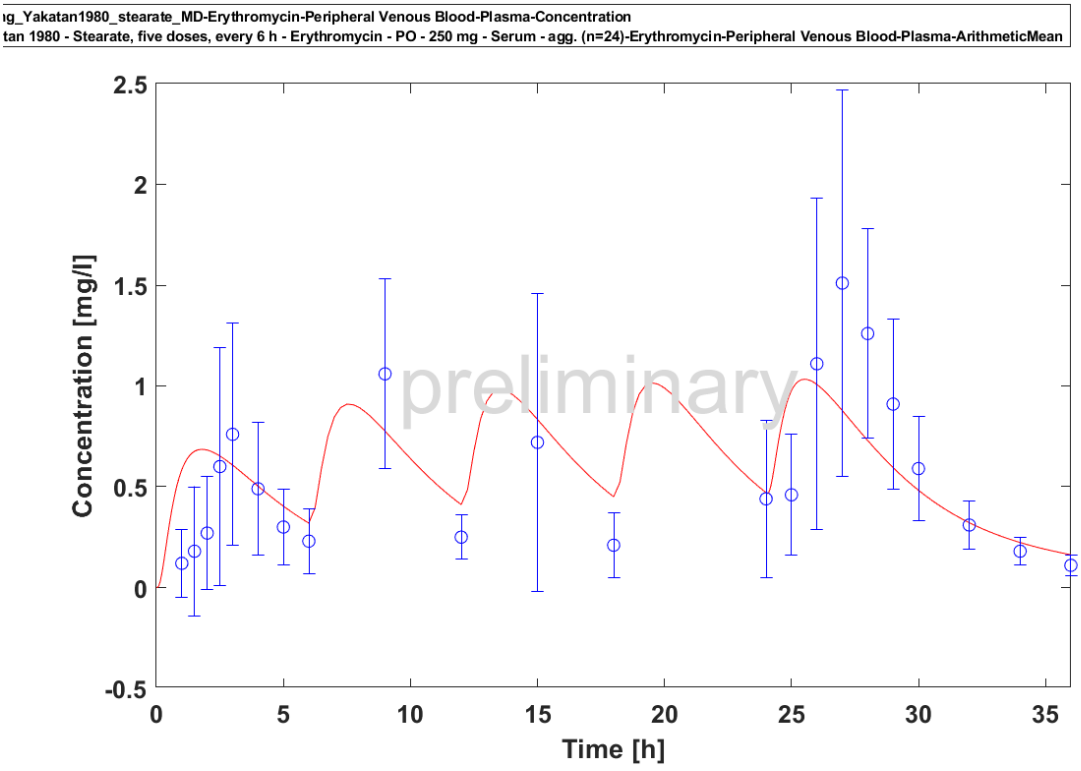
Time Profile Analysis



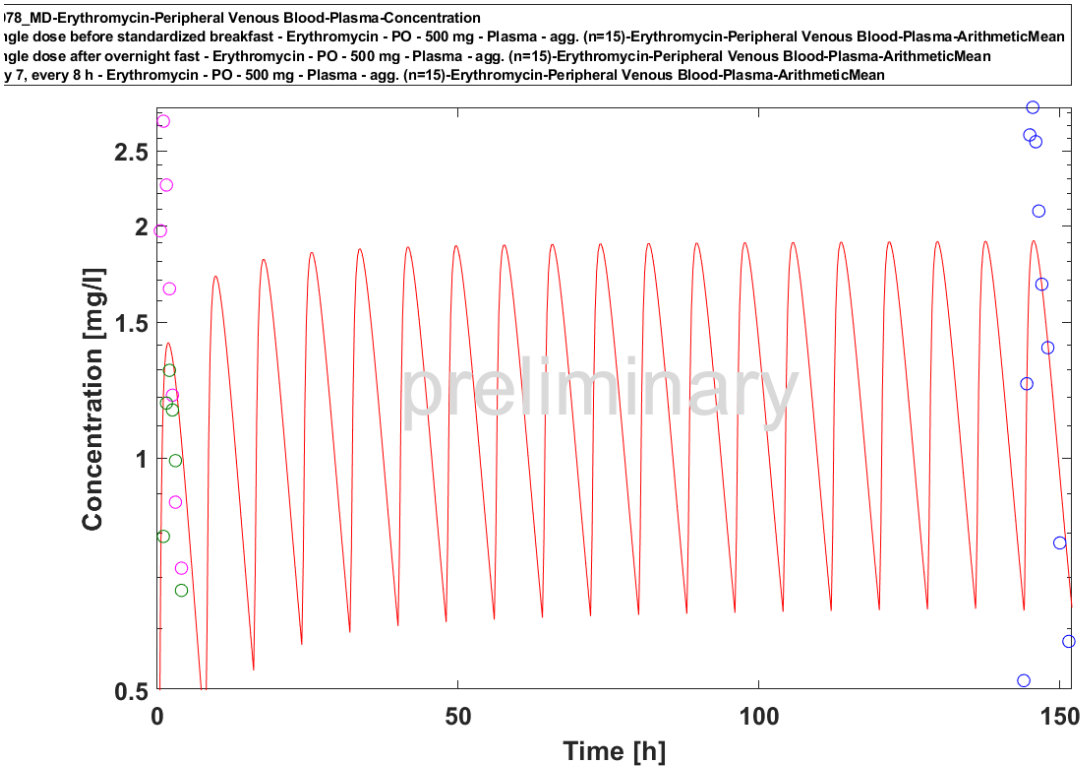
Time Profile Analysis 1



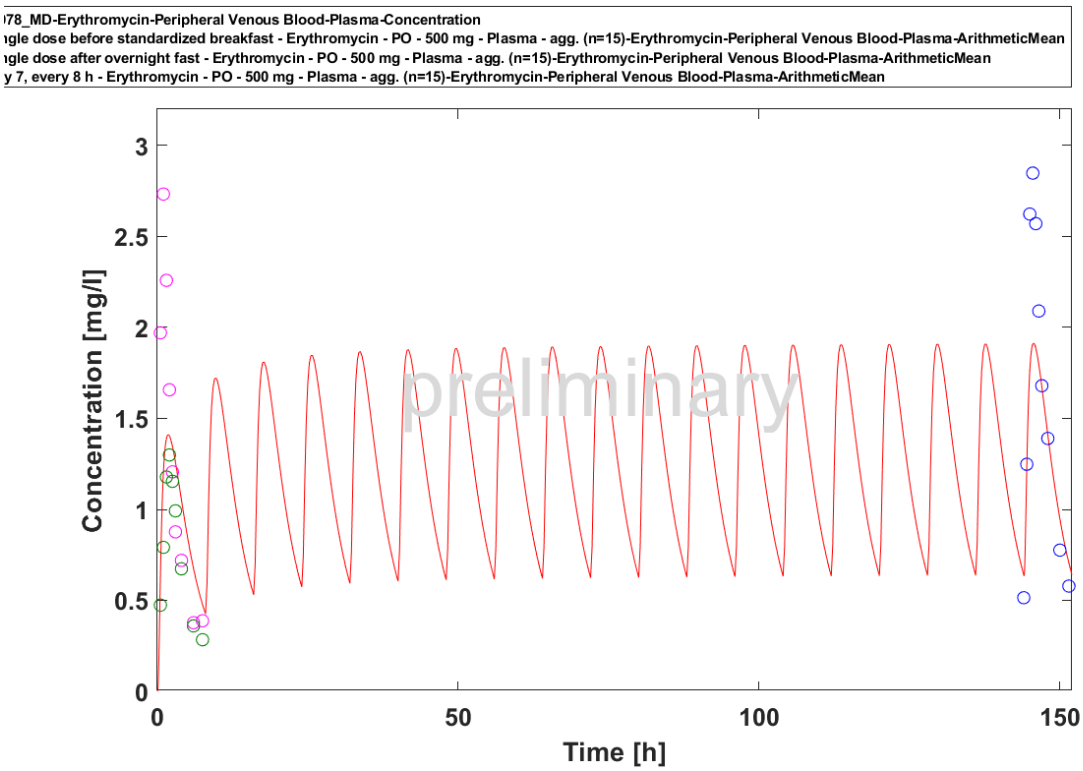
Time Profile Analysis



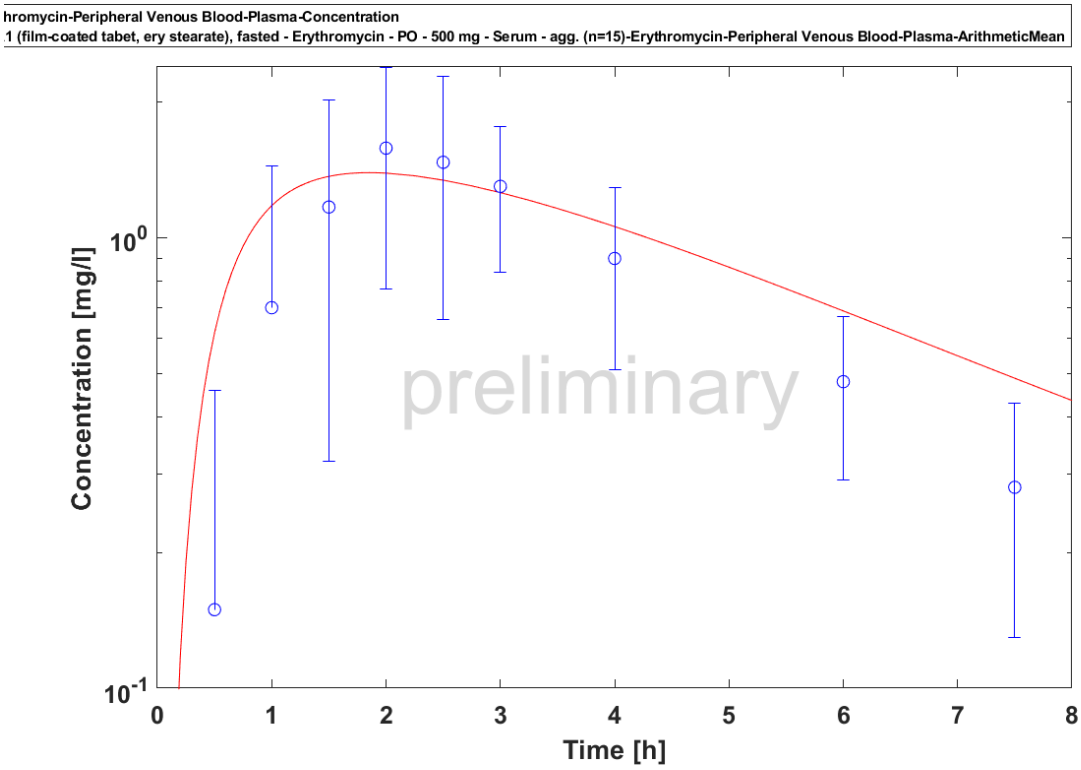
Time Profile Analysis 1



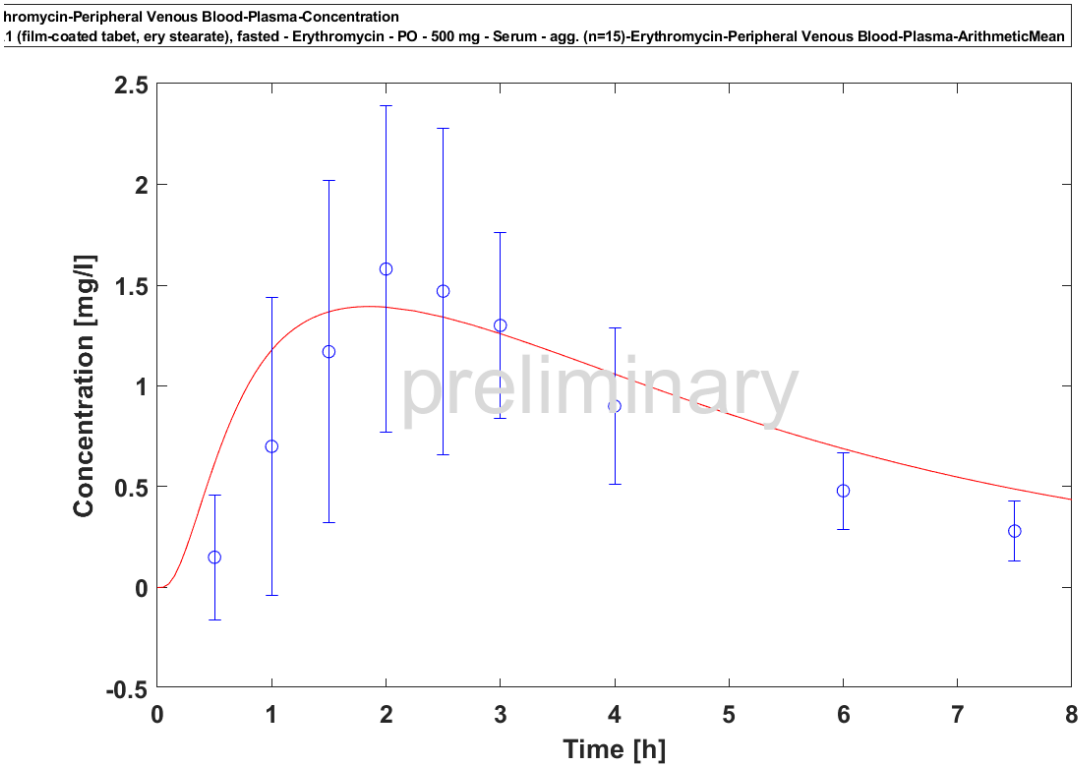
Time Profile Analysis



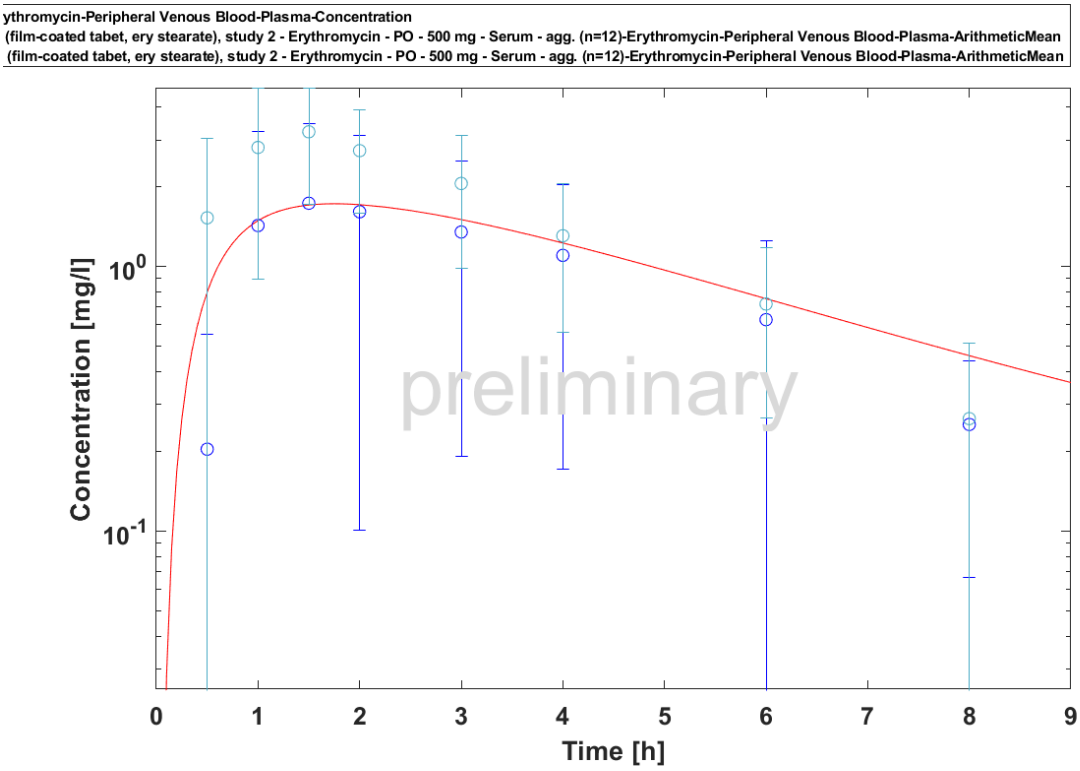
Time Profile Analysis 1



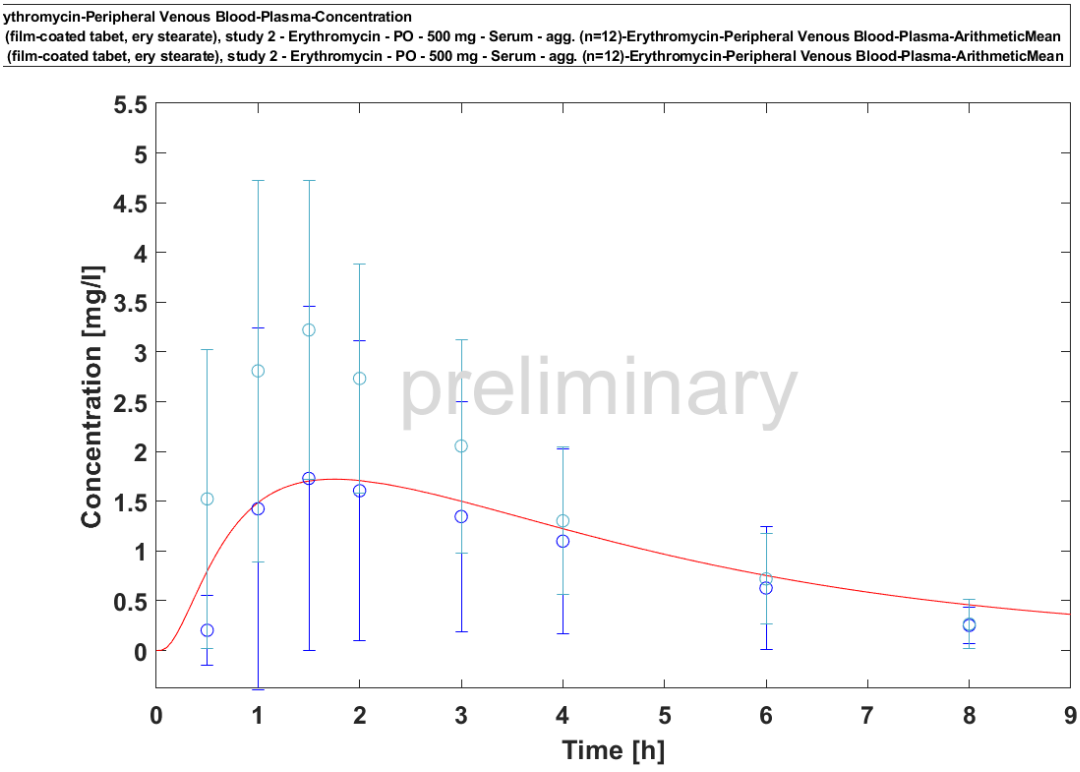
Time Profile Analysis



Time Profile Analysis 1



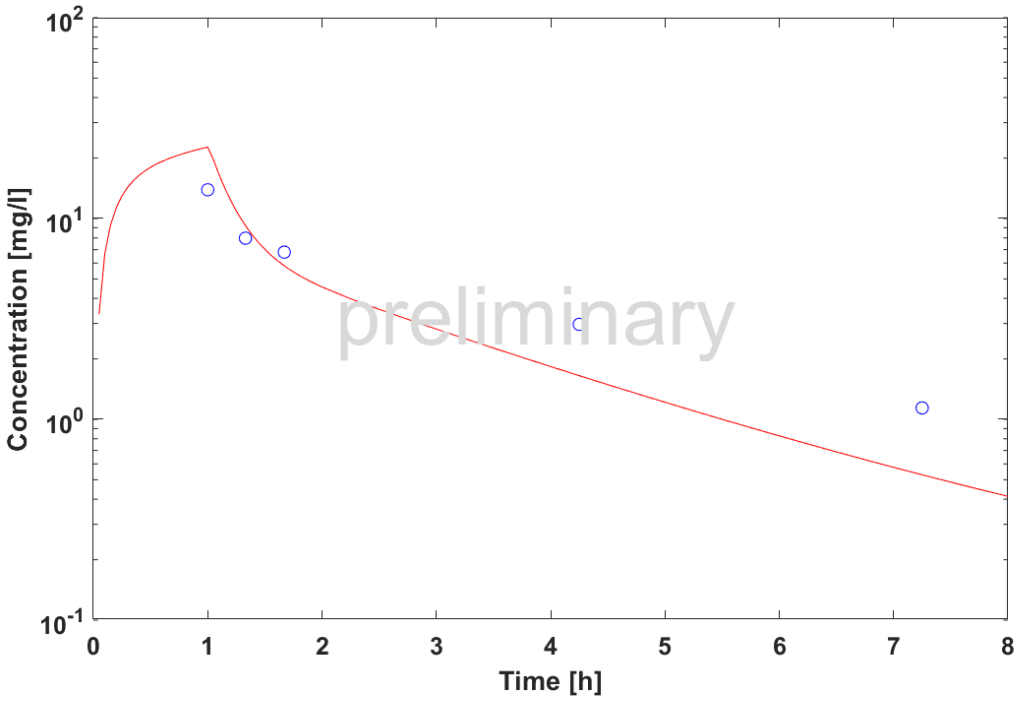
Time Profile Analysis



Time Profile Analysis 1

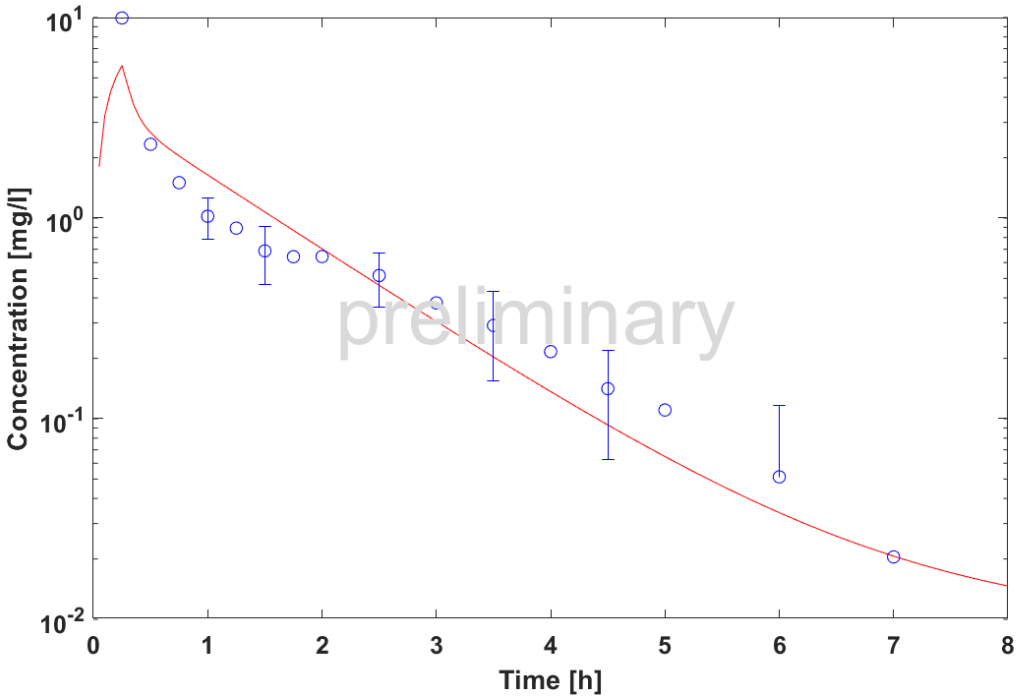
3.3.2 Model Verification

mg_Huppertz2011-Erythromycin-Peripheral Venous Blood-Plasma-Concentration
z 2011 - Treatment group, erythromycin - Erythromycin - IV - 1000 mg - Plasma - agg. (n=12)-Erythromycin-Peripheral Venous Blood-Plasma-GeometricMean

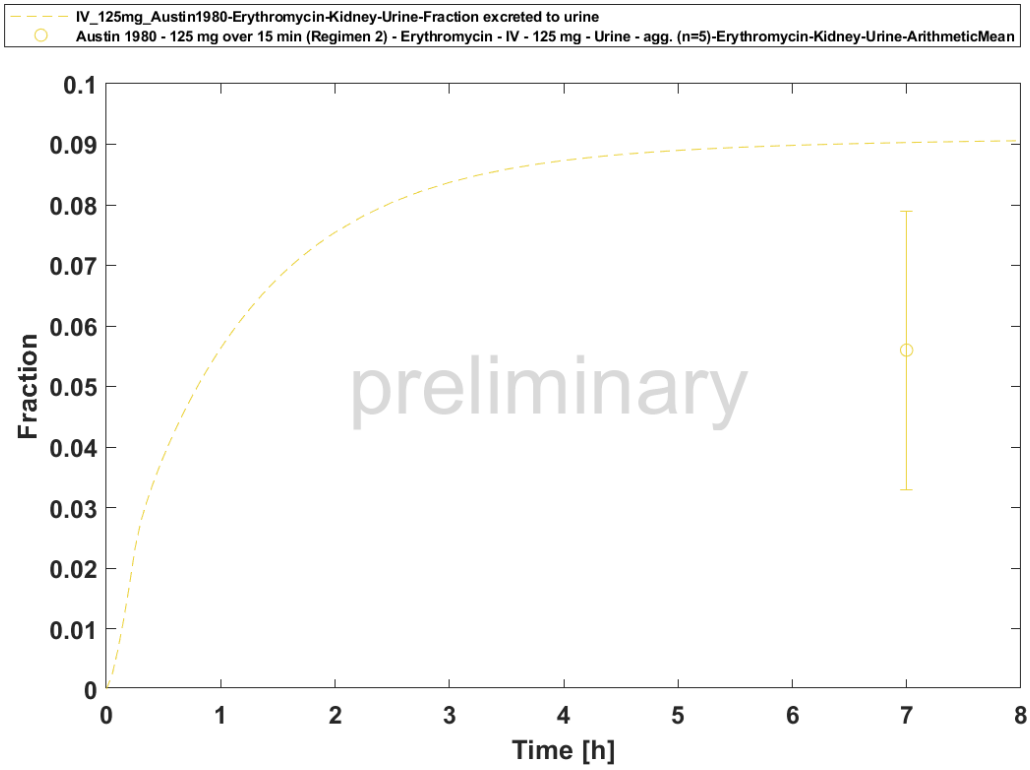


Time Profile Analysis

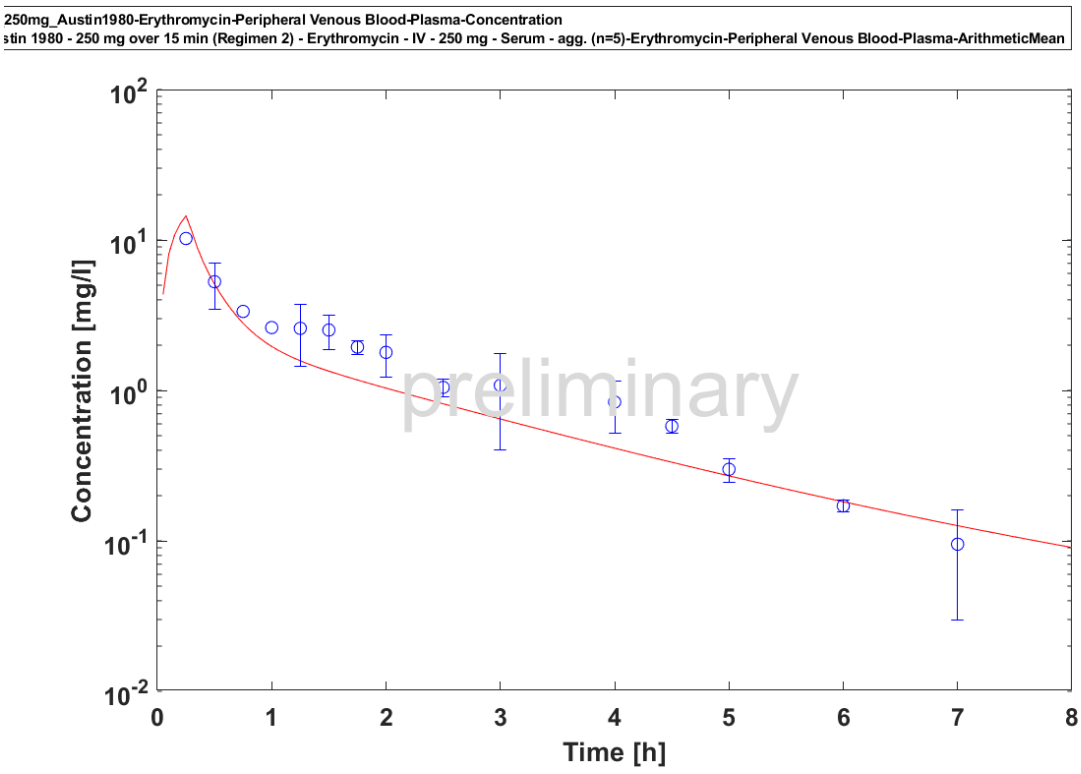
125mg_Austin1980-Erythromycin-Peripheral Venous Blood-Plasma-Concentration
stin 1980 - 125 mg over 15 min (Regimen 2) - Erythromycin - IV - 125 mg - Serum - agg. (n=5)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean



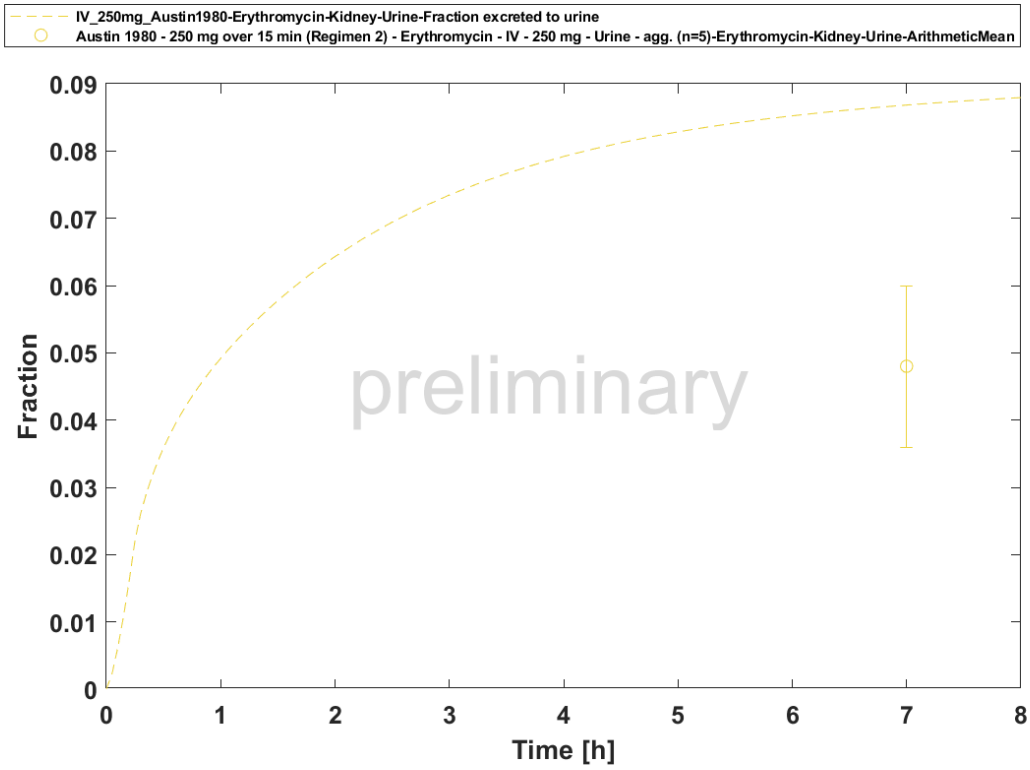
Time Profile Analysis



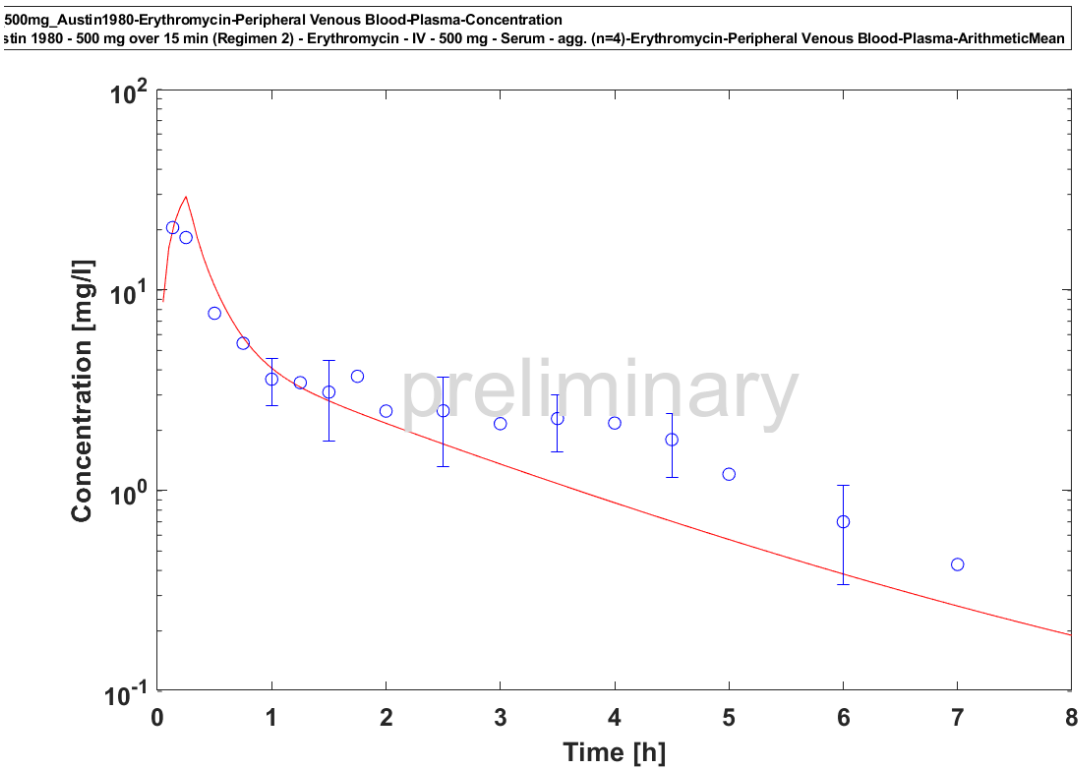
Time Profile Analysis 1



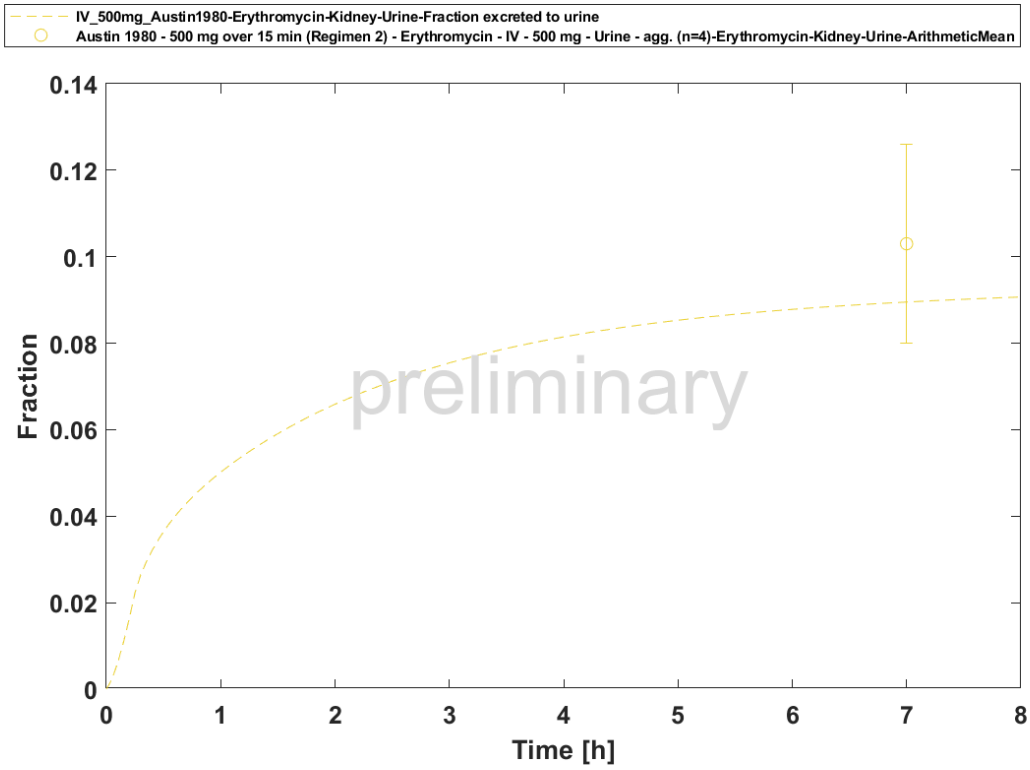
Time Profile Analysis



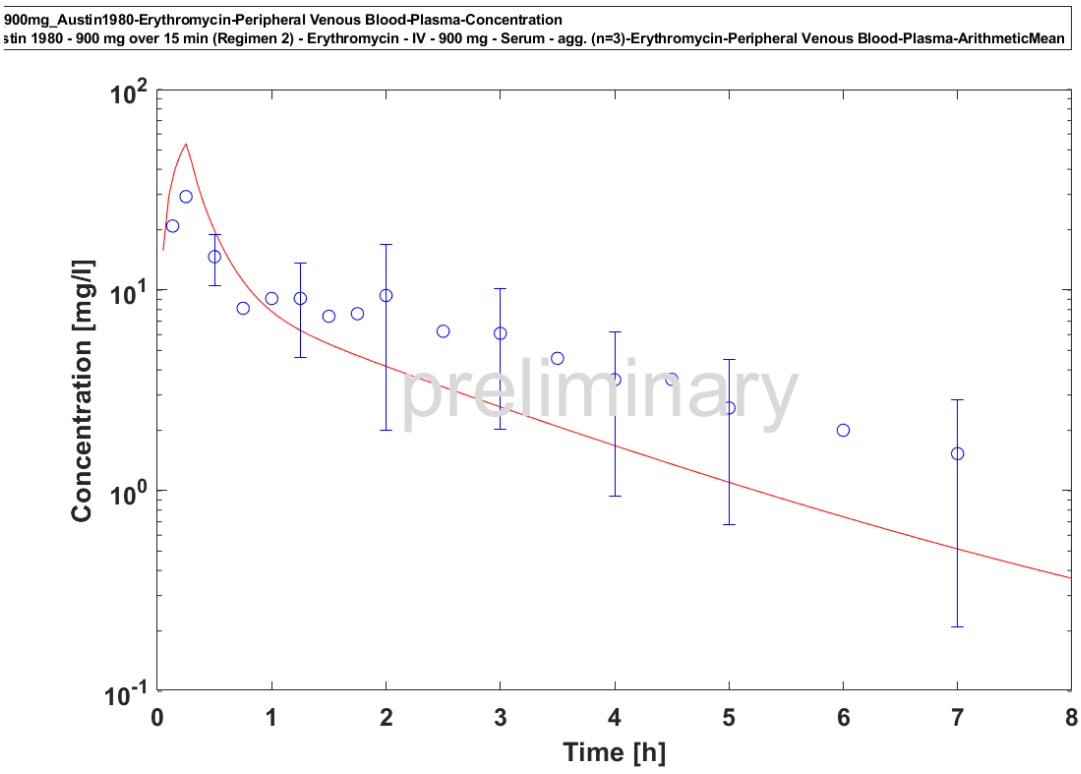
Time Profile Analysis 1



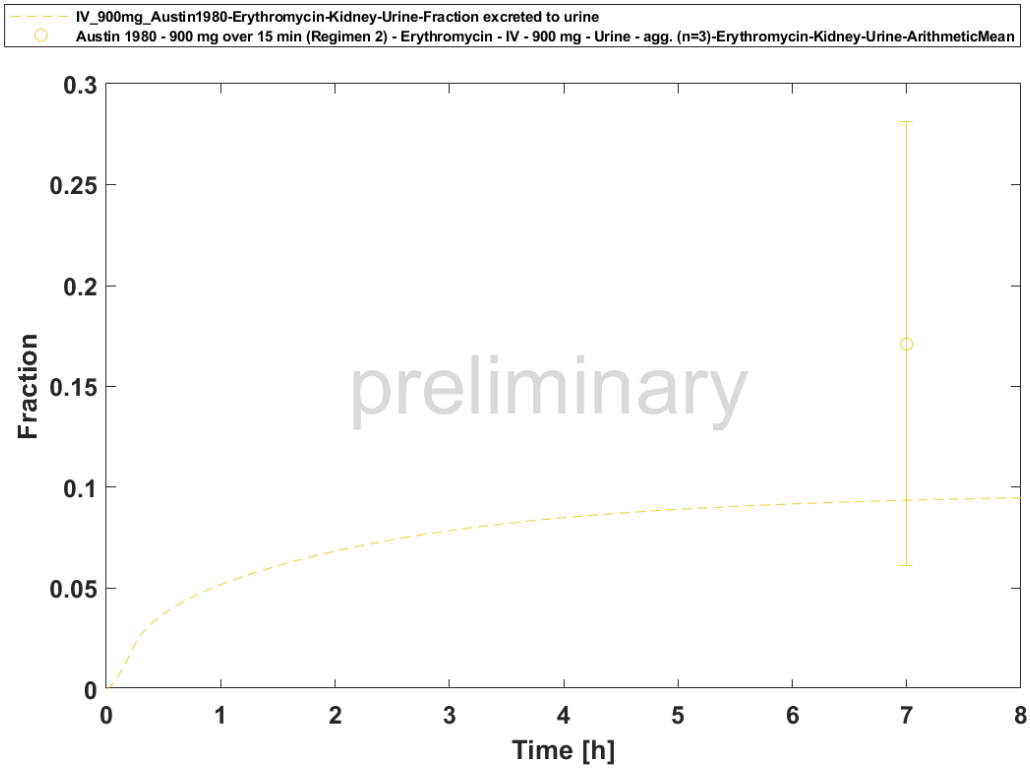
Time Profile Analysis



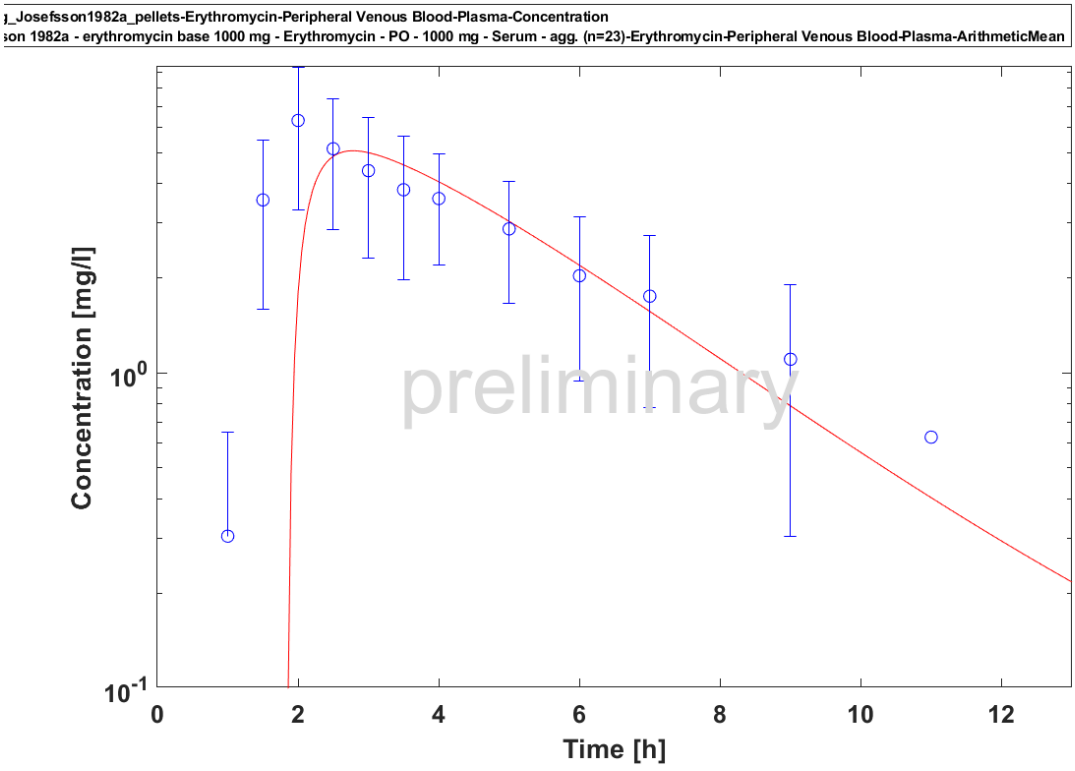
Time Profile Analysis 1



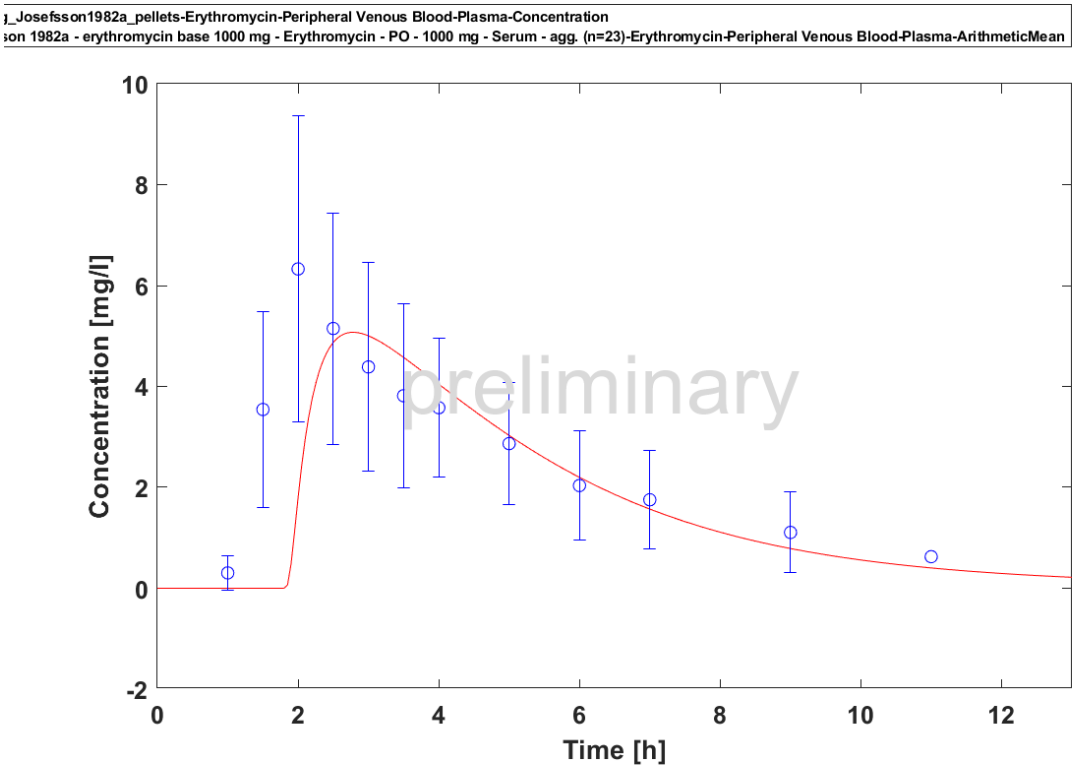
Time Profile Analysis



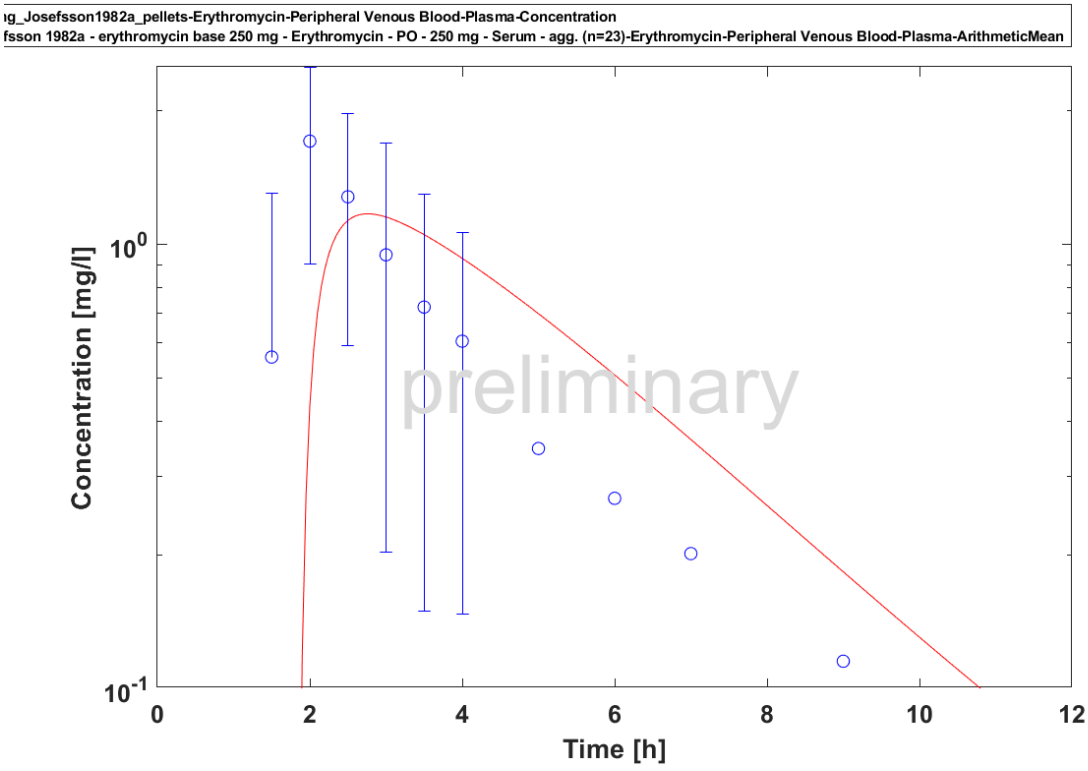
Time Profile Analysis 1



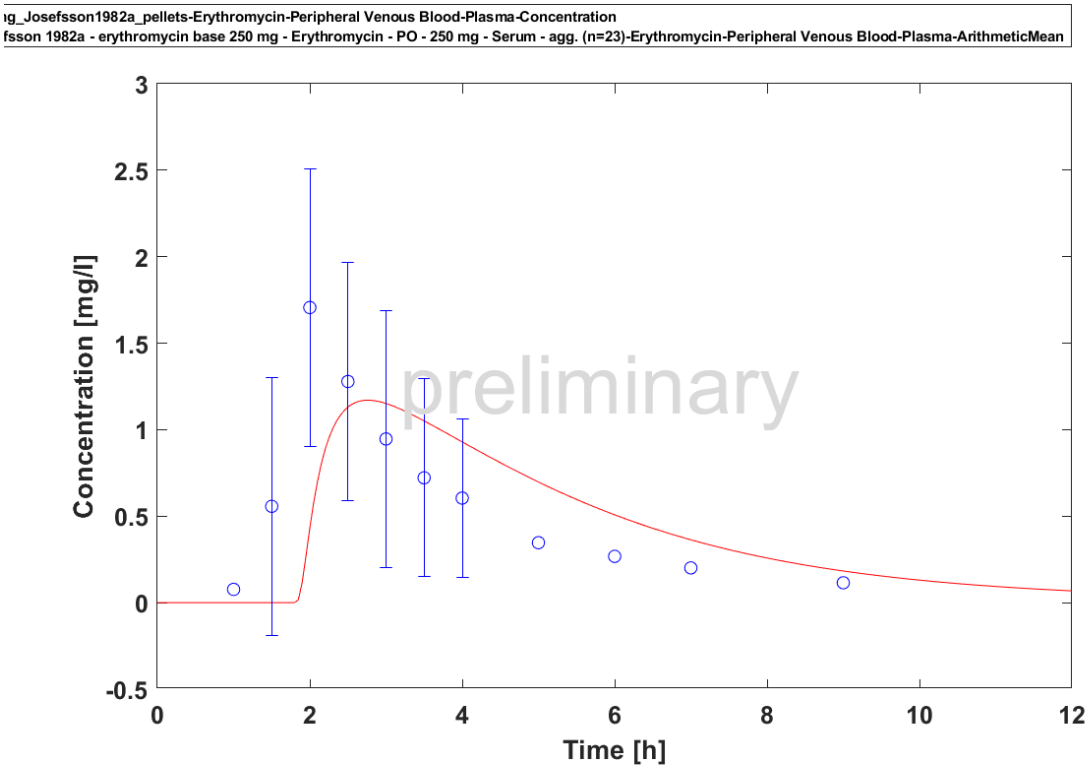
Time Profile Analysis



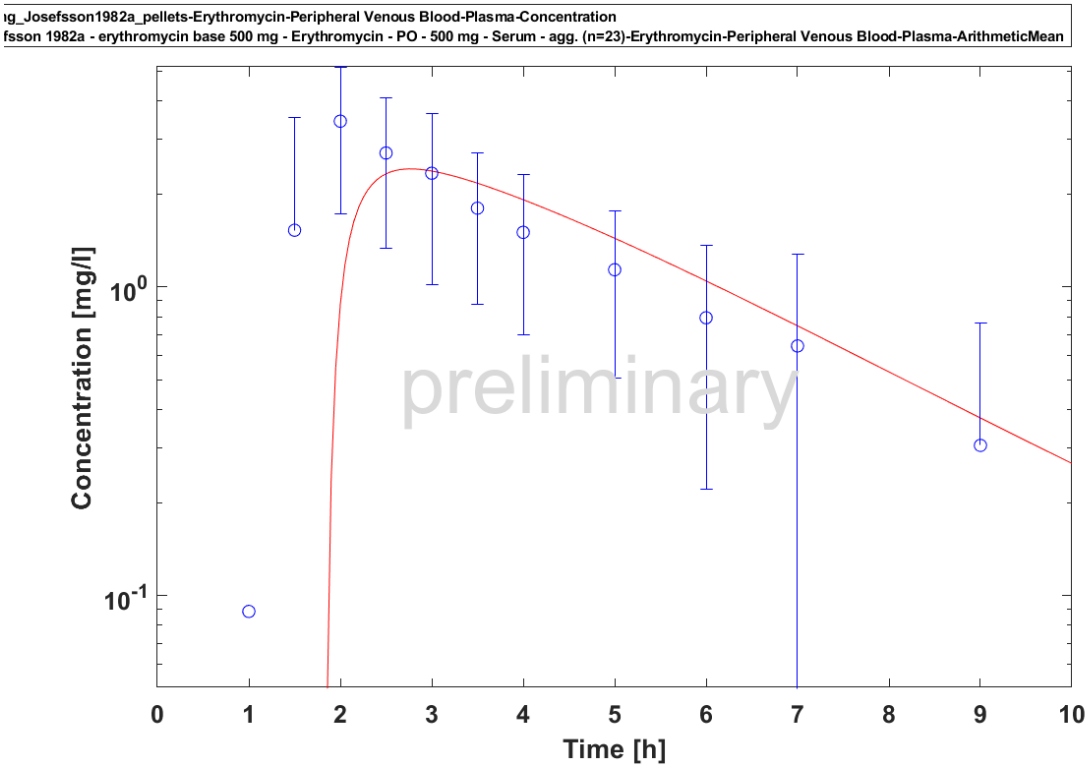
Time Profile Analysis 1



Time Profile Analysis

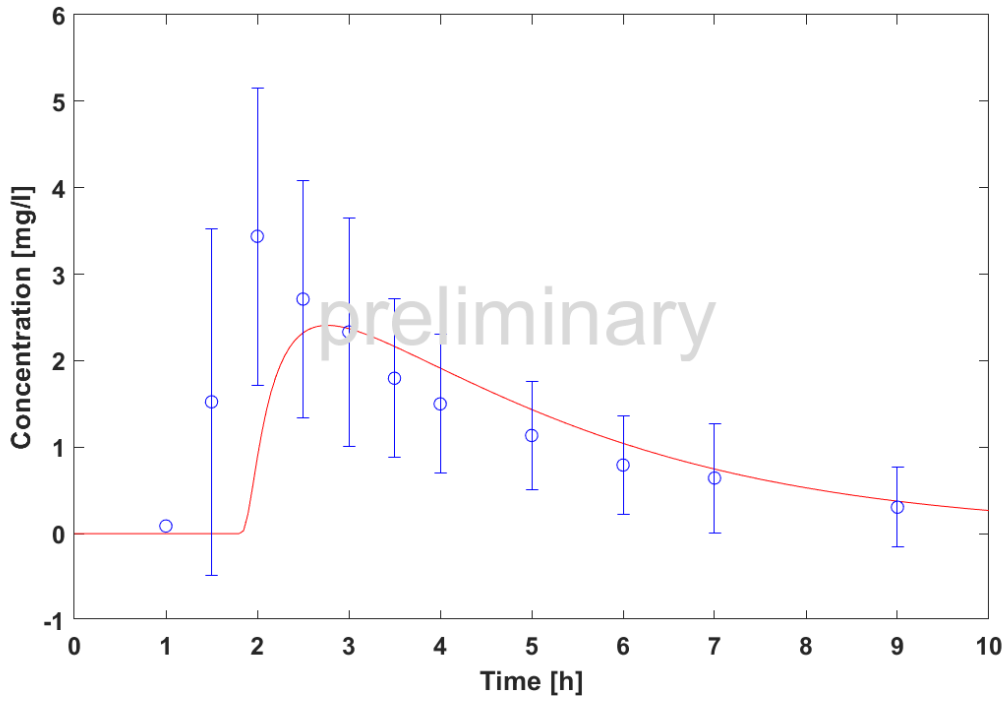


Time Profile Analysis 1



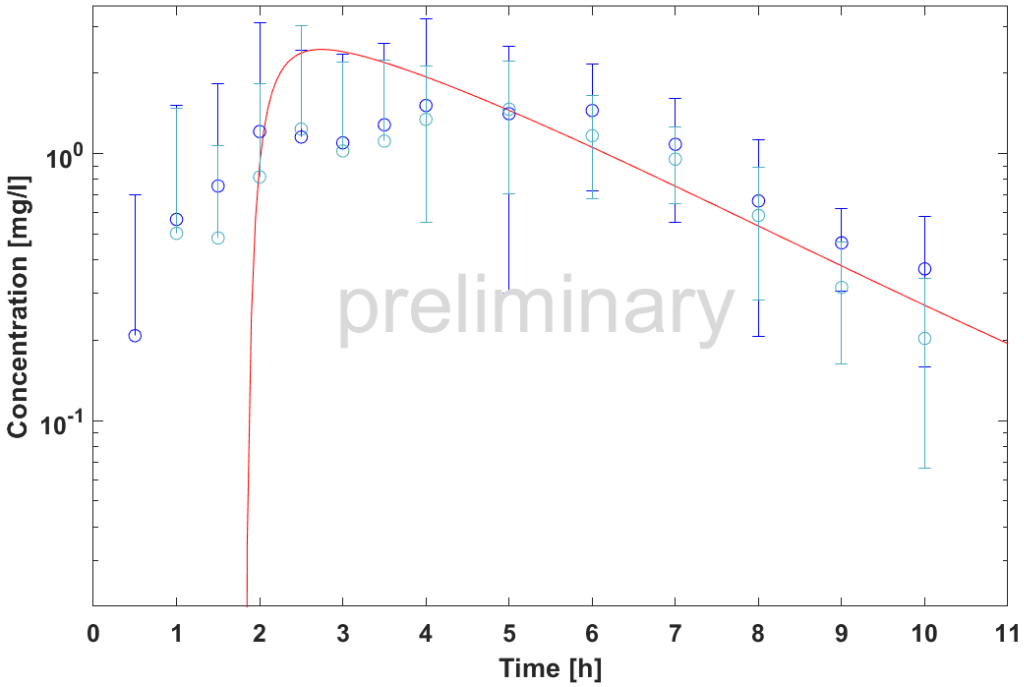
Time Profile Analysis

ıg_Josefsson1982a_pellets-Erythromycin-Peripheral Venous Blood-Plasma-Concentration
fsson 1982a - erythromycin base 500 mg - Erythromycin - PO - 500 mg - Serum - agg. (n=23)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean



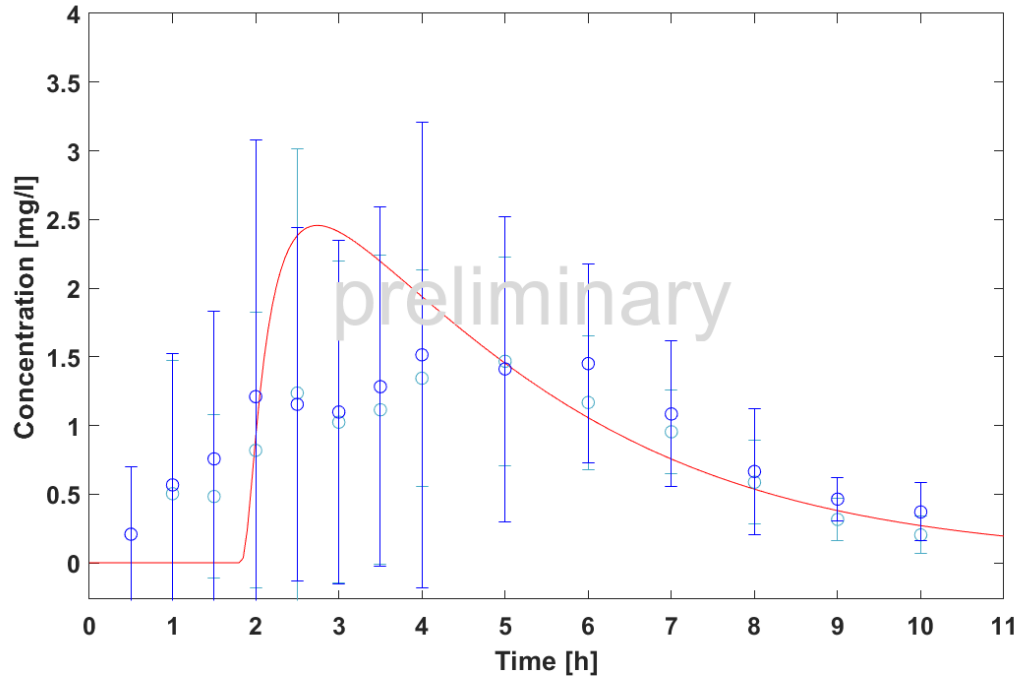
Time Profile Analysis 1

reiner1984_pellets-Erythromycin-Peripheral Venous Blood-Plasma-Concentration
984 - Part I, base (enteric-coated pellets) - Erythromycin - PO - 500 mg - Serum - agg. (n=14)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean
984 - Part II, base (enteric-coated pellets) - Erythromycin - PO - 500 mg - Serum - agg. (n=10)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean



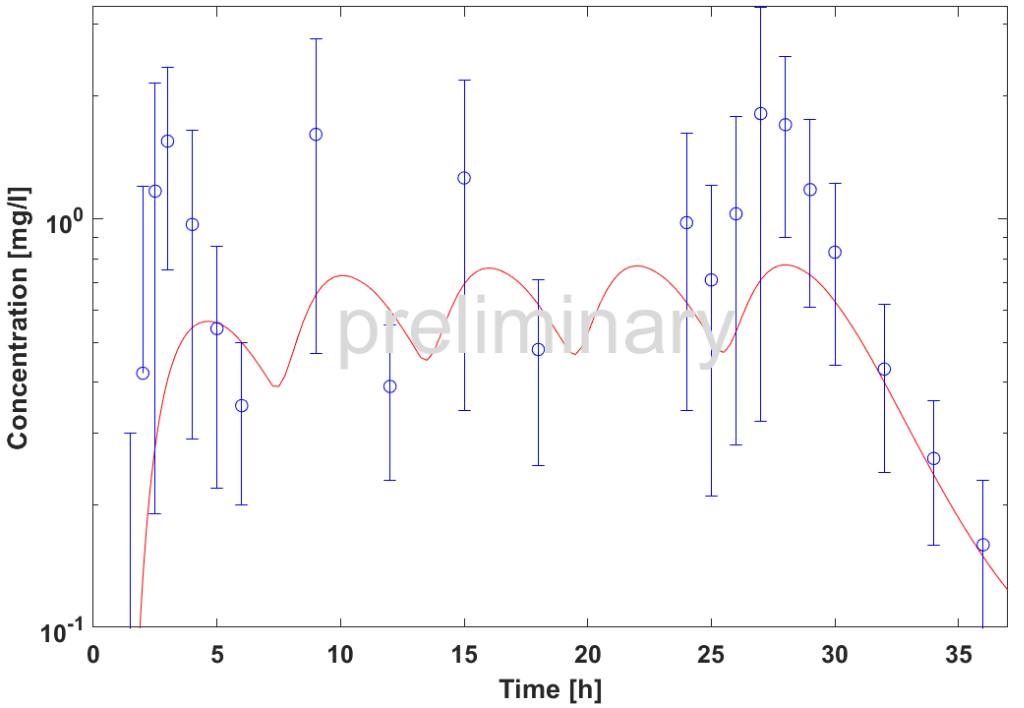
Time Profile Analysis

reiner1984_pellets-Erythromycin-Peripheral Venous Blood-Plasma-Concentration
984 - Part II, base (enteric-coated pellets) - Erythromycin - PO - 500 mg - Serum - agg. (n=10)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean
984 - Part I, base (enteric-coated pellets) - Erythromycin - PO - 500 mg - Serum - agg. (n=14)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean

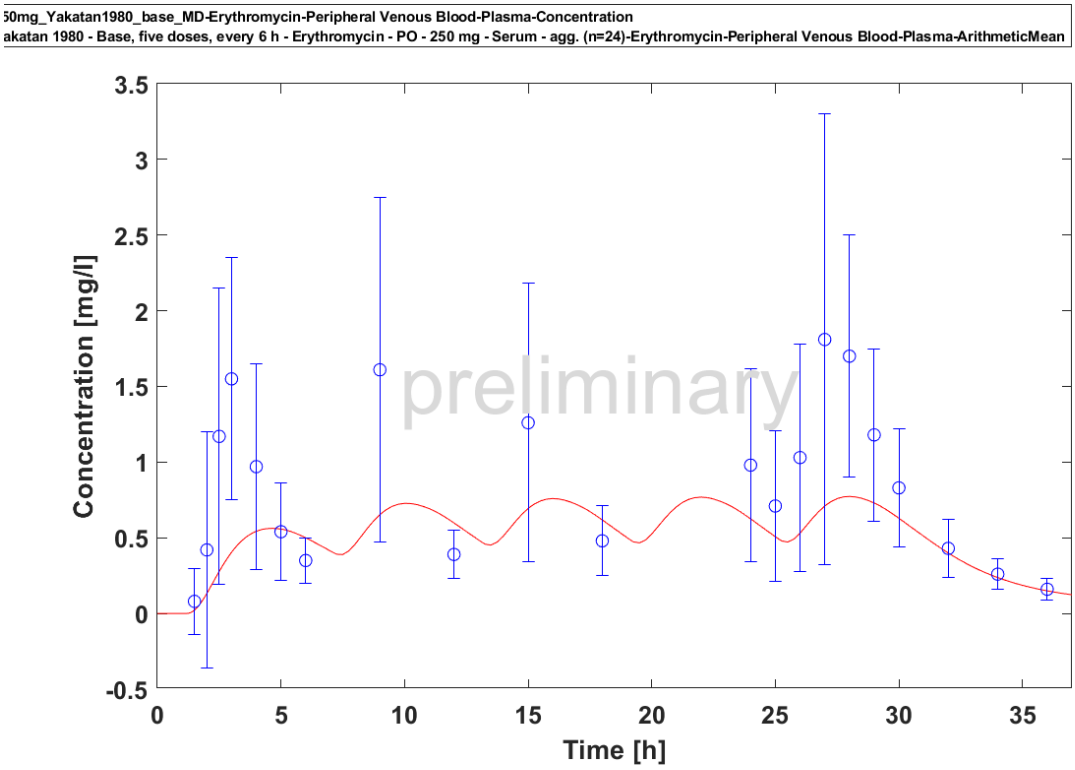


Time Profile Analysis 1

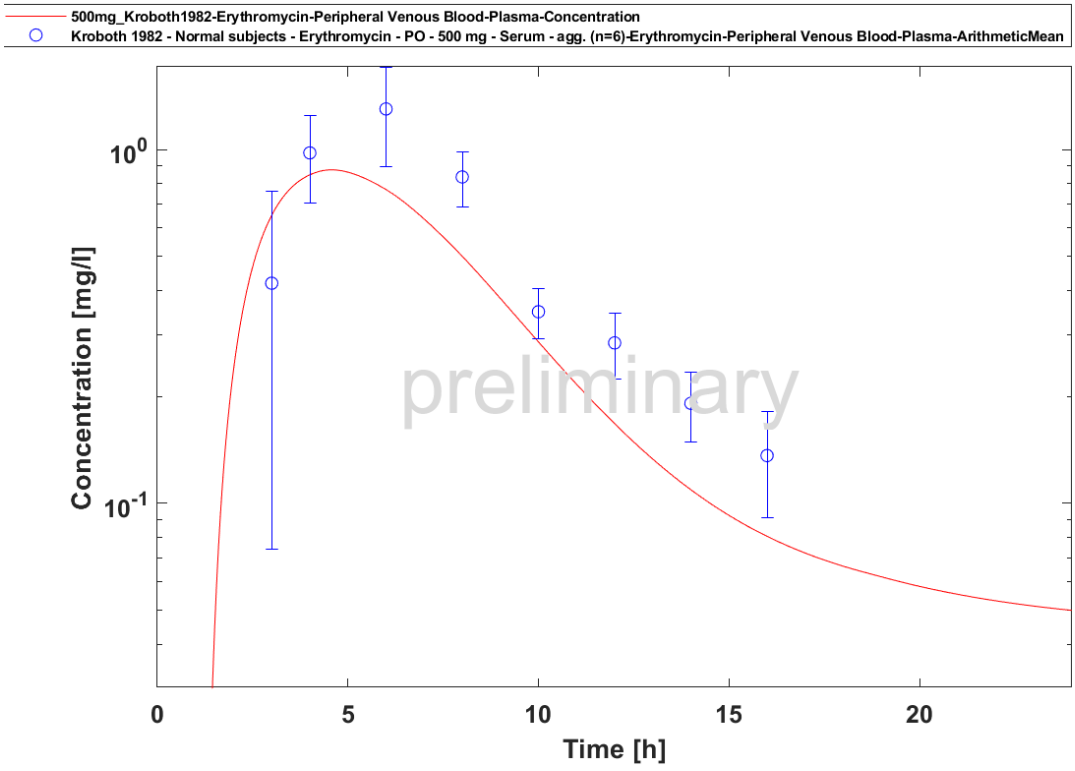
50mg_Yakatan1980_base_MD-Erythromycin-Peripheral Venous Blood-Plasma-Concentration
akatan 1980 - Base, five doses, every 6 h - Erythromycin - PO - 250 mg - Serum - agg. (n=24)-Erythromycin-Peripheral Venous Blood-Plasma-ArithmeticMean



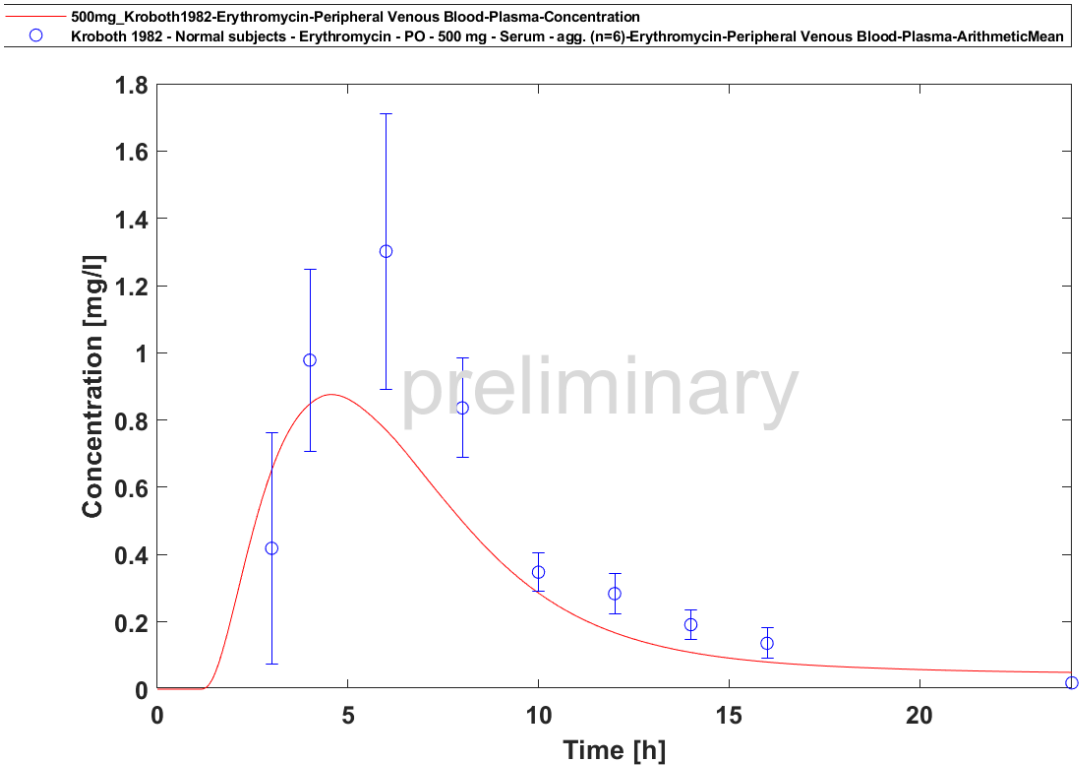
Time Profile Analysis



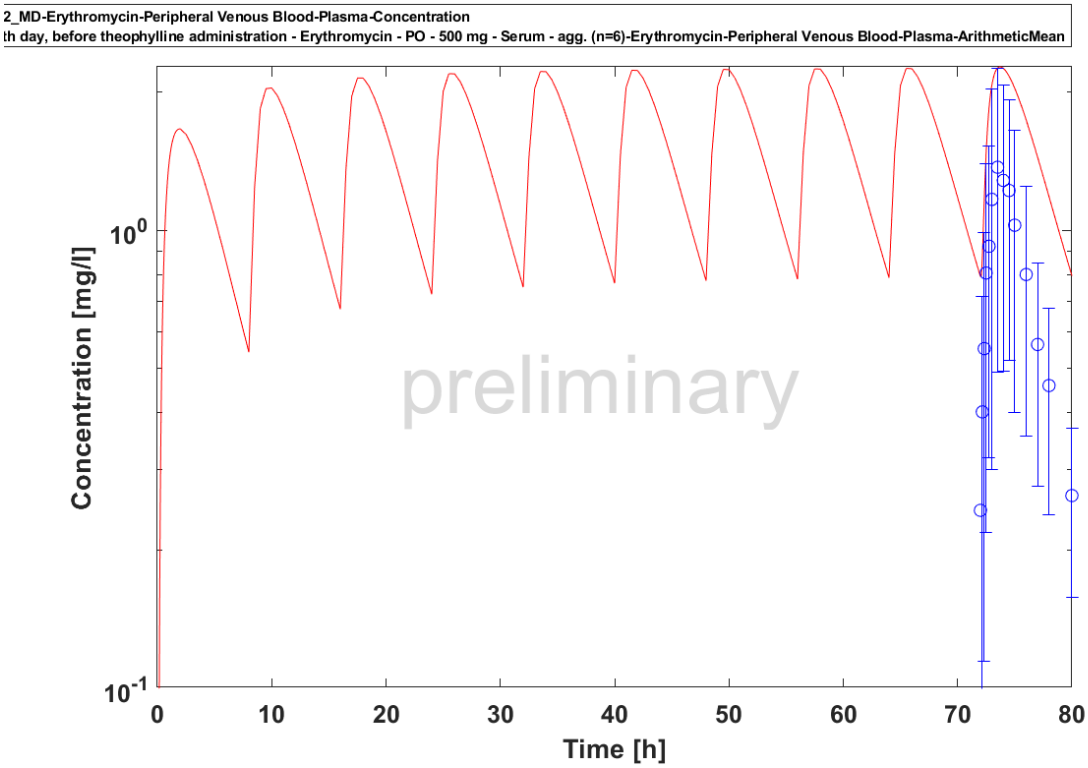
Time Profile Analysis 1



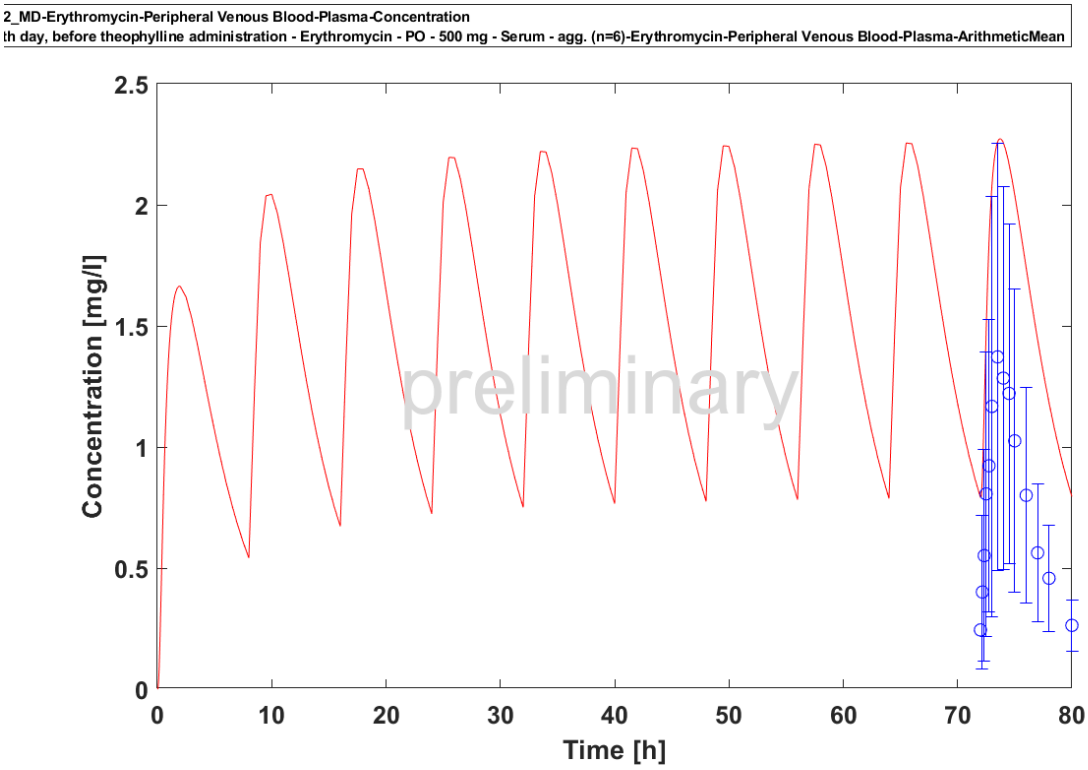
Time Profile Analysis



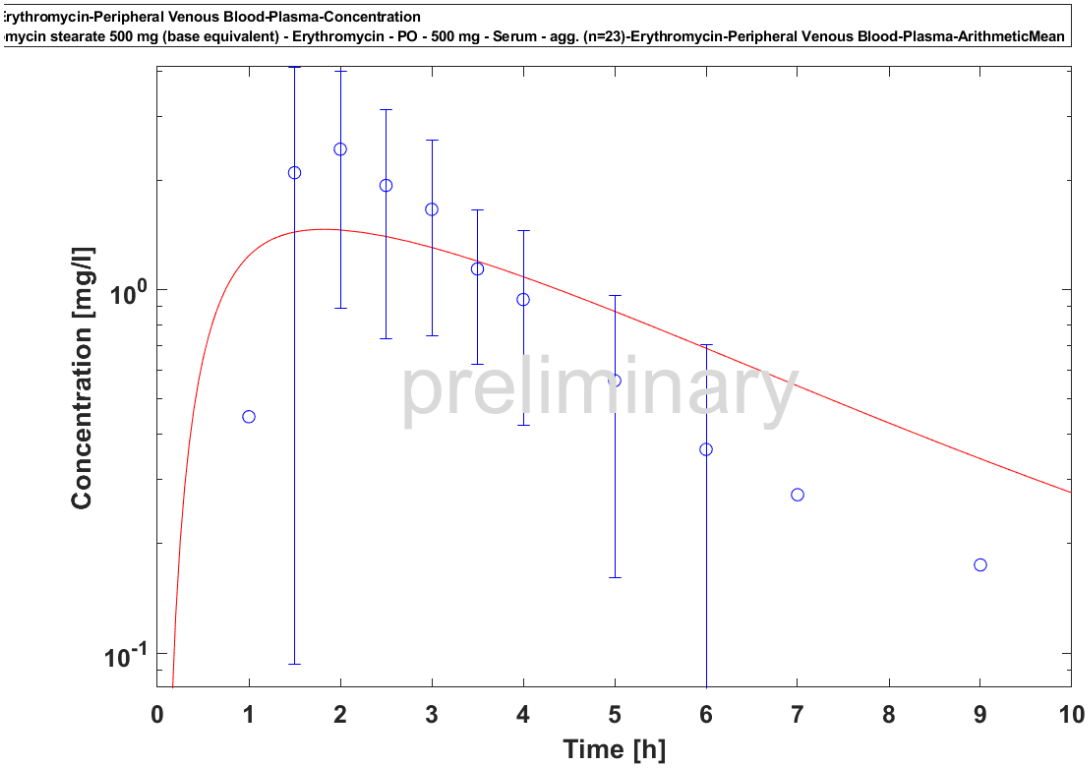
Time Profile Analysis 1



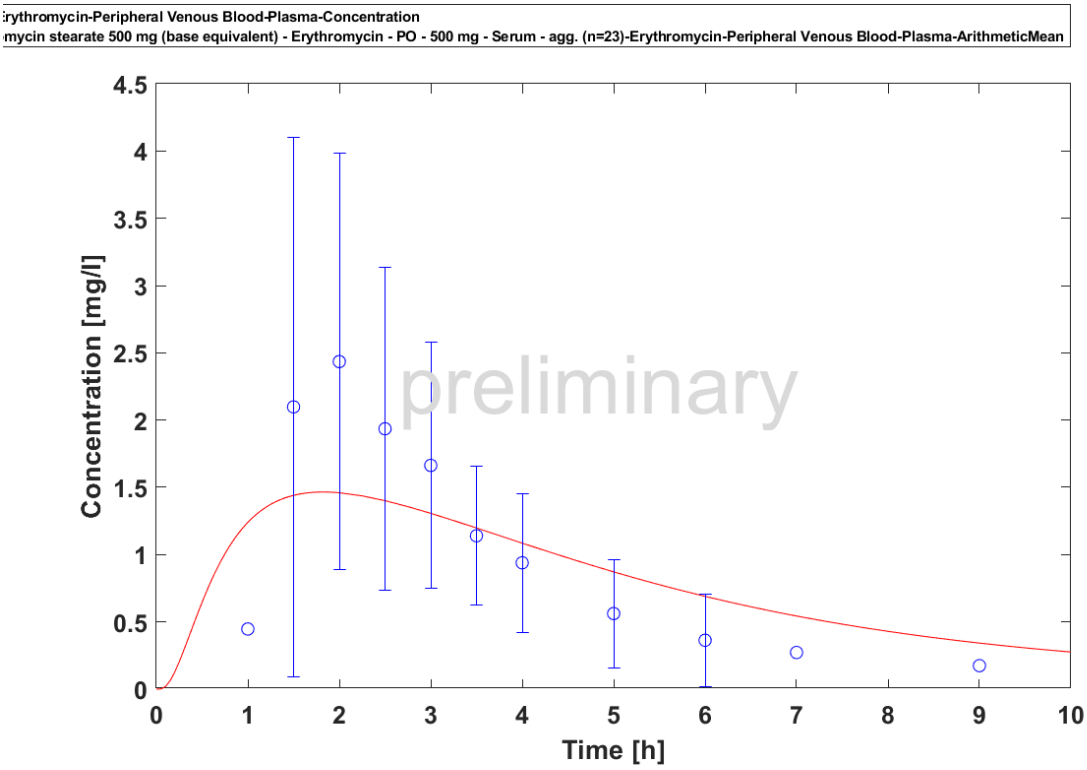
Time Profile Analysis



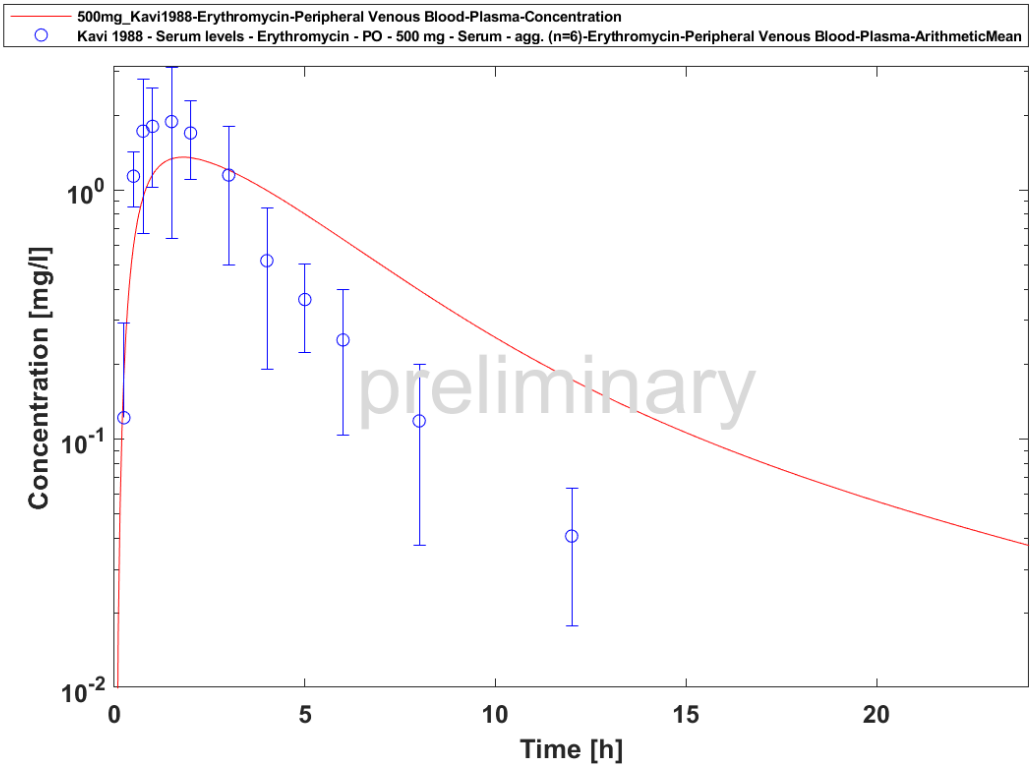
Time Profile Analysis 1



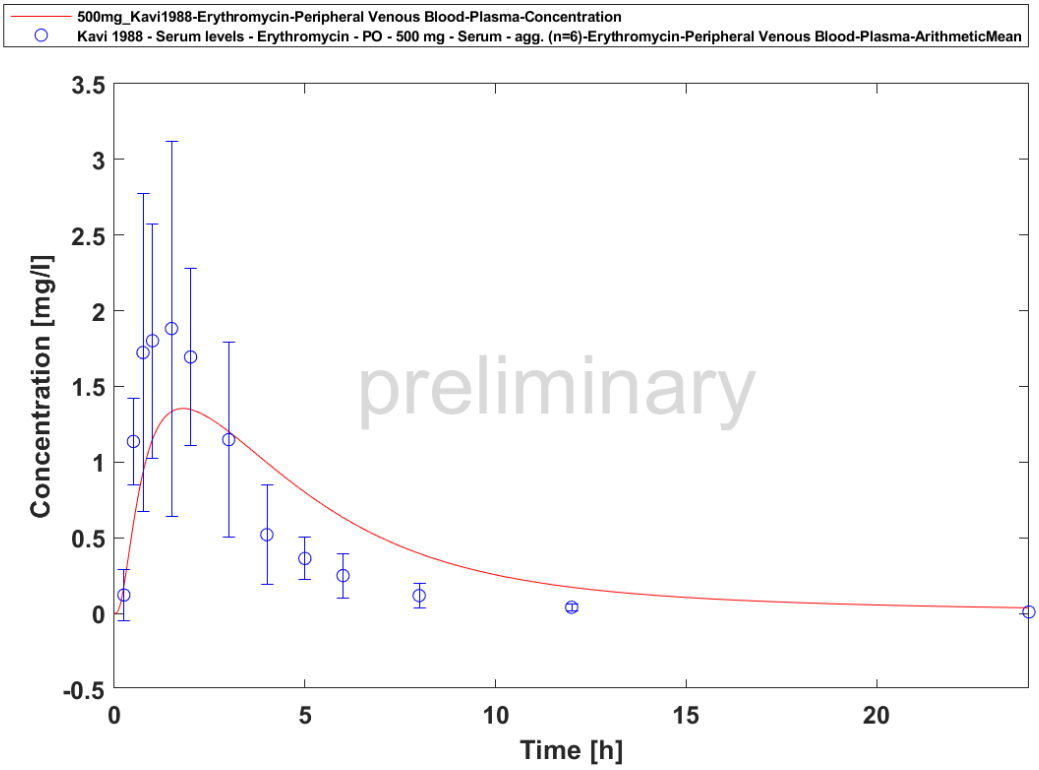
Time Profile Analysis



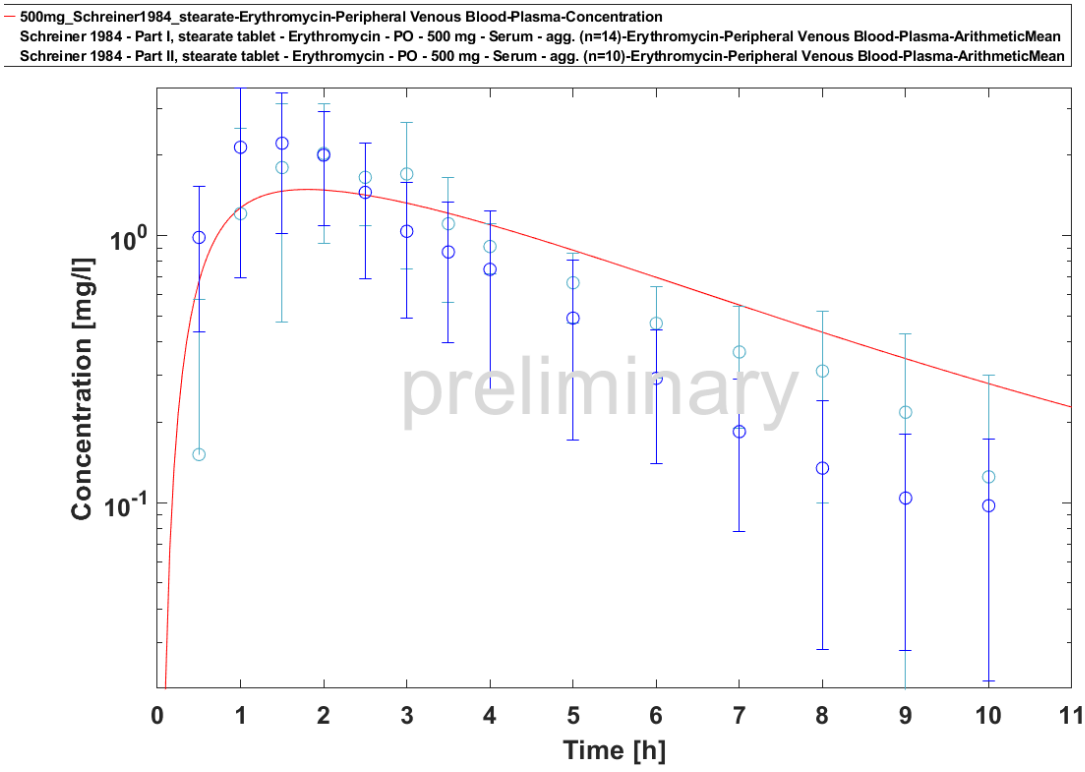
Time Profile Analysis 1



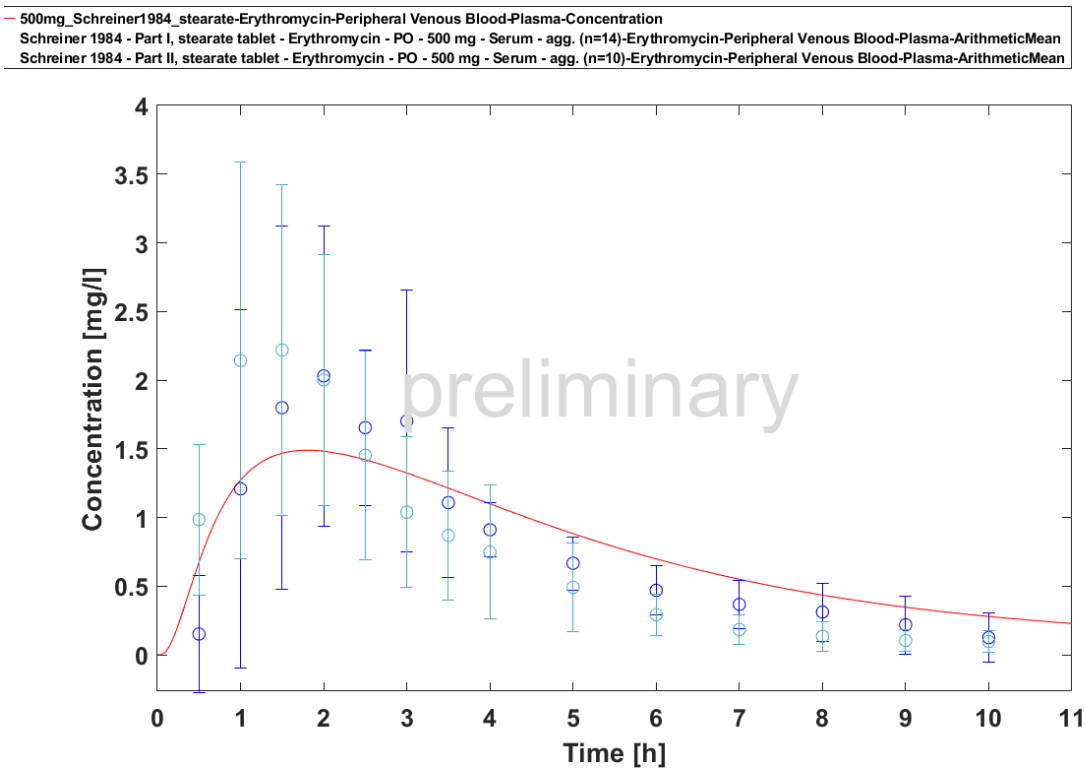
Time Profile Analysis



Time Profile Analysis 1



Time Profile Analysis



Time Profile Analysis 1

4 Conclusion

The final erythromycin PBPK model applies metabolism by CYP3A4, hepatic plasma clearance, glomerular filtration and mechanism-based inhibition of CYP3A4. Overall, the model adequately describes the oral pharmacokinetics of erythromycin in healthy adults receiving different single or multiple doses of several oral dosage forms.

5 References

Acocella 1968 Acocella G, Mattiussi R, Nicolis FB, Pallanza R, Tenconi LT. Biliary excretion of antibiotics in man. *Gut* 1968, 9(5): 536-545.

Akiyoshi 2013 Akiyoshi T, Ito M, Murase S, Miyazaki M, Guengerich FP, Nakamura K, Yamamoto K, Ohtani H. Mechanism-based inhibition profiles of erythromycin and clarithromycin with cytochrome P450 3A4 genetic variants. *Drug Metab Pharmacokinet* 2013, 28(5): 411-415.

Atkinson 2005 Atkinson A, Kenny JR, Grime K. Automated Assessment of Time-Dependent Inhibition of Human CYP Enzymes using LC-MS-MS Analysis. *Drug Metab Dispos* 2005, 33(11): 1637-1347.

Aueviriyavit 2010 Aueviriyavit S, Kobayashi K, Chiba K. Species differences in mechanism-based inactivation of CYP3A in humans, rats and mice. *Drug Metab Pharmacokinet* 2010, 25(1): 93-100.

Austin 1980 Austin KL, Mather LE, Philpot CR, McDonald PJ. Intersubject and dose-related variability after intravenous administration of erythromycin. *Br J Clin Pharmacol* 1980, 10(3): 273-379.

Barre 1987 Barre J, Mallat A, Rosenbaum J, Deforges L, Houin G, Dhumeaux D, Tillement JP. Pharmacokinetics of erythromycin in patients with severe cirrhosis. Respective influence of decreased serum binding and impaired liver metabolic capacity. *Br J Clin Pharmacol* 1987, 23(6): 753-757.

Birkett 1990 Birkett DJ, Robson RA, Grgurinovich N, Tonkin A. Single oral dose pharmacokinetics of erythromycin and roxithromycin and the effects of chronic dosing. *Ther Drug Monit* 1990, 12(1): 65-71.

Capobianco 1994 Capobianco JO, Goldman RC. Macrolide transport in Escherichia coli strains having normal and altered OmpC and/or OmpF porins. *Int J Antimicrob Agents* 1994, 4(3): 183-189.

Carlier 1987 Carlier MB, Zenebergh A, Tulkens PM. Cellular uptake and subcellular distribution of roxithromycin and erythromycin in phagocytic cells. *J Antimicrob Chemother* 1987, 20 Suppl B, 47-56.

Chan 2000 Chan WK, Delucchi AB. Resveratrol, a red wine constituent, is a mechanism-based inactivator of cytochrome P450 3A4. *Life Sci* 2000, 67(25): 3103-3112.

Chelvan 1979 Chelvan P, Hamilton-Miller JM, Brumfitt W. Biliary excretion of erythromycin after parenteral administration. *Br J Clin Pharmacol* 1979, 8(3): 233-235.

Chen 2011 Chen Y, Liu L, Monshouwer M, Fretland AJ. Determination of time-dependent inactivation of CYP3A4 in cryopreserved human hepatocytes and assessment of human drug-drug interactions. *Drug Metab Dispos* 2011, 39(11): 2085-2092.

Chun 1977 Chun AHC, Seitz JA. Pharmacokinetics and biological availability of erythromycin. *Infection* 1977, 5(1): 14-22.

Cui 2000 Cui X, Nelson DR, Strobel HW. A novel human cytochrome P450 4F isoform (CYP4F11): cDNA cloning, expression, and genomic structural characterization. *Genomics* 2000, 68(2): 161-166.

DiSanto 1981 DiSanto AR, Chodos DJ. Influence of study design in assessing food effects on absorption of erythromycin base and erythromycin stearate. *Antimicrob Agents Chemother* 1981, 20(2): 190-196.

drugbank (https://www.drugbank.ca/drugs/DB00199), accessed on 05-14-2018.

Huppertz 2011 Huppertz A, Breuer J, Fels LM, Schultze-Mosgau M, Sutter G, Klein S, et al. Evaluation of possible drug–drug interaction between gadoxetic acid and erythromycin as an inhibitor of organic anion transporting peptides (OATP). *J Magn Reson Imaging* 2011, 33(2): 409-416.

Iliopoulou 1982 Iliopoulou A, Aldhous ME, Johnston A, Turner P. Pharmacokinetic interaction between theophylline and erythromycin. *Br J Clin Pharmacol* 1982, 14(4): 495-499.

Ishikawa 2017 Ishikawa Y, Akiyoshi T, Imaoka A, Ohtani H. Inactivation kinetics and residual activity of CYP3A4 after treatment with erythromycin. *Biopharm Drug Dispos* 2017, 38(7): 420-425.

Jones 1969 Jones PH, Rowley EK, Weiss AL, Bishop DL, Chun AH. (1969). Insoluble erythromycin salts. *J Pharm Sci* 1969, 58(3): 337-339.

Josefsson 1982 Josefsson K, Bergan T, Magni L. Dose-related pharmacokinetics after oral administration of a new formulation of erythromycin base. *Br J Clin Pharmacol* 1982, 13(5): 685-691.

Kalsotra 2004 Kalsotra A, Turman CM, Kikuta Y, Strobel HW. Expression and characterization of human cytochrome P450 4F11: Putative role in the metabolism of therapeutic drugs and eicosanoids. *Toxicol Appl Pharmacol* 2004, 199(3): 295-304.

Kanamitsu 2000 Kanamitsu SI, Ito K, Green CE, Tyson CA, Shimada N, Sugiyama Y. Prediction of in vivo interaction between triazolam and erythromycin based on in vitro studies using human liver microsomes and recombinant human CYP3A4. *Pharm Res* 2000, 17(4): 419-426.

Kavi 1988 Kavi J, Webberley JM, Andrews JM, Wise R. A comparison of the pharmacokinetics and tissue penetration of spiramycin and erythromycin. *J Antimicrob Chemother* 1988, 22 Suppl B: 105-110.

Kivistö 1997 Kivistö KT, Lamberg TS, Kantola T, Neuvonen PJ. Plasma buspirone concentrations are greatly increased by erythromycin and itraconazole. *Clin Pharmacol Ther* 1997, 62(3): 348-354.

Kosaka 2017 Kosaka M, Kosugi Y, Hirabayashi H. Risk assessment using cytochrome P450 time-dependent inhibition assays at single time and concentration in the early stage of drug discovery. *J Pharm Sci* 2017, 106(9): 2839-2846.

Kozakai 2013 Kozakai K, Yamada Y, Oshikata M, Kawase T, Suzuki E, Haramaki Y, Taniguchi H. Cocktail-substrate approach-based high-throughput assay for evaluation of direct and time-dependent inhibition of multiple cytochrome P450 isoforms. *Drug Metab Pharmacokinet* 2013, 29(2): 198-207.

Kroboth 1982 Kroboth PD, Brown A, Lyon JA, Kroboth FJ, Juhl RP. Pharmacokinetics of single-dose erythromycin in normal and alcoholic liver disease subjects. *Antimicrob Agents Chemother* 1982, 21(1): 135-140.

Kuepfer 2016 Kuepfer L, Niederalt C, Wendl T, Schlender JF, Willmann S, Lippert J, Block M, Eissing T, Teutonico D. Applied concepts in PBPK modeling: how to build a PBPK/PD model. *CPT Pharmacometrics Syst Pharmacol* 2016, 5(10): 516-531.

Lancaster 2012 Lancaster CS, Bruun GH, Peer CJ, Mikkelsen TS, Corydon TJ, Gibson AA, Hu S, Orwick SJ, Mathijssen RH, Figg WD, Baker SD, Sparreboom A. OATP1B1 polymorphism as a determinant of erythromycin disposition. *Clin Pharmacol Ther* 2012, 92(5): 642-650.

Larrey 1983 Larrey D, Tinel M, and Pessayre D. Formation of inactive cytochrome P-450 Fe(II)-metabolite complexes with several erythromycin derivatives but not with josamycin and midecamycin in rats. *Biochem Pharmacol* 1983, 32(9): 1487-1493.

Lien 1974 Lien EJ, Kuwahara J, Koda RT. Diffusion of drugs into prostatic fluid and milk. *Drug Intelligence & Clinical Pharmacy* 1974, 8(8): 470-475.

Malmborg 1978 Malmborg AS. Absorption of erythromycin stearate after oral administration. *Curr Med Res Opin* 1978, 5 Sup 2: 15-18.

Manna 1998 Manna PK, Basu SK. Preparation and Evaluation of Erythromycin Fumarate-a New Derivative of Erythromycin. *Drug Dev Ind Pharm* 1998, 24(9): 879-882.

Mather 1981 Mather LE, Austin KL, Philpot CR, McDonald PJ. Absorption and bioavailability of oral erythromycin. *Br J Clin Pharmacol* 1981, 12(2): 131-140.

Mao 2011 Mao J, Mohutsky MA, Harrelson JP, Wrighton SA, Hall SD. Prediction of CYP3A-mediated drug-drug interactions using human hepatocytes suspended in human plasma. *Drug Metab Dispos* 2011, 39(4): 591-602.

Mao 2016 Mao J, Tay S, Khojasteh CS, Chen Y, Hop CE, Kenny JR. Evaluation of time dependent inhibition assays for marketed oncology drugs: comparison of human hepatocytes and liver microsomes in the presence and absence of human plasma. *Pharm Res* 2016, 33(5): 1204-1219.

McConn 2004 McConn DJ, Lin YS, Allen K, Kunze KL, Thummel KE. Differences in the inhibition of cytochromes P450 3A4 and 3A5 by metabolite-inhibitor complex-forming drugs. *Drug Metab Dispos* 2004, 32(10): 1083-1091.

McFarland 1997 McFarland JW, Berger CM, Froshauer SA, Hayashi SF, Hecker SJ, Jaynes BH, et al. Quantitative structure− activity relationships among macrolide antibacterial agents: in vitro and in vivo potency against Pasteurella multocida. *J Med Chem* 1997, 40(9): 1340-1346.

McGinnity 2006 McGinnity DF, Berry AJ, Kenny JR, Grime K, Riley RJ. Evaluation of time-dependent cytochrome P450 inhibition using cultured human hepatocytes. *Drug Metab Dispos* 2006, 34(8): 1291-1300.

Michaels 2014 Michaels S, Wang MZ. The revised human liver cytochrome P450 "Pie": absolute protein quantification of CYP4F and CYP3A enzymes using targeted quantitative proteomics. *Drug Metab Dispos* 2014, 42(8): 1241-1251.

Miglioli 1990 Miglioli PA, Pivetta P, Strazzabosco M, Orlando R, Okolicsanyi L, Palatini P. Effect of age on single- and multiple-dose pharmacokinetics of erythromycin. *Eur J Clin Pharmacol* 1990, 39(2): 161-164.

Miller 1984 Miller MF, Martin JR, Johnson P, Ulrich JT, Rdzok EJ, Billing P. Erythromycin uptake and accumulation by human polymorphonuclear leukocytes and efficacy of erythromycin in killing ingested Legionella pneumophila. *J Infect Dis* 1984, 149(5): 714-718.

Mordi 2000 Mordi MN, Pelta MD, Boote V, Morris GA, Barber J. Acid-catalyzed degradation of clarithromycin and erythromycin B: a comparative study using NMR spectroscopy. *J Med Chem* 2000, 43(3): 467-474.

Obach 2007 Obach RS, Walsky RL, Venkatakrishnan K. Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. *Drug Metab Dispos* 2007, 35(2): 246-255.

Open Systems Pharmacology Documentation. (https://docs.open-systems-pharmacology.org/), accessed on 07-30-2019.

Pasic 1987 Pasic J, Jackson SH, Johnston A, Peverel-Cooper CA, Turner P, Downey K, Chaput de Saintonge DM. The interaction between chronic oral slow-release theophylline and single-dose intravenous erythromycin. *Xenobiotica* 1987, 17(4):493-497.

PK-Sim Ontogeny Database Version 7.3. (https://github.com/Open-Systems-Pharmacology/OSPSuite.Documentation/blob/38cf71b384cfc25cfa0ce4d2f3addfd32757e13b/PK-Sim%20Ontogeny%20Database%20Version%207.3.pdf), accessed on 07-30-2019.

Polasek 2006 Polasek TM, Miners JO. Quantitative prediction of macrolide drug-drug interaction potential from in vitro studies using testosterone as the human cytochrome P4503A substrate. *Eur J Clin Pharmacol* 2006, 62(3): 203-208.

Posti 1983 Posti J, Salonen M. Effect of formulation factors and food intake on the bioavailability of erythromycin stearate tablets. *Int J Pharm* 1983, 17(2-3): 225-235.

Riley 1997 Riley RJ, Howbrook D. In vitro analysis of the activity of the major human hepatic CYP enzyme (CYP3A4) using [N-methyl-14C]-erythromycin. *J Pharmacol Toxicol Methods* 1997, 38(4): 189-193.

Ring 2005 Ring BJ, Patterson BE, Mitchell MI, Vandenbranden M, Gillespie J, Bedding AW, Jewell H, Payne CD, Forgue ST, Eckstein J, Wrighton SA, Phillips DL. Effect of tadalafil on cytochrome P450 3A4-mediated clearance: studies in vitro and in vivo. *Clin Pharmacol Ther* 2005, 77(1): 63-75.

Schreiner 1984 Schreiner A, Digranes A. Absorption of erythromycin stearate and enteric-coated erythromycin base after a single oral dose immediately before breakfast. *Infection* 1984, 12(5): 345-348.

Sun 2010 Sun H, Frassetto LA, Huang Y, Benet LZ. Hepatic clearance, but not gut availability, of erythromycin is altered in patients with end-stage renal disease. *Clin Pharmacol Ther* 2010, 87(4): 465-472.

Teng 2010 Teng WC, Oh JW, New LS, Wahlin MD, Nelson SD, Ho HK, Chan EC. Mechanism-based inactivation of cytochrome P450 3A4 by lapatinib. *Mol Pharmacol* 2010, 78(4): 693-703.

Uehara 2015 Uehara S, Murayama N, Nakanishi Y, Nakamura C, Hashizume T, Zeldin DC, Yamazaki H, Uno Y. Immunochemical quantification of cynomolgus CYP2J2, CYP4A and CYP4F enzymes in liver and small intestine. *Xenobiotica* 2015, 45(2): 124-130.

Ung 2009 Ung D, Parkman HP, Nagar S. Metabolic interactions between prokinetic agents domperidone and erythromycin: an in vitro analysis. *Xenobiotica* 2009, 39(10): 749-756.

U.S. Patent 2,761,859. Aqueous soluble salts of erythromycin. Issued September 4, 1956. https://patents.google.com/patent/US2761859A/en, accessed on 07-17-2018.

Wang 1997 Wang RW, Newton DJ, Scheri TD, Lu AY. Human cytochrome P450 3A4-catalyzed testosterone 6 beta-hydroxylation and erythromycin N-demethylation. Competition during catalysis. *Drug Metab Dispos* 1997, 25(4): 502-507.

Watanabe 2007 Watanabe A, Nakamura K, Okudaira N, Okazaki O, Sudo K. Risk assessment for drug-drug interaction caused by metabolism-based inhibition of CYP3A using automated in vitro assay systems and its application in the early drug discovery process. *Drug Metab Dispos* 2007, 35(7): 1232-1238.

Willmann 2007 Willmann S, Höhn K, Edginton A, Sevestre M, Solodenko J, Weiss W, Lippert J, Schmitt W. Development of a physiology-based whole-body population model for assessing the influence of individual variability on the pharmacokinetics of drugs. *J Pharmacokinet Pharmacodyn* 2007, 34(3): 401-431.

Xu 2009 Xu L, Chen Y, Pan Y, Skiles GL, Shou M. Prediction of human drug-drug interactions from time-dependent inactivation of CYP3A4 in primary hepatocytes using a population-based simulator. *Drug Metab Dispos* 2009, 37(12): 2330-2339.

Yakatan 1980 Yakatan GJ, Poynor WJ, Breeding SA, Lankford CE, Dighe SV, Martin AN, Doluisio JT. Single- and multiple-dose bioequivalence of erythromycin pharmaceutical alternatives. *J Clin Pharmacol* 1980, 20(11): 625-638.

Yamano 2001 Yamano K, Yamamoto K, Katashima M, Kotaki H, Takedomi S, Matsuo H, Ohtani H, Sawada Y, Iga T. Prediction of midazolam-CYP3A inhibitors interaction in the human liver from in vivo/in vitro absorption, distribution, and metabolism data. *Drug Metab Dispos* 2001, 29(4 Pt 1): 443-452.

Zhang 2006 Zhang X, Gorski J, Lucksiri A, Chien J, Quinney S, Jones D, Hall S. OIV-B-4; Physiologically-based pharmacokinetic models for the inhibition of midazolam clearance by erythromycin and diltiazem. *Clin Pharmacol Ther* 2006, 79: P34-P34.

Zhang 2009 Zhang X, Jones DR, Hall SD. Prediction of the effect of erythromycin, diltiazem, and their metabolites, alone and in combination, on CYP3A4 inhibition. *Drug Metab Dispos* 2009, 37(1):150-160.

Zimmerlin 2011 Zimmerlin A, Trunzer M, Faller B. CYP3A time-dependent inhibition risk assessment validated with 400 reference drugs. *Drug Metab Dispos* 2011, 39(6): 1039-1046.