

Supplemental Materials

Minimal Physiologically Based Pharmacokinetic Modeling of Atenolol and Metoprolol Absorption in Malnourished Rats

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Journal Name: European Journal of Drug Metabolism and Pharmacokinetics

Supplementary Table S1 Noncompartmental comparisons

Parameter	ATN		MET	
	Control	Malnourished	Control	Malnourished
AUC _{0-inf} (µg.min/mL)	7318 ± 40	7951 ± 950	3094 ± 446*	5584 ± 1503
AUC _(0-tlast) (µg.min/mL)	5684 ± 2465	7241 ± 498	2364 ± 570*	3969 ± 498
CL/F (mL/min/kg)	34.16 ± 0.19	31.74 ± 3.73	102.4 ± 15.6*	58.7 ± 13.9
C _{max} (µg /mL)	24.02 ± 4.91	23.46 ± 2.41	14.34 ± 1.78*	23.04 ± 1.55
k _{el} (min ⁻¹)	0.0096 ± 0.0022	0.017 ± 0.013	0.0067 ± 0.0011	0.0038 ± 0.0019
T _{last} (min)	390 ± 140	480 ± 0	300 ± 98*	420 ± 0
T _{max} (min)	240 ± 69	240 ± 49	120 ± 0*	93.8 ± 7.5
V _d /F (mL/kg)	3662 ± 870	2945 ± 2242	15345 ± 993	20408 ± 12826

*significantly different from MET malnourished group (p ≤ 0.05)

Supplementary Table S2 Physiologic parameters of tissues

Parameter	Volume (V, mL/kg)	Blood Flow (Q, mL/min/kg)
Lung	3.27 ^a	179.91 ^c
Liver	33.81 ^a	75.348 ^c
Kidney	6.16 ^a	29.00 ^c
Blood	58.82 ^b	179.91 ^c

^a Calculated based on Li et al. (2020), ^b Calculated based on Shah and Betts (2012), ^c Calculated based on Li et al. (2020) and adjusted according to Risberg et al. (1987)

Supplementary Table S3 Final estimated pharmacokinetic parameters for ATN jointly fitted model obtained by naïve pooling

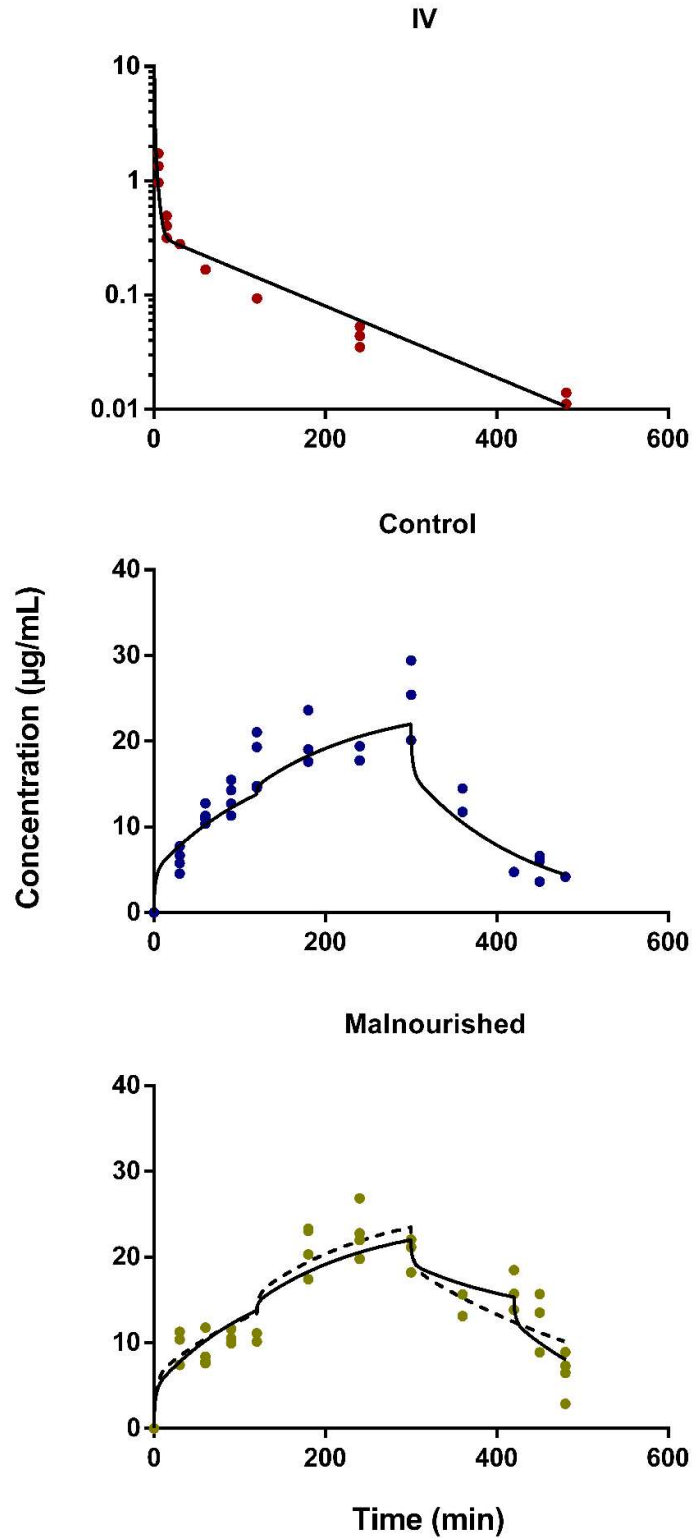
Parameter	Definition	Estimate (CV %)
k_{01} (mg/min/kg)	Apparent zero-order absorption rate constant 1 (t = 0 to 120 min)	1.32 (8.83)
k_{02}^* (mg/min/kg)	Apparent zero-order absorption rate constant 2 (t = 120 to 300 min)	1.61 (9.51)
$k_{03} C^a$ (mg/min/kg)	Apparent zero-order absorption rate constant 3 (t = 300 to 360 min)	0
$k_{03} M$ (mg/min/kg)	Apparent zero-order absorption rate constant 3 (t = 300 to 420 min)	0.817 (19.3)
f_{d1}^b	Fractional distribution parameter for Tissue 1	0.312 (18.2)
K_{p1}^c	Tissue-to-plasma partition coefficient for Tissue 1	1.79 (9.68)
CL (mL/min/kg)	Total clearance	16.3 (6.68)
V_{ss}^d (L/kg)	The volume of distribution at steady-state	1.75 (9.25)
σ_{slope}	Variance parameter	0.284 (8.37)
F C ^e	The apparent absolute bioavailability	0.390
F M ^e	The apparent absolute bioavailability	0.631

^a Assumed to be 0, ^b $f_{d1} = f_{d2}$, ^c $K_{p1} = K_{p2}$, ^d calculated based on $V_{ss} = (V_b + V_1 \cdot K_{p1} + V_2 \cdot K_{p2} + V_3 \cdot K_{p3}) / \text{body weight}$ as second parameter, ^e calculated based on $F = (AUC_{PO} \cdot \text{Dose}_{IV}) / (AUC_{IV} \cdot \text{Dose}_{PO})$, C: control group, M: malnourished group, *significantly higher than $k_{03} M$ ($p < 0.05$)

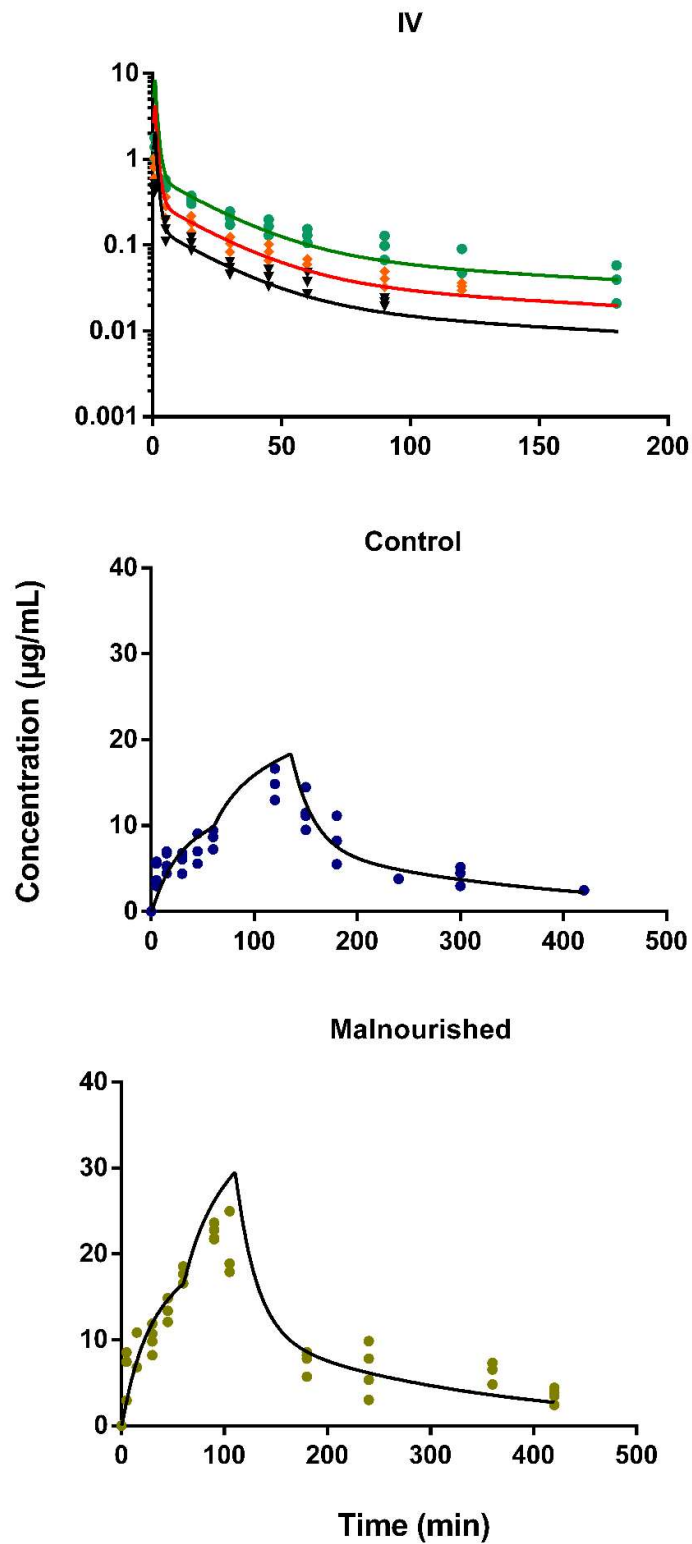
Supplementary Table S4 Final estimated pharmacokinetic parameters for MET jointly fitted model obtained by naïve pooling

Parameter	Definition	Estimate (CV %)
$k_{01} C^a$ (mg/min/kg)	Apparent zero-order absorption rate constant 1 (t = 0 to 60 min)	4 (10.7)
$k_{01} M^b$ (mg/min/kg)		7 (11.8)
$k_{02} C^c$ (mg/min/kg)	Apparent zero-order absorption rate constant 2 (t = 60 to 135 min)	6 (16.05)
$k_{02} M^{d,*}$ (mg/min/kg)	Apparent zero-order absorption rate constant 3 (t = 60 to 110 min)	11.1 (17.9)
f_{d1}	Fractional distribution parameter for Tissue 1	0.82 (2.98)
f_{d2}^e	Fractional distribution parameter for Tissue 2	0.18 (13.5)
K_{p1}^f	Tissue-to-plasma partition coefficient for Tissue 2	5.40 (19.1)
CL _{int} (mL/min/kg)	Intrinsic clearance	40.4 (8.56)
V_{ss}^g (L/kg)	The volume of distribution at steady-state	7.062 (13.3)
σ_{slope}	Variance parameter	0.55 (7.17)
F C ^h	The apparent absolute bioavailability	0.492
F M ^h	The apparent absolute bioavailability	0.815

^a limited to 2.5 to 4, ^b limited to 5.5 to 7, ^c limited to 4 to 6, ^d limited to 10 to 11.5, ^e calculated as second parameter $f_{d2} = 1 - f_{d1}$, ^f $K_{p1} = K_{p2}$, ^g calculated based on $V_{ss} = (V_b + V_1 \cdot K_{p1} + V_2 \cdot K_{p2} + V_3 \cdot K_{p3}) / \text{body weight}$ as second parameter, ^h calculated based on $F = (AUC_{PO} \cdot \text{Dose}_{IV}) / (AUC_{IV} \cdot \text{Dose}_{PO})$, the average of the F values obtained by comparing the oral profiles with each reference IV dose (0.5, 1, and 2 mg/kg), C: control group, M: malnourished group, *significantly higher than $k_{01} C$ ($p < 0.05$)

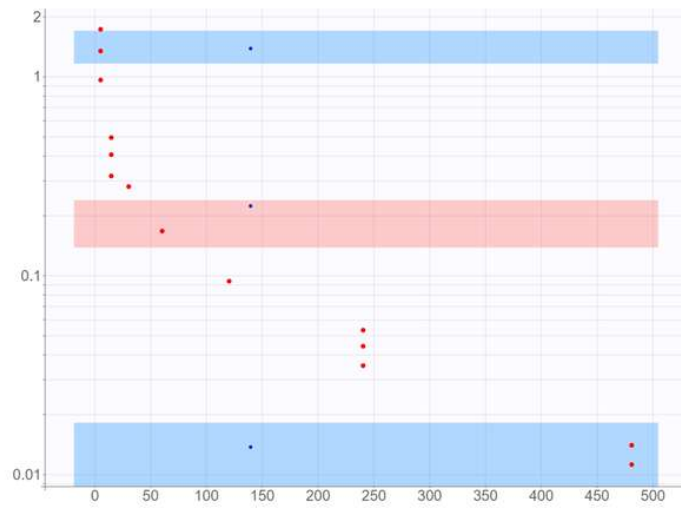


Supplementary Fig. S1 Joint fittings of ATN plasma concentration for IV and oral doses using by the mPBPK model (Figure 1). Symbols show observations and lines depict fitting with the mPBPK model. The dashed line shows the fitting without k_{03}

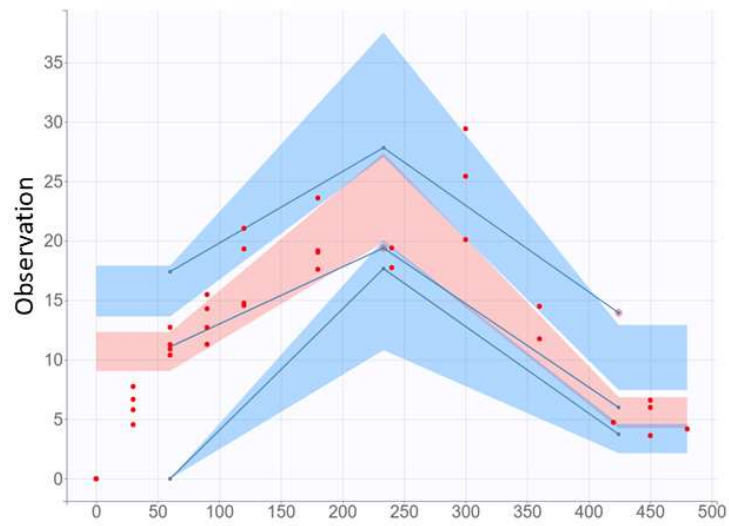


Supplementary Fig. S2 Joint fittings of MET plasma concentration for IV and oral doses using by the mPBPK model (Figure 2). Symbols are observations and lines depict fitting with the mPBPK model

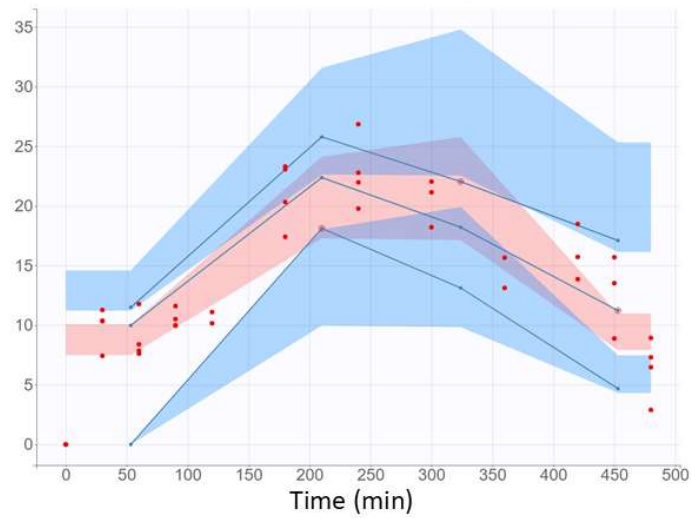
IV



Control

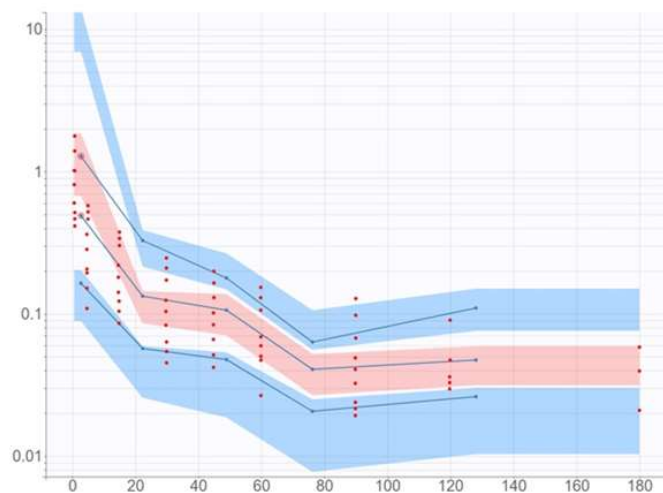


Malnourished

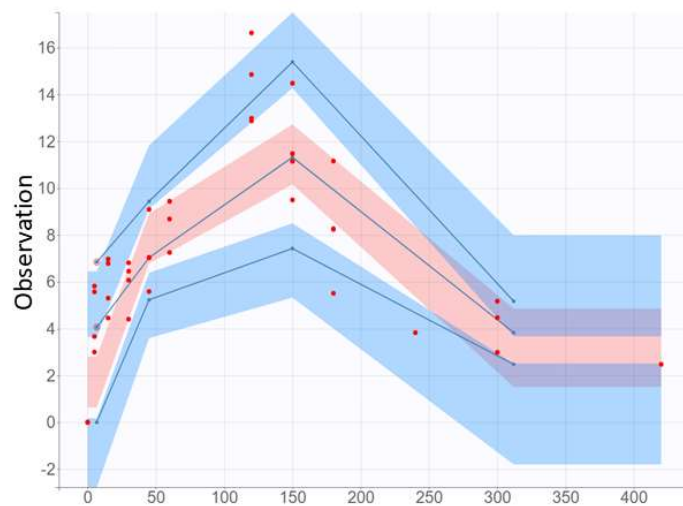


Supplementary Fig. S3 Visual predictive check plot for ATN

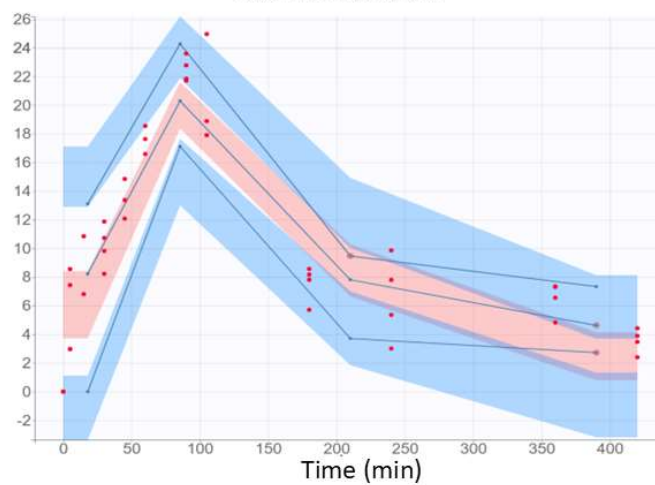
IV



Control



Malnourished



Supplementary Fig. S4 Visual predictive check plot for MET

Monolix code for population analysis of ATN IV profile

DESCRIPTION: Model for ATN IV

[LONGITUDINAL]

input={fd1,Kp1,CL,Group}

Group = {use=regressor}

PK:

depot(target=B, p=1/58.82)

EQUATION:

Vb = 58.82

V1 = 37.08

V2 = 897.94

Vkid = 6.16

Qco = 179.91

Qkid = 29.00

Rb_H = 1.000

Kpkid = 3.0

Kp2 = Kp1

fd2 = fd1

fdt = fd1+fd2

;Initial Conditions

t_0 = 0

B_0 = 0

T1_0 = 0

T2_0 = 0

K_0 = 0

AUC_0 = 0

;ODEs

odeType=stiff

;Blood Compartment

ddt_B = ((Qco-Qkid)*fd1*T1*Rb_H/Kp1+(Qco-Qkid)*fd2*T2*Rb_H/Kp2+Qkid*K*Rb_H/Kpkid-((Qco-Qkid)*fd1*Rb_H+(Qco-Qkid)*fd2*Rb_H+Qkid*Rb_H)*B)/(Vb*Rb_H)

ddt_T1 = ((Qco-Qkid)*fd1*Rb_H*B-(Qco-Qkid)*fd1*T1*Rb_H/Kp1)/V1

ddt_T2 = ((Qco-Qkid)*fd2*Rb_H*B-(Qco-Qkid)*fd2*T2*Rb_H/Kp2)/V2


```
ddt_K = (Qkid*Rb_H*B-Qkid*K*Rb_H/Kpkid-CL*B)/Vkid  
ddt_AUC = B
```

```
OUTPUT:  
output = {B}  
table = {AUC}
```

Monolix code for population analysis of ATN control group

```
DESCRIPTION: Model for ATN H  
[LONGITUDINAL]  
input={k01,k02,Group}  
Group = {use=regressor}
```

```
PK:
```

```
EQUATION:
```

```
Vb = 58.82  
V1 = 37.08  
V2 = 897.94  
Vkid = 6.16
```

```
Qco = 179.91  
Qkid = 29.00
```

```
Rb_H = 1.000
```

```
Kpkid = 3.0
```

```
tfirst= 120  
tmax = 300
```

```
fd1 = 0.134  
fd2 = 0.134  
Kp1 = 1.43  
Kp2 = 1.43  
CL = 16.04
```

```
;Initial Conditions  
t_0 = 0
```

```

B_0 = 0
T1_0 = 0
T2_0 = 0
K_0 = 0
AUC_0 = 0

;ODEs
odeType=stiff

if (t <= tfirst)
input_H = k01*250000

elseif (t>tfirst&t<=tmax)
input_H = k02*250000
else
input_H = 0
end

;Blood Compartment
ddt_B = (input_H+(Qco-Qkid)*fd1*T1*Rb_H/Kp1+(Qco-
Qkid)*fd2*T2*Rb_H/Kp2+Qkid*K*Rb_H/Kpkid-((Qco-Qkid)*fd1*Rb_H+(Qco-
Qkid)*fd2*Rb_H+Qkid*Rb_H)*B)/(Vb*Rb_H)
ddt_T1 = ((Qco-Qkid)*fd1*Rb_H*B-(Qco-Qkid)*fd1*T1*Rb_H/Kp1)/V1
ddt_T2 = ((Qco-Qkid)*fd2*Rb_H*B-(Qco-Qkid)*fd2*T2*Rb_H/Kp2)/V2
ddt_K = (Qkid*Rb_H*B-Qkid*K*Rb_H/Kpkid-CL*B)/Vkid
ddt_AUC = B

OUTPUT:
output = {B}
table = {AUC}

```

Monolix code for population analysis of ATN malnourished group

DESCRIPTION: Model for ATN M

```

[LONGITUDINAL]
input={k01,k02,k03,Group}
Group = {use=regressor}

```

PK:

```

EQUATION:
Vb = 58.82

```

V1 = 37.08

V2 = 897.94

Vkid = 6.16

Qco = 179.91

Qkid = 29.00

Rb_M = 1.014

Kpkid = 3.0

tfirst= 120

tmax = 300

tmal = 420

fd1 = 0.134

fd2 = 0.134

Kp1 = 1.43

Kp2 = 1.43

CL = 16.04

;Initial Conditions

t_0 = 0

B_0 = 0

T1_0 = 0

T2_0 = 0

K_0 = 0

AUC_0 = 0

;ODEs

odeType=stiff

if (t <= tfirst)

input_M = k01*250000

elseif (t>tfirst&t<=tmax)

input_M = k02*250000

elseif (t>tmax&t<=tmal)

input_M = k03*250000

else

input_M = 0

end

;Blood Compartment

```

ddt_B = (input_M+(Qco-Qkid)*fd1*T1*Rb_M/Kp1+(Qco-
Qkid)*fd2*T2*Rb_M/Kp2+Qkid*K*Rb_M/Kpkid-((Qco-Qkid)*fd1*Rb_M+(Qco-
Qkid)*fd2*Rb_M+Qkid*Rb_M)*B)/(Vb*Rb_M)
ddt_T1 = ((Qco-Qkid)*fd1*Rb_M*B-(Qco-Qkid)*fd1*T1*Rb_M/Kp1)/V1
ddt_T2 = ((Qco-Qkid)*fd2*Rb_M*B-(Qco-Qkid)*fd2*T2*Rb_M/Kp2)/V2
ddt_K = (Qkid*Rb_M*B-Qkid*K*Rb_M/Kpkid-CL*B)/Vkid
ddt_AUC = B

```

OUTPUT:

output = {B}

table = {AUC}

Monolix code for population analysis of MET IV profile

DESCRIPTION: Model for MET IV

[LONGITUDINAL]

input={fd1,Kp1,CL}

PK:

depot(target=B, p=1/58.82)

EQUATION:

Vb = 58.82

V1 = 9.43

V2 = 897.94

Vliver = 33.81

Qco = 179.91

Qliver = 75.34

Kp2 = Kp1

Kpliver = 62.25

Rb_H = 1.70

;Initial Conditions

t_0 = 0

B_0 = 0

T1_0 = 0

T2_0 = 0

L_0 = 0

AUC_0 = 0

;ODEs

odeType=stiff

;Blood Compartment

ddt_B = (((Qco-Qliver)*fd1*T1*Rb_H/Kp1+(Qco-Qliver)*(1-fd1)*T2*Rb_H/Kp2+Qliver*L*Rb_H/Kpliver)-((Qco-Qliver)*fd1*Rb_H+(Qco-Qliver)*(1-fd1)*Rb_H+Qliver*Rb_H)*B)/(Vb*Rb_H)

ddt_T1 = ((Qco-Qliver)*fd1*Rb_H*B-(Qco-Qliver)*fd1*T1*Rb_H/Kp1)/V1

ddt_T2 = ((Qco-Qliver)*(1-fd1)*Rb_H*B-(Qco-Qliver)*(1-fd1)*T2*Rb_H/Kp2)/V2

ddt_L = (Qliver*Rb_H*B-Qliver*L*Rb_H/Kpliver-CL*L*Rb_H/Kpliver)/Vliver

ddt_AUC = B

OUTPUT:

output = {B}

table = {AUC}

Monolix code for population analysis of MET control group

DESCRIPTION: Model for MET H

[LONGITUDINAL]

input={k01_H,k02_H,b,Group}

Group = {use=regressor}

PK:

EQUATION:

Vb = 58.82

V1 = 9.43

V2 = 897.94

Vliver = 33.81

Qco = 179.91

Qliver = 75.34

Kpliver = 62.25

Rb_H = 1.508

tfirst = 60

tmax_H = 135

fd1 = 0.464

Kp1 = 4.033

Kp2 = 4.033

CL = 148.63

;Initial Conditions

t_0 = 0

B_0 = 0

T1_0 = 0

T2_0 = 0

L_0 = 0

AUC_0 = 0

;ODEs

odeType=stiff

if (t <= tfirst)

input_PO= k01_H*312327.4

```
elseif (t>tfirst&t<=tmax_H)
input_PO= k02_H*312327.4
```

```
else
input_PO = 0
```

```
end
```

```
;Blood Compartment
```

```
ddt_B = (((Qco-Qliver)*fd1*T1*Rb_H/Kp1+(Qco-Qliver)*(1-
fd1)*T2*Rb_H/Kp2+Qliver*L*Rb_H/Kpliver)-((Qco-Qliver)*fd1*Rb_H+(Qco-Qliver)*(1-
fd1)*Rb_H+Qliver*Rb_H)*B)/(Vb*Rb_H)
ddt_T1 = ((Qco-Qliver)*fd1*Rb_H*B-(Qco-Qliver)*fd1*T1*Rb_H/Kp1)/V1
ddt_T2 = ((Qco-Qliver)*(1-fd1)*Rb_H*B-(Qco-Qliver)*(1-fd1)*T2*Rb_H/Kp2)/V2
ddt_L = (input_PO+Qliver*Rb_H*B-Qliver*L*Rb_H/Kpliver-CL*b*L*Rb_H/Kpliver)/Vliver
ddt_AUC = B
```

```
OUTPUT:
```

```
output = {B}
```

```
table = {AUC}
```

Monolix code for population analysis of MET malnourished group

DESCRIPTION: Model for MET M

[LONGITUDINAL]

input={k01_M,k02_M,b,Group}

Group = {use=regressor}

PK:

EQUATION:

Vb = 58.82

V1 = 9.43

V2 = 897.94

Vliver = 33.81

Qco = 179.91

Qliver = 75.34

Kpliver = 62.25

Rb_H = 1.607

tfirst = 60

tmax_M = 110

fd1 = 0.464

Kp1 = 4.033

Kp2 = 4.033

CL = 148.63

;Initial Conditions

t_0 = 0

B_0 = 0

T1_0 = 0

T2_0 = 0

L_0 = 0

AUC_0 = 0

;ODEs

odeType=stiff

if (t <= tfirst)

input_PO= k01_M*312327.4

elseif (t>tfirst&t<=tmax_M)

input_PO= k02_M*312327.4


```

else
input_PO = 0

```

```

end

```

```

;Blood Compartment

```

```

ddt_B = (((Qco-Qliver)*fd1*T1*Rb_H/Kp1+(Qco-Qliver)*(1-
fd1)*T2*Rb_H/Kp2+Qliver*L*Rb_H/Kpliver)-((Qco-Qliver)*fd1*Rb_H+(Qco-Qliver)*(1-
fd1)*Rb_H+Qliver*Rb_H)*B)/(Vb*Rb_H)
ddt_T1 = ((Qco-Qliver)*fd1*Rb_H*B-(Qco-Qliver)*fd1*T1*Rb_H/Kp1)/V1
ddt_T2 = ((Qco-Qliver)*(1-fd1)*Rb_H*B-(Qco-Qliver)*(1-fd1)*T2*Rb_H/Kp2)/V2
ddt_L = (input_PO+Qliver*Rb_H*B-Qliver*L*Rb_H/Kpliver-CL*b*L*Rb_H/Kpliver)/Vliver
ddt_AUC = B

```

```

OUTPUT:

```

```

output = {B}

```

```

table = {AUC}

```

ADAPT-5 code for ATN

```

*****
C                                ADAPT                                *
C                                Version 5                            *
C*****
C                                *
C                                MODEL                                *
C                                *
C                                *
C    This file contains Fortran subroutines into which the user      *
C    must enter the relevant model equations and constants.          *
C    Consult the User's Guide for details concerning the format for  *
C    entered equations and definition of symbols.                    *
C                                *
C    1. Symbol-   Parameter symbols and model constants              *
C    2. DiffEq-   System differential equations                      *
C    3. Output-   System output equations                            *
C    4. Varmod-   Error variance model equations                    *
C    5. Covmod-   Covariate model equations (ITS,MLEM)               *
C    6. Popinit-  Population parameter initial values (ITS,MLEM)     *
C    7. Prior -   Parameter mean and covariance values (ID,NPD,STS) *
C    8. Sparam-   Secondary parameters                               *
C    9. Amat -    System state matrix                                *
C                                *
C*****
C#####
C
    Subroutine SYMBOL
    Implicit None

    Include 'globals.inc'
    Include 'model.inc'

CC
C-----
C
C    Enter as Indicated
C
C---c-----
C
    NDEqs   = 15 ! Enter # of Diff. Eqs.
    NSParam = 6  ! Enter # of System Parameters.
    NVparam = 1  ! Enter # of Variance Parameters.
    NSecPar = 1  ! Enter # of Secondary Parameters.
    NSecOut = 0  ! Enter # of Secondary Outputs (not used).
    Ieqsol  = 1  ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT
only.
    Descr   = 'mPBPK model with two tissue compartments for ATN joint
fitting'

CC
C-----
C

```

```

C   Enter Symbol for Each System Parameter (eg. Psym(1)='Kel')
C
C-----C-----
C
      Psym(1)='fd1'
      Psym(2)='Kp1'
      Psym(3)='CL'

      Psym(4)='k01'
      Psym(5)='k02'

      Psym(6)='k03'

CC
C-----
C
C   Enter Symbol for Each Variance Parameter {eg: PVsym(1)='Sigma'}
C
C-----C-----
C
      PVsym(1)='Sigma'

CC
C-----
C
C   Enter Symbol for Each Secondary Parameter {eg: PSsym(1)='CLt'}
C
C-----C-----
C
      PSsym(1)='Vss'

C-----
C
C-----
C
C
      Return
      End

C#####
C

      Subroutine DIFFEQ(T,X,XP)
      Implicit None

      Include 'globals.inc'
      Include 'model.inc'

      Real*8 T,X(MaxNDE),XP(MaxNDE)

      Real*8 fd1,fd2,fdt,Vss
      Real*8 Kp1,Kp2,Kpkid
      Real*8 Qco,Qkid
      Real*8 CL,CL_M
      Real*8 k01,k02,k03

```

```

Real*8 Vb,V1,V2,Vkid
Real*8 Rb_H,Rb_M
Real*8 input_H,input_M
Real*8 tfirst,tmax,tmal

CC
C-----
C
C   Enter Differential Equations Below   {e.g.  XP(1) = -P(1)*X(1)  }
C
C-----C-----
C

      fd1      = P(1)
      Kp1      = P(2)
      CL       = P(3)

      k01      = P(4)
      k02      = P(5)

      k03      = P(6)

      Vb       = 58.82      !mL/kg
      V1       = 37.08      !mL/kg
      V2       = 897.94     !mL/kg
      Vkid     = 6.16       !mL/kg

      Qco      = 179.91     !mL/min/kg
      Qkid     = 29.00      !mL/min/kg

      Rb_H     = 1.000
      Rb_M     = 1.014

      Kpkid    = 3.0
      Kp2      = Kp1

      fdt      = fd1+fd2
      fd2      = fd1

      tfirst   = 120        !min
      tmax     = 300        !min

      tmal     = 420        !min

C   ATN iv

C   1. Blood Compartment
      IF (fdt>1) THEN
      XP(1)=0
      ELSE

      XP(1) = (R(1)+(Qco-Qkid)*fd1*X(2)*Rb_H/Kp1
c + (Qco-Qkid)*fd2*X(3)*Rb_H/Kp2+Qkid*X(4)*Rb_H/Kpkid
c - ((Qco-Qkid)*fd1*Rb_H+
c (Qco-Qkid)*fd2*Rb_H+Qkid*Rb_H)*X(1))/(Vb*Rb_H)

      ENDIF

```

```

C      2. Tissue Compartment 1
      XP(2) = ((Qco-Qkid)*fd1*Rb_H*X(1) -
c      (Qco-Qkid)*fd1*X(2)*Rb_H/Kp1)/V1

C      3. Tissue Compartment 2
      XP(3) = ((Qco-Qkid)*fd2*Rb_H*X(1)
c      -(Qco-Qkid)*fd2*X(3)*Rb_H/Kp2)/V2

C      4. Kidney
      XP(4) = (Qkid*Rb_H*X(1)
c      -Qkid*X(4)*Rb_H/Kpkid-CL*X(1))/Vkid

C      Healthy Rats

      IF (T<=tfirst) THEN
      input_H = k01*Bolus(1,1)

      ELSE IF (T>tfirst.and.T<=tmax) THEN
      input_H = k02*Bolus(1,2)

      ELSE
      input_H = 0

      END IF

C      2. Blood Compartment
      IF(fdt>1) THEN
      XP(5)=0
      ELSE

      XP(5) = (input_H+(Qco-Qkid)*fd1*X(6)*Rb_H/Kp1
c      +(Qco-Qkid)*fd2*X(7)*Rb_H/Kp2+Qkid*X(8)*Rb_H/Kpkid
c      -((Qco-Qkid)*fd1*Rb_H
c      +(Qco-Qkid)*fd2*Rb_H+Qkid*Rb_H)*X(5))/(Vb*Rb_H)

      ENDIF

C      3. Tissue Compartment 1
      XP(6) = ((Qco-Qkid)*fd1*Rb_H*X(5) -
c      (Qco-Qkid)*fd1*X(6)*Rb_H/Kp1)/V1

C      4. Tissue Compartment 2
      XP(7) = ((Qco-Qkid)*fd2*Rb_H*X(5)
c      -(Qco-Qkid)*fd2*X(7)*Rb_H/Kp2)/V2

C      5. Kidney
      XP(8) = (Qkid*Rb_H*X(5)
c      -Qkid*X(8)*Rb_H/Kpkid-CL*X(5))/Vkid

C      Malnourished Rats

      IF (T<=tfirst) THEN
      input_M = k01*Bolus(1,2)

      ELSE IF (T>tfirst.and.T<=tmax) THEN
      input_M = k02*Bolus(1,2)

```

```

        ELSE IF (T>tmax.and.T<=tmal) THEN
input_M = k03*Bolus(1,2)

        ELSE

input_M = 0

        END IF

C      2. Blood Compartment
XP(9) = (input_M+(Qco-Qkid)*fd1*X(10)*Rb_M/Kp1
c +(Qco-Qkid)*fd2*X(11)*Rb_M/Kp2+Qkid*X(12)*Rb_M/Kpkid
c -( (Qco-Qkid)*fd1*Rb_M
c +(Qco-Qkid)*fd2*Rb_M+Qkid*Rb_M)*X(9) ) / (Vb*Rb_M)

C      3. Tissue Compartment 1
XP(10) = ( (Qco-Qkid)*fd1*Rb_M*X(9)-
c (Qco-Qkid)*fd1*X(10)*Rb_M/Kp1) /V1

C      4. Tissue Compartment 2
XP(11) = ( (Qco-Qkid)*fd2*Rb_M*X(9)
c -(Qco-Qkid)*fd2*X(11)*Rb_M/Kp2) /V2

C      5. Kidney
XP(12) = (Qkid*Rb_M*X(9)
c -Qkid*X(12)*Rb_M/Kpkid-CL*X(9) ) /Vkid

C      AUC Calculation

XP(13) = X(1)
XP(14) = X(5)
XP(15) = X(9)

C-----
C
C-----
C
C
C      Return
C      End

C#####
C

Subroutine OUTPUT(Y,T,X)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 Y(MaxNOE),T,X(MaxNDE)

CC

```

```

C-----
C
C   Enter Output Equations Below    {e.g.  Y(1) = X(1)/P(2)  }
C
C---c-----
C
      Y(1) = X(1)
      Y(2) = X(5)
      Y(3) = X(9)

      Y(4) = X(13)
      Y(5) = X(14)
      Y(6) = X(15)

C-----
C
C-----
C
C
      Return
      End

C#####
C

      Subroutine VARMOD(V,T,X,Y)
      Implicit None

      Include 'globals.inc'
      Include 'model.inc'

      Real*8 V(MaxNOE),T,X(MaxNDE),Y(MaxNOE)

CC
C-----
C
C   Enter Variance Model Equations Below
C
C      {e.g. V(1) = (PV(1) + PV(2)*Y(1))**2 }
C
C---c-----
C
      V(1:6) = (0.0001+PV(1)*Y(1:6))**2

C-----
C
C-----
C
C
      Return
      End

C#####
C

      Subroutine COVMOD(Pmean, ICmean, PC)

```

```

Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 PC(MaxNCP)
Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)

CC
C-----
C
C   Enter # of Covariate Parameters
C
C---c-----
C

      NCparam = 0      ! Enter # of Covariate Parameters.

CC
C-----
C
C   Enter Symbol for Covariate Params {eg: PCSym(1)='CLRenal'}
C
C---c-----
C

CC
C-----
C
C   For the Model Params. that Depend on Covariates Enter the Equation
C
C       {e.g. Pmean(1) = PC(1)*R(2) }
C
C---c-----
C

C-----
C
C-----
C
C
C
      Return
      End

C#####
C

      Subroutine POPINIT(PmeanI,ICmeanI,PcovI,ICcovI, PCI)
C   Initial parameter values for population program parameters (ITS,
MLEM)

Implicit None

Include 'globals.inc'
Include 'model.inc'

```



```

        Integer I,J
        Real*8 PmeanI (MaxNSP+MaxNDE) , ICmeanI (MaxNDE)
        Real*8 PcovI (MaxNSP+MaxNDE,MaxNSP+MaxNDE) ,
ICcovI (MaxNDE,MaxNDE)
        Real*8 PCI (MaxNCP)

CC
C-----
C
C   Enter Initial Values for Population Means
C
C       {   e.g. PmeanI(1) = 10.0       }
C
C-----c-----
C

CC
C-----
C
C   Enter Initial Values for Pop. Covariance Matrix (Lower Triang.)
C
C       {   e.g. PcovI(2,1) = 0.25       }
C
C-----c-----
C

CC
C-----
C
C   Enter Values for Covariate Model Parameters
C
C       {   e.g. PCI(1) = 2.0           }
C
C-----c-----
C

C-----
C
C-----
C
C
C
C       Return
C       End

C#####

Subroutine PRIOR(Pmean,Pcov,ICmean,ICcov)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I,J
Real*8 Pmean (MaxNSP+MaxNDE) , ICmean (MaxNDE)

```

```

Real*8 Pcov(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcov(MaxNDE,MaxNDE)

CC
C-----
C
C   Enter Nonzero Elements of Prior Mean Vector
C
C       { e.g. Pmean(1) = 10.0      }
C
C-----C-----
C

CC
C-----
C
C   Enter Nonzero Elements of Covariance Matrix (Lower Triang.)
C
C       { e.g. Pcov(2,1) = 0.25      }
C
C-----C-----
C

C-----
C
C-----
C
C
C
C       Return
C       End

C#####
C

Subroutine SPARAM(PS,P,IC)
Implicit None

Include 'globals.inc'

Real*8 PS(MaxNSECP), P(MaxNSP+MaxNDE), IC(MaxNDE)

CC
C-----
C
C   Enter Equations Defining Secondary Paramters
C
C       { e.g. PS(1) = P(1)*P(2)      }
C
C-----C-----
C

PS(1) = (58.82+P(2)*37.08+P(2)*897.4+3.0*6.16)/1000 !L/kg

C-----
C

```

```

C-----
C
C
      Return
      End

C#####
C

      Subroutine AMAT(A)
      Implicit None

      Include 'globals.inc'
      Include 'model.inc'

      Integer I,J
      Real*8 A(MaxNDE,MaxNDE)

      DO I=1,Ndeqs
        Do J=1,Ndeqs
          A(I,J)=0.0D0
        End Do
      End Do

CC
C-----
C
C   Enter non zero elements of state matrix {e.g. A(1,1) = -P(1) }
C
C---c-----
C

C-----
C
C-----
C
C
      Return
      End

C#####
C

```

ADAPT-5 code for MET

```

*****
C                                ADAPT                                *
C                                Version 5                            *
C*****
C                                *
C                                MODEL                                *
C                                *
C                                *
C    This file contains Fortran subroutines into which the user      *
C    must enter the relevant model equations and constants.          *
C    Consult the User's Guide for details concerning the format for  *
C    entered equations and definition of symbols.                    *
C                                *
C    1. Symbol-   Parameter symbols and model constants              *
C    2. DiffEq-   System differential equations                      *
C    3. Output-   System output equations                            *
C    4. Varmod-   Error variance model equations                    *
C    5. Covmod-   Covariate model equations (ITS,MLEM)               *
C    6. Popinit-  Population parameter initial values (ITS,MLEM)     *
C    7. Prior -   Parameter mean and covariance values (ID,NPD,STS) *
C    8. Sparam-   Secondary parameters                               *
C    9. Amat -    System state matrix                                *
C                                *
C*****
C#####
C
C    Subroutine SYMBOL
C    Implicit None
C
C    Include 'globals.inc'
C    Include 'model.inc'
C
CC
C-----
C
C    Enter as Indicated
C
C-----c-----
C
C
C    NDEqs   = 25  ! Enter # of Diff. Eqs.
C    NSParam = 7   ! Enter # of System Parameters.
C    NVparam = 1   ! Enter # of Variance Parameters.
C    NSecPar = 6   ! Enter # of Secondary Parameters.
C    NSecOut = 0   ! Enter # of Secondary Outputs (not used).
C    Ieqsol  = 1   ! Model type: 1 - DIFFEQ, 2 - AMAT, 3 - OUTPUT
C
C    only.
C    Descr   = 'mPBPK model with two tissue compartments for MET joint
C    fitting'

```

```

CC
C-----
C
C   Enter Symbol for Each System Parameter (eg. Psym(1)='Kel')
C
C---c-----
C
C
C       Psym(1)='fd1'
C       Psym(2)='Kp1'
C       Psym(3)='CL'
C
C       Psym(4)='k01dummy_H'
C       Psym(5)='k02dummy_H'
C
C       Psym(6)='k01dummy_M'
C       Psym(7)='k02dummy_M'
C
C
CC
C-----
C
C   Enter Symbol for Each Variance Parameter {eg: PVsym(1)='Sigma'}
C
C---c-----
C
C
C       PVsym(1)='Sigma'
C
C
CC
C-----
C
C   Enter Symbol for Each Secondary Parameter {eg: PSsym(1)='CLt'}
C
C---c-----
C
C
C       PSsym(1)='k01_H'
C       PSsym(2)='k02_H'
C
C       PSsym(3)='k01_M'
C       PSsym(4)='k02_M'
C
C       PSsym(5)='fd2'
C       PSsym(6)='Vss'
C
C-----
C
C-----
C
C
C       Return
C       End
C
C#####
C

```

```

Subroutine DIFFEQ(T,X,XP)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Real*8 T,X(MaxNDE),XP(MaxNDE)

Real*8 fd1,fd2,fdt
Real*8 Kp1,Kp2,Kpliver
Real*8 Vb,V1,V2,Vliver,Vss
Real*8 Qco,Qliver,CL
Real*8 Rb_H_iv,Rb_H,Rb_M
Real*8 k01_H,k02_H,tfirst_H,tmax_H,input_H
Real*8 k01_M,k02_M,tfirst_M,tmax_M,input_M

```

CC

```

C-----
C
C   Enter Differential Equations Below   {e.g.  XP(1) = -P(1)*X(1) }
C
C-----c-----
C

```

```

fd1      = P(1)
Kp1      = P(2)
CL       = P(3)

k01_H    = 0.0025+(0.0015*P(4))/(0.0015+P(4))
k02_H    = 0.0040+(0.0020*P(5))/(0.0020+P(5))

k01_M    = 0.0055+(0.0015*P(6))/(0.0015+P(6))
k02_M    = 0.0100+(0.0015*P(7))/(0.0015+P(7))

Vb       = 58.82      !mL/kg
V1       = 9.43       !mL/kg
V2       = 897.94     !mL/kg
Vliver   = 33.81      !mL/kg

Qco      = 179.91     !mL/min/kg
Qliver   = 75.348     !mL/min/kg

Kpliver  = 62.25

Rb_H_iv  = 1.70
Rb_H     = 1.508
Rb_M     = 1.607

tfirst_H = 60        !min
tfirst_M = 60        !min

tmax_H   = 135       !min
tmax_M   = 110       !min

Kp2 = Kp1

fd2 = 1-fd1
fdt = fd1+fd2

```

```

C      IV 0.5

C      1. Blood Compartment
      IF (fdt>1) THEN
        XP(1) = 0
      ELSE

        XP(1) = (R(1)+(Qco-Qliver)*fd1*Rb_H_iv*X(2)/Kp1
c +(Qco-Qliver)*fd2*Rb_H_iv*X(3)/Kp2
c +Qliver*X(4)*Rb_H_iv/Kpliver
c -((Qco-Qliver)*fd1*Rb_H_iv+(Qco-Qliver)
c *fd2*Rb_H_iv+Qliver*Rb_H_iv)*X(1))/(Vb*Rb_H_iv)

      ENDIF

C      2. Tissue Compartment 1
      XP(2) = ((Qco-Qliver)*fd1*Rb_H_iv*X(1)
c -(Qco-Qliver)*fd1*Rb_H_iv*X(2)/Kp1)/V1

C      3. Tissue Compartment 2
      XP(3) = ((Qco-Qliver)*fd2*Rb_H_iv*X(1)
c -(Qco-Qliver)*fd2*Rb_H_iv*X(3)/Kp2)/V2

C      4. Liver Compartment
      XP(4) = (Qliver*Rb_H_iv*X(1)
c - Qliver*X(4)*Rb_H_iv/Kpliver-CL*X(4)*Rb_H_iv/Kpliver)/Vliver

C      IV 1.0

C      1. Blood Compartment
      IF (fdt>1) THEN
        XP(5) = 0
      ELSE

        XP(5) = (R(2)+(Qco-Qliver)*fd1*Rb_H_iv*X(6)/Kp1
c +(Qco-Qliver)*fd2*Rb_H_iv*X(7)/Kp2
c +Qliver*X(8)*Rb_H_iv/Kpliver
c -((Qco-Qliver)*fd1*Rb_H_iv+(Qco-Qliver)
c *fd2*Rb_H_iv+Qliver*Rb_H_iv)*X(5))/(Vb*Rb_H_iv)

      ENDIF

C      2. Tissue Compartment 1
      XP(6) = ((Qco-Qliver)*fd1*Rb_H_iv*X(5)
c -(Qco-Qliver)*fd1*Rb_H_iv*X(6)/Kp1)/V1

C      3. Tissue Compartment 2
      XP(7) = ((Qco-Qliver)*fd2*Rb_H_iv*X(5)
c -(Qco-Qliver)*fd2*Rb_H_iv*X(7)/Kp2)/V2

C      4. Liver Compartment
      XP(8) = (Qliver*Rb_H_iv*X(5)
c - Qliver*X(8)*Rb_H_iv/Kpliver-CL*X(8)*Rb_H_iv/Kpliver)/Vliver

```

```

C      IV 2.0

C      1. Blood Compartment
      IF (fdt>1) THEN
        XP(9) = 0
      ELSE

        XP(9) = (R(3)+(Qco-Qliver)*fd1*Rb_H_iv*X(10)/Kp1
c +(Qco-Qliver)*fd2*Rb_H_iv*X(11)/Kp2
c +Qliver*X(12)*Rb_H_iv/Kpliver
c -((Qco-Qliver)*fd1*Rb_H_iv+(Qco-Qliver)
c *fd2*Rb_H_iv+Qliver*Rb_H_iv)*X(9))/(Vb*Rb_H_iv)

        ENDIF

C      2. Tissue Compartment 1
      XP(10) = ((Qco-Qliver)*fd1*Rb_H_iv*X(9)
c -(Qco-Qliver)*fd1*Rb_H_iv*X(10)/Kp1)/V1

C      3. Tissue Compartment 2
      XP(11) = ((Qco-Qliver)*fd2*Rb_H_iv*X(9)
c -(Qco-Qliver)*fd2*Rb_H_iv*X(11)/Kp2)/V2

C      4. Liver Compartment
      XP(12) = (Qliver*Rb_H_iv*X(9)
c - Qliver*X(12)*Rb_H_iv/Kpliver-CL*X(12)*Rb_H_iv/Kpliver)/Vliver

C      Healthy Rats

      IF (T<=tfirst_H) THEN
        input_H= k01_H*Bolus(1,1)

      ELSE IF (T>tfirst_H.and.T<=tmax_H) THEN
        input_H= k02_H*Bolus(1,1)

      ELSE
        input_H= 0

      END IF

C      1. Blood Compartment
      IF (fdt>1) THEN
        XP(13) = 0
      ELSE

        XP(13) = ((Qco*fd1*X(14)*Rb_H/Kp1+Qco*fd2*X(15)*Rb_H/Kp2
c +Qliver*X(16)*Rb_H/Kpliver)
c -(Qco*fd1*Rb_H+Qco*fd2*Rb_H+Qliver*Rb_H)
c *X(13))/(Vb*Rb_H)

        ENDIF

C      2. Tissue Compartment 1
      XP(14) = (Qco*fd1*Rb_H*X(13)-Qco*fd1*X(14)*Rb_H/Kp1)/V1

C      3. Tissue Compartment 2

```



```

XP(15) = (Qco*fd2*Rb_H*X(13)
c -Qco*fd2*X(15)*Rb_H/Kp2)/V2

C    4. Liver Compartment
      XP(16) = (input_H+Qliver*Rb_H*X(13)
c - Qliver*X(16)*Rb_H/Kpliver-CL*X(16)*Rb_H/Kpliver)/Vliver

C    Malnoursihed Rats

      IF (T<=tfirst_M) THEN
input_M= k01_M*Bolus(1,2)

      ELSE IF (T>tfirst_M.and.T<=tmax_M) THEN
input_M= k02_M*Bolus(1,2)

      ELSE
input_M= 0

      END IF

C    1. Blood Compartment
      IF (fdt>1) THEN
XP(17) = 0
      ELSE

      XP(17) = ((Qco*fd1*X(18)*Rb_M/Kp1+Qco*fd2*X(19)*Rb_M/Kp2
c +Qliver*X(20)*Rb_M/Kpliver)
c -(Qco*fd1*Rb_M+Qco*fd2*Rb_M+Qliver*Rb_M)
c *X(17))/(Vb*Rb_M)

      ENDIF

C    2. Tissue Compartment 1
      XP(18) = (Qco*fd1*Rb_M*X(17)-Qco*fd1*X(18)*Rb_M/Kp1)/V1

C    3. Tissue Compartment 2
      XP(19) = (Qco*fd2*Rb_M*X(17)
c -Qco*fd2*X(19)*Rb_M/Kp2)/V2

C    4. Liver Compartment
      XP(20) = (input_M+Qliver*Rb_M*X(17)
c - Qliver*X(20)*Rb_M/Kpliver-CL*X(20)*Rb_M/Kpliver)/Vliver

C    Calculation of AUC

      XP(21) = X(1)/Rb_H_iv
      XP(22) = X(5)/Rb_H_iv
      XP(23) = X(9)/Rb_H_iv

      XP(24) = X(13)/Rb_H

      XP(25) = X(17)/Rb_M

C-----
C
C-----
C

```

```

C
    Return
    End

C#####

C

    Subroutine OUTPUT(Y,T,X)
    Implicit None

    Include 'globals.inc'
    Include 'model.inc'

    Real*8 Y(MaxNOE),T,X(MaxNDE)

CC
C-----
C
C   Enter Output Equations Below   {e.g.  Y(1) = X(1)/P(2)  }
C
C-----C-----
C

    Y(1) = X(1)
    Y(2) = X(5)
    Y(3) = X(9)

    Y(4) = X(13)

    Y(5) = X(17)

C   Calculation of AUC

    Y(6) = X(21)
    Y(7) = X(22)
    Y(8) = X(23)

    Y(9) = X(24)

    Y(10) = X(25)

C-----
C
C-----
C
C
C
    Return
    End

C#####
C

    Subroutine VARMOD(V,T,X,Y)
    Implicit None

    Include 'globals.inc'

```

```

      Include 'model.inc'

      Real*8 V(MaxNOE),T,X(MaxNDE),Y(MaxNOE)

CC
C-----
C
C   Enter Variance Model Equations Below
C
C       {e.g. V(1) = (PV(1) + PV(2)*Y(1))**2 }
C
C-----C-----
C
      V(1:10) = (0.0001+PV(1)*Y(1:10))**2

C-----
C
C-----
C
C
      Return
      End

C#####
C

      Subroutine COVMOD(Pmean, ICmean, PC)
      Implicit None

      Include 'globals.inc'
      Include 'model.inc'

      Real*8 PC(MaxNCP)
      Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)

CC
C-----
C
C   Enter # of Covariate Parameters
C
C-----C-----
C
      NCparam = 0      ! Enter # of Covariate Parameters.

CC
C-----
C
C   Enter Symbol for Covariate Params {eg: PCsym(1)='CLRenal'}
C
C-----C-----
C

CC
C-----
C

```

```

C   For the Model Params. that Depend on Covariates Enter the Equation
C
C       {e.g. Pmean(1) =  PC(1)*R(2) }
C
C-----C-----
C
C-----C-----
C
C-----C-----
C
C
C       Return
C       End

C#####
C
C       Subroutine POPINIT(PmeanI,ICmeanI,PcovI,ICcovI, PCI)
C   Initial parameter values for population program parameters (ITS,
C   MLEM)

C       Implicit None

C       Include 'globals.inc'
C       Include 'model.inc'

C       Integer I,J
C       Real*8 PmeanI(MaxNSP+MaxNDE), ICmeanI(MaxNDE)
C       Real*8 PcovI(MaxNSP+MaxNDE,MaxNSP+MaxNDE),
C   ICcovI(MaxNDE,MaxNDE)
C       Real*8 PCI(MaxNCP)

CC
C-----C-----
C
C   Enter Initial Values for Population Means
C
C       { e.g. PmeanI(1) = 10.0      }
C
C-----C-----
C
C
CC
C-----C-----
C
C   Enter Initial Values for Pop. Covariance Matrix (Lower Triang.)
C
C       { e.g. PcovI(2,1) = 0.25      }
C
C-----C-----
C
C
CC

```

```

C-----
C
C   Enter Values for Covariate Model Parameters
C
C       {   e.g. PCI(1) = 2.0       }
C
C-----c-----
C
C
C-----
C
C-----
C
C
C
C       Return
C       End
C
C#####
C
C       Subroutine PRIOR(Pmean,Pcov,ICmean,ICcov)
C       Implicit None
C
C       Include 'globals.inc'
C       Include 'model.inc'
C
C       Integer I,J
C       Real*8 Pmean(MaxNSP+MaxNDE), ICmean(MaxNDE)
C       Real*8 Pcov(MaxNSP+MaxNDE,MaxNSP+MaxNDE), ICcov(MaxNDE,MaxNDE)
C
CC
C-----
C
C   Enter Nonzero Elements of Prior Mean Vector
C
C       {   e.g. Pmean(1) = 10.0       }
C
C-----c-----
C
C
C
CC
C-----
C
C   Enter Nonzero Elements of Covariance Matrix (Lower Triang.)
C
C       {   e.g. Pcov(2,1) = 0.25       }
C
C-----c-----
C
C
C
C-----
C
C-----
C
C-----
C
C
C

```

```

Return
End

C#####
C

Subroutine SPARAM(PS,P,IC)
Implicit None

Include 'globals.inc'

Real*8 PS(MaxNSECP), P(MaxNSP+MaxNDE), IC(MaxNDE)

CC
C-----
C
C   Enter Equations Defining Secondary Paramters
C
C       {   e.g.   PS(1) = P(1)*P(2)   }
C
C-----c-----
C
C
C       PS(1)  = 0.0025+(0.0015*P(4))/(0.0015+P(4))      !µg/min/kg
C       PS(2)  = 0.0040+(0.0020*P(5))/(0.0020+P(5))      !µg/min/kg
C       PS(3)  = 0.0055+(0.0015*P(6))/(0.0015+P(6))      !µg/min/kg
C       PS(4)  = 0.0100+(0.0015*P(7))/(0.0015+P(7))      !µg/min/kg
C
C       PS(5)  = 1-P(1)
C       PS(6)  = (58.82+9.43*P(2)+897.94*P(2)+33.81*62.25)/1000 !L/kg
C
C-----
C
C-----
C
C
C       Return
C       End

C#####
C

Subroutine AMAT(A)
Implicit None

Include 'globals.inc'
Include 'model.inc'

Integer I,J
Real*8 A(MaxNDE,MaxNDE)

DO I=1,Ndeqs
  DO J=1,Ndeqs
    A(I,J)=0.0D0
  End Do
End Do

CC

```

```

C-----
C
C   Enter non zero elements of state matrix   {e.g.  A(1,1) = -P(1)  }
C
C---c-----
C

C-----
C
C-----
C
C
C
      Return
      End

C#####
C

```

Model Fitting in ADAPT

The model fittings of blood concentration-time profile were conducted by using the maximum likelihood method in ADAPT 5. The variance model was identified as:

$$V_i = (\sigma_{\text{intercept}} + \sigma_{\text{slope}} Y_i)^2$$

where V_i is the variance of the i th data point, Y_i is the i th estimated concentration by model, variance parameters $\sigma_{\text{intercept}}$ and σ_{slope} are variance model parameters. The goodness-of-fit (GoF) of the model was evaluated by visual inspection, Akaike Information Criterion (AIC), Schwarz Criterion (SC), and Coefficient of Variation (CV %) of the estimates.