# Delineating the Complex Interplay of Enzymes and Transporters Governing the Absorption and Disposition of Atorvastatin and the Metabolites Using Physiologically Based Pharmacokinetic (PBPK) Modeling

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## ABSTRACT

Atorvastatin (ATS) is widely used to treat high cholesterol and potentially lower the risk of cardiac complications. Absorption and disposition of atorvastatin and the metabolites 2-hydroxy atorvastatin (2-OH ATS), 4-hydroxy atorvastatin (4-OH ATS) and atorvastatin lactone (AL) is governed by complex interplay of multiple enzymes and transporters.

## OBJECTIVES

i. To develop a mechanistic physiologically based pharmacokinetic (PBPK) model delineating the role of enzymes and transporters in the absorption and disposition of atorvastatin, using a middle-out approach by incorporating the *in vitro* and *in vivo* data representing all the key mechanisms that reproduced the observed clinical data of atorvastatin and the metabolites 2-OH ATS, 4-OH ATS and AL across the dose range of 10-80 mg.

ii. To verify the role of individual enzymes and transporters by using drug-drug interaction (DDI) studies with rifampicin, gemfibrozil and itraconazole.

iii. To verify the observed food effect.

## METHODS

**PBPK modeling:** PBPK modeling was performed using simulation software GastroPlus Ver. 9.8.2.

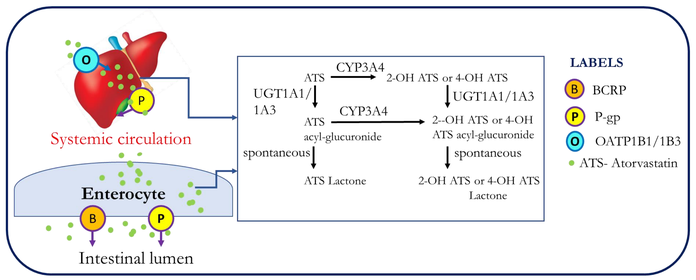


**Figure 1: Schematic View of Enzymes and Transporters Involved in Absorption and Disposition of Atorvastatin in Human Body**

**Table 1: Input Parameters used for Atorvastatin PBPK Model Development**

| Parameter | Value | Reference |
|---|---|---|
| Molecular weight | 558.64 | PubChem |
| LogP | 4.1 | (Poli 2007) (Gertz et al. 2011) |
| Ionisation constant (pKa) | 11.05 (acid); 4.46 (acid) | (Gertz et al. 2011) |
| Human Jejunal $P_{eff}$ ($\times 10^{-4}$ cm/s) | 3.97 | (Human jejunal $P_{eff}$ value is estimated from $P_{app(A-B)}$ data in Caco2) (Wu et al. 2000) |
| Blood Plasma concentration ratio ($R_{b/p}$) | 0.55 | (Morse et al. 2019) |
| Fraction unbound in plasma ($F_{up}$) % | 2.0 | CPBR-FDA-1996 |
| **Enzyme-mediated kinetics** | | |
| CYP3A4 (Formation of 2-OH AA) | | |
| $K_m$ (µM) | 29.7 | (Jacobsen et al. 2000) |
| $V_{max}$ (pmol/min/pmol of isoform) | 14.7 | Optimized value |
| CYP3A4 (Formation of 4-OH AA) | | |
| $K_m$ (µM) | 25.6 | (Jacobsen et al. 2000) |
| $V_{max}$ (pmol/min/pmol of isoform) | 3.0 | Optimized value |
| UGT1A1(Formation of AL) | | |
| $K_m$ (µM) | 2 | (Schirris et al. 2015) |
| $V_{max}$ (pmol/sec/mg protein) | 0.15 | Optimized value |
| UGT1A3 (Formation of AL) | | |
| $K_m$ (µM) | 4 | (Schirris et al. 2015) |
| $V_{max}$ (pmol/sec/mg protein) | 0.3 | Optimized value |

| Parameter | Value | Reference |
|---|---|---|
| **Transporter-mediated kinetics** | | |
| OATP1B1(liver-basolateral) | | |
| Km (µM) | 0.77 | (Karlgren et al. 2012) |
| Vmax (mg/s) | 0.1 | Optimized value |
| OATP1B3 (liver-basolateral) | | |
| Km (µM) | 0.73 | (Karlgren et al. 2012) |
| Vmax (mg/s) | 0.1 | Optimized value |
| P-gp | | |
| Km (µM) | 10.7 | (Deng et al. 2021) |
| Vmax (mg/s) (gut-apical) | 0.01 | Optimized value |
| Vmax (mg/s) (liver-apical) | 0.005 | Optimized value |
| BCRP (gut-apical) | | |
| Km (µM) | 82.4 | (Deng et al. 2021) |
| Vmax (mg/s) | 0.05 | Optimized value |
| Renal Clearance | Fup*GFR | Assumed value (since renal elimination is <1%) |
| $CL_{PD}$ (mL/min/million cells) | 0.02 | (Zhang 2015) |

## RESULTS

### PBPK Model Development and Verification of Atorvastatin and Its Metabolites

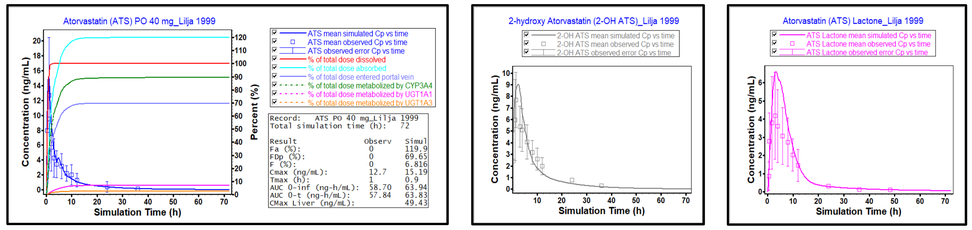


**Figure 2: Cp-time Profile for a 40 mg Oral Dose of Atorvastatin in Healthy Subjects Under Fasting Conditions**

Simulated blue line (—) and observed blue squares (■) of atorvastatin (A), simulated grey line (—) and observed grey squares (■) of 2-hydroxy atorvastatin (B), and simulated pink line (—) and observed pink squares (■) of atorvastatin lactone (C) (Lilja et al. 1999). The plot also displays simulated total amount of dose dissolved (red), absorbed (blue), entered portal vein (purple), metabolized by CYP3A4 (green), UGT1A1 (pink), and UGT1A3 (orange) are shown as a percent of total administered dose (A).

### Model Verification: Comparison of Predicted vs. Observed PK Data of Atorvastatin and Its Metabolites

| Reference | Dose (mg) | Condition | PK Parameter | Observed# | Simulated | Sim/Obs Ratio |
|---|---|---|---|---|---|---|
| (Lilja et al. 1999) | 40 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 12.7 ± 7.8 | 15.2 | **1.2** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 61.4 ± 36.2 | 63.9 | **1.04** |
| (Nagard et al. 2021) | 10 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 2.0 | 2.01 | **1.01** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 11.0 | 12.3 | **1.12** |
| (Fukazawa et al. 2004) | 10 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 4.6 (3.2-6.6) | 3.85 | **0.84** |
| | | | $AUC_{(0\text{-}48)}$ (ng.hr/mL) | 19.0 (12.6-28.6) | 19.2 | **1.01** |
| (Backman et al. 2005) | 20 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 8.2 | 7.05 | **0.86** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 35.2 | 31.5 | **0.89** |
| (McKeand et al. 2018) | 20 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 7.8 (3.7-18.9) | 8.33 | **1.07** |
| | | | $AUC_{(0\text{-}24)}$ (ng.hr/mL) | 36 (19-96) | 32.1 | **0.89** |
| (Shin et al. 2011) | 20 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 5 (3.0-13.9) | 6.7 | **1.33** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 38.6 (31.9-51.6) | 29.8 | **0.77** |
| (Khalilieh et al. 2017) | 20 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 7.87 | 6.9 | **0.88** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 39.9 | 30.5 | **0.76** |
| (Dingemanse et al. 2014) | 20 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 4.2 | 6.43 | **1.53** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 35.07 | 29.2 | **0.83** |
| (Malm-Erjefalt et al. 2015) | 40 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 12.1 (3.1–51.1) | 16.9 | **1.4** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 61.6(22.1–158.4) | 63.2 | **1.03** |
| (Whitfield et al. 2011) | 40 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 16.7 | 17.04 | **1.02** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 62.8 | 64.4 | **1.03** |
| (Backman et al. 2005) | 40 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 15.8 ± 5.4 | 15.1 | **0.96** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 64.0 ± 21.3 | 64.4 | **1.01** |
| (Bullman et al. 2011) | 40 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 14.3 | 14.3 | **1.0** |
| | | | $AUC_{(0\text{-}24)}$ (ng.hr/mL) | 62 | 56.5 | **0.91** |
| | 40 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 12.1 | 14.2 | **1.17** |
| | | | $AUC_{(0\text{-}24)}$ (ng.hr/mL) | 63.4 | 56.2 | **0.89** |
| (Machado et al. 2014) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 27.1 | 22.1 | **0.82** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 146.8 | 128.9 | **0.88** |
| (Chung et al. 2006) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 29.5 | 35 | **1.19** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 167 | 135.3 | **0.81** |
| Lipitor (ANDA-Ranbaxy) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 33.2 | 36 | **1.08** |
| | | | $AUC_{(0\text{-}96)}$ (ng.hr/mL) | 131.1 | 139.4 | **1.06** |
| Lipitor (ANDA-Apotex) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 35.8 | 36 | **1.01** |
| | | | $AUC_{(0\text{-}48)}$ (ng.hr/mL) | 158.5 | 139.2 | **0.88** |
| Lipitor (ANDA-Sandoz) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 35.5 | 35 | **0.99** |
| | | | $AUC_{(0\text{-}60)}$ (ng.hr/mL) | 139 | 137.3 | **0.99** |

| Reference | Metabolite | PK Parameter | Observed# | Simulated | Sim/Obs Ratio |
|---|---|---|---|---|---|
| (Lilja et al. 1999) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 7.7 ± 2.4 | 9.05 | **1.18** |
| | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 77.5 ± 24 | 70.5 | **0.91** |
| | atorvastatin lactone | $C_{max}$ (ng/mL) | 4.2 ± 2.4 | 6.6 | **1.57** |
| | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 53 ± 27.3 | 64.6 | **1.22** |
| (Nagard et al. 2021) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 1.8 | 2.05 | **1.14** |
| | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 23.5 | 17.4 | **0.74** |
| (McKeand et al. 2018) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 4.0 (1.6-9.2) | 5.25 | **1.3** |
| | | $AUC_{(0\text{-}24)}$ (ng.hr/mL) | 39 (19-79) | 38 | **0.97** |
| (Dingemanse et al. 2014) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 3.02 | 4.15 | **1.37** |
| | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 43 | 33.1 | **0.77** |
| (Whitfield et al. 2011) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 12.3 | 9.9 | **0.81** |
| | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 86.8 | 71.6 | **0.82** |
| | atorvastatin lactone | $C_{max}$ (ng/mL) | 4.2 | 6.4 | **1.52** |
| | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 50.7 | 60.9 | **1.2** |
| (Bullman et al. 2011) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 10.3 (9-11.7) | 8.6 | **0.84** |
| | | $AUC_{(0\text{-}24)}$ (ng.hr/mL) | 75 (67-83.6) | 62.2 | **0.83** |
| | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 10.5 (9-12) | 8.6 | **0.82** |
| | | $AUC_{(0\text{-}24)}$ (ng.hr/mL) | 85.2 (75-97) | 62.4 | **0.73** |
| Lipitor (ANDA-Ranbaxy) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 29.9 | 17 | **0.57** |
| | | $AUC_{(0\text{-}96)}$ (ng.hr/mL) | 168.6 | 137.3 | **0.81** |
| | 4-OH atorvastatin | $C_{max}$ (ng/mL) | 0.93 | 1.7 | **1.9** |
| | | $AUC_{(0\text{-}96)}$ (ng.hr/mL) | 19.2 | 18.1 | **0.94** |
| Lipitor (ANDA-Apotex) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 28.5 | 17 | **0.6** |
| | | $AUC_{(0\text{-}48)}$ (ng.hr/mL) | 173.3 | 132.7 | **0.77** |
| | 4-OH atorvastatin | $C_{max}$ (ng/mL) | 0.98 | 1.69 | **1.7** |
| | | $AUC_{(0\text{-}48)}$ (ng.hr/mL) | 16.7 | 17.5 | **1.05** |
| Lipitor (ANDA-Sandoz) | 2-OH atorvastatin | $C_{max}$ (ng/mL) | 25.3 | 17 | **0.7** |
| | | $AUC_{(0\text{-}60)}$ (ng.hr/mL) | 150.1 | 133 | **0.9** |
| | 4-OH atorvastatin | $C_{max}$ (ng/mL) | 0.9 | 1.67 | **1.9** |
| | | $AUC_{(0\text{-}60)}$ (ng.hr/mL) | 16.2 | 17.5 | **1.1** |

IR = Immediate release #Observed represent values from the average of individual values and represent mean ± SD and the range enclosed in brackets, as specified in the summary of tabulated PK parameters in the respective publications.

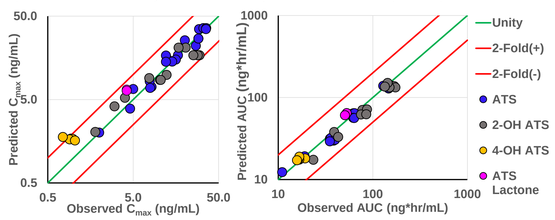


**Figure 3: Observed vs Predicted Values for $C_{max}$ and AUC of Atorvastatin and Its Metabolites**

### Model Verification: Food Effect

| Reference | Dose (mg) | Condition | PK Parameter | Observed# | Simulated | Sim/Obs Ratio |
|---|---|---|---|---|---|---|
| Lipitor (ANDA-Ranbaxy) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 33.2 | 36 | **1.08** |
| | | | $AUC_{(0\text{-}96)}$(ng.hr/mL) | 131.1 | 139.4 | **1.06** |
| | | Fed | $C_{max}$ (ng/mL) | 29.03 | 27 | **0.93** |
| | | | $AUC_{(0\text{-}96)}$(ng.hr/mL) | 128.9 | 138.7 | **1.08** |
| Lipitor (ANDA-Apotex) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 35.8 | 36 | **1.01** |
| | | | $AUC_{(0\text{-}48)}$ (ng.hr/mL) | 158.5 | 139.2 | **0.88** |
| | | Fed | $C_{max}$ (ng/mL) | 29.02 | 27 | **0.92** |
| | | | $AUC_{(0\text{-}48)}$ (ng.hr/mL) | 146.6 | 129.6 | **0.88** |
| (Chung et al. 2006) | 80 mg, IR tablet | Fasted | $C_{max}$ (ng/mL) | 29.5 | 35 | **1.19** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 167 | 135.3 | **0.81** |
| | | Fed | $C_{max}$ (ng/mL) | 20.1 | 25.7 | **1.28** |
| | | | $AUC_{(0\text{-}inf)}$ (ng.hr/mL) | 148 | 133.8 | **0.9** |

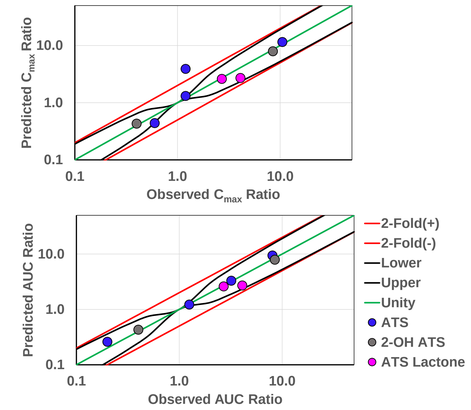


**Figure 4: Observed vs Predicted DDI Ratios for $C_{max}$ and AUC of Atorvastatin and Its Metabolites 2-OH ATS and ATS Lactone with Rifampicin, Itraconazole and Gemfibrozil**

Circles represent the DDI ratios for $C_{max}$ and $AUC_{0\text{-}t}$ respectively Blue (●) ATS, Grey (●) 2-OH ATS, and Pink (●) ATS Lactone. Red lines (—) represent 2-fold prediction error, and black lines (—) represent fold prediction error per Guest's criteria.

## CONCLUSION

- Overall, our mechanistic model well captured the observed plasma profiles of 10 mg, 20 mg, 40 mg, and 80 mg doses and the predicted values for $C_{max}$ and AUC were mostly within Bioequivalence (BE) limits (0.8 – 1.25-fold) of observed data for atorvastatin and within 2-fold for its metabolites.
- Food moderately affected the $C_{max}$ (~10-25% reduction) by delaying the gastric emptying but had minimal effect on AUC.
- The simulated DDI ratios of $C_{max}$ and AUCs for ATS and its metabolites with rifampicin and gemfibrozil are in excellent agreement with the observed data.
- In the DDI study with itraconazole, the simulated DDI ratio of AUC was well predicted, however for the $C_{max}$ the simulated DDI ratio did not fall within the Guest limits.

## REFERENCES

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