Challenges in Predicting Colonic Luminal and Tissue Concentrations of Mesalamine and Acetyl Mesalamine Using Physiologically Based Biopharmaceutics Modeling

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Absorption, Biopharmaceutics Modeling, Colonic luminal concentration, Mesalamine, NAT1, Tissue concentration, PBBM

# Graphical abstract

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# Abstract

Mesalamine is a standard first-line therapy for managing chronic inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis. Despite its established efficacy, the precise mechanism of action within enterocytes remains unclear. This study aimed to develop and validate Physiologically Based Biopharmaceutics Models (PBBM) for mesalamine (5-ASA) and its metabolite, acetyl mesalamine (Ac-5-ASA), to predict drug concentrations in plasma, colonic lumen, and colonic tissue of healthy subjects and compare the results to measured concentrations. Using the Simcyp Simulator (V22), the models accurately predicted plasma concentrations for various formulations, including intravenous, oral immediate-release and controlled release formulations within a two-fold range. Results also captured the intestinal and hepatic metabolism converting mesalamine to acetyl mesalamine. However, significant discrepancies were observed in predicting luminal and tissue concentrations, with underpredictions for Claversal and Pentasa formulations reaching factors of up to 506 and 55 for 5-ASA and Ac-5-ASA in colonic tissue, respectively. These discrepancies highlight limitations in current modeling approaches, particularly in simulating drug accumulation within enterocytes. Despite these challenges, this investigation highlights both the potential benefits and the complexities of using PBBMs. Future work should focus on generating definitive N-acetyl transferase (NAT1) abundance data with an *in-vitro in-vivo* extrapolation link, improving approaches to better explore local drug concentrations in the gastrointestinal tract, and addressing the gap in accurately predicting luminal and tissue concentrations in the colon.

# Introduction

Inflammatory bowel diseases (IBD) like Crohn’s disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of the gastrointestinal tract. Globally, the estimated number of IBD cases rose from 3.32 million in 1990 to 4.90 million in 2019, marking a 47.45% increase over this period[1, 2]. These conditions are debilitating and presently have no cure. Mesalamine if the standard first-line therapy for UC, which only affects the colon. Mesalamine, also known as 5-ASA, is effective in inducing or maintaining remission in mild-to-moderate cases of UC. Nonetheless, the precise mechanism of its action remains unclear[3, 4]. 5-ASA has demonstrated efficacy both in oral administration and rectal therapy, which can be in the form of suspension (enema), suppository, gel, or foam. Additionally, combining oral and rectal formulations has also proven to be effective[5]. Several oral 5-ASA formulations are available, including immediate and controlled-release versions. The development of controlled-release formulations was prompted by the documented ineffectiveness of simple 5-ASA tablets or capsules when taken orally[6-9]. Marketed formulations such as Salofalk, Apriso, Claversal, and Pentasa, are designed to release the drug in the distal part of the small intestine and the colon. Although 5-ASA is not well absorbed due to its poor intestinal permeability, its uptake is facilitated by the extensive transit time and surface area of the small bowel, leading to substantial drug absorption in the small intestine[10]. However, this also makes it difficult to achieve therapeutic drug levels in the distal gut, particularly in the colonic tissue, which are the primary target for treatment[10]. The absorbed 5-ASA is metabolized by the N-acetyl transferase (NAT1) enzyme, converting it to N-acetyl-5-ASA (Ac-5-ASA) in both the gut epithelium and the liver[11]. Although Ac-5-ASA retains limited therapeutic activity, it can be secreted into the intestinal lumen through transporter-mediated efflux[12].

Traditionally, plasma concentrations have served as a surrogate for local tissue concentrations due to technical challenges in direct measurement[13]. However, locally acting gastrointestinal (GI) products present a unique challenge where the plasma concentration is not directly aligned with the clinical effect site, leading to a disconnect between the pharmacokinetic (PK) profile and therapeutic effectiveness. Upon administration, the active drug ingredient is released from the product, either in a dissolved state, making it immediately accessible at the site of action, or in an undissolved state, requiring dissolution in the GI tract before becoming accessible. The extent and rate of drug availability at the site of action are primarily influenced by the dissolution process and GI tract transit, rather than by plasma concentration[14, 15]. Nevertheless, a small number of studies have reported systemic-driven colonic tissue concentrations, indicating potential systemic influences on the concentrations of the drug within the colonic tissues[16, 17]. The US FDA has recommended that additional pharmacokinetic metrics for generic product development beyond AUC and Cmax, such as partial AUC, mean residence time, and steady-state Cmax, can help detect significant differences in 5-ASA release profiles between test and reference products at the site of action. By evaluating these metrics, along with *in vitro* dissolution characteristics, the FDA can assess drug availability at target sites[18, 19]. Studies addressing these questions have primarily relied on indirect methods such as assessing plasma kinetics, excretion through renal, fecal, or ileostomy routes, or utilizing radiographic or scintigraphic techniques with markers. Specifically, the concentration of 5-ASA from various formulations at specific sites within the intestine is still mostly unknown [20]. However, given that 5-ASA exerts its anti-inflammatory effects at the tissue site in GIT, understanding its local concentration is crucial.

Application of Physiologically Based Biopharmaceutics Modeling (PBBM) or Physiologically Based Pharmacokinetics (PBPK) modeling for biopharmaceutics applications has become more frequently used due to its regulatory acceptance, potential to waive clinical studies, and resulting cost and time savings in drug product development[13, 21, 22]. PBBM can be used throughout the drug development process to optimize the *in vivo* performance of the drug product, enabling absorption and dose optimization, risk assessment, and support regulatory decision-making. Several software packages, such as GastroPlus, Simcyp, PK-Sim, and GI-Sim, are available to predict the *in vivo* performance of a drug product[21, 23-36]. The scope of PBPK/PBBM extends beyond predicting plasma drug concentrations; several studies have demonstrated their use in predicting drug concentrations at various sites of action, including the brain, bone, adipose tissue, heart, lungs, muscle, and tumor tissues[37-40]. However, no study has yet demonstrated the predictive capability for local tissue and luminal drug concentrations, nor has there been a comparison with available clinical data for gastrointestinal (GI) locally acting compounds.

The primary objective of our study was to predict the concentrations of 5-ASA and Ac-5-ASA in the colonic lumen and colonic tissue for the Claversal 500 and Pentasa 500 formulations using the Simcyp simulator and compare the results with observed clinical data. We collected and used systemic data on 5-ASA and Ac-5-ASA from a total of 12 clinical trials, including 10 from literature and 2 based on in-house data, to develop and validate the model. The model was specifically designed to account for the metabolic conversion of 5-ASA to Ac-5-ASA by the NAT1 enzyme, and its accuracy was confirmed against systemic clinical data.

# Materials and Methods

## Modelling Workflow

The PBBM of 5-ASA and Ac-5-ASA were developed and validated against clinical data in healthy subjects available in the literature. NAT1 enzyme was incorporated to capture the conversion of 5-ASA to Ac-5-ASA. The validated model was then used to predict drug concentrations in plasma, colonic lumen, and colonic tissue of healthy subjects following oral intake of Claversal 500 and Pentasa 500 formulations. Additionally, predictive accuracy was evaluated using USP II and USP IV dissolution inputs for Pentasa 500 and 1000. The detailed modeling workflow is depicted in **Figure 1**.



**Figure 1.** Detailed modeling workflow for the development of the PBBM of 5-ASA and Ac-5-ASA, encompassing NAT1 enzyme kinetics, USP II and USP IV dissolution inputs, and the model scheme, designed to predict luminal and tissue concentrations in the colon using Simcyp simulator.

## Software

The Simcyp PBPK Simulator (Version 22 Release 1, Certara UK Limited, Sheffield, UK) was used to build the model for 5-ASA and its metabolite Ac-5-ASA in healthy subjects. Clinical plasma concentration-time data from the literature were digitized with WebPlotDigitizer (version 4.6, A Rohatgi, 2022)[41]. Noncompartmental analyses were performed using PKPlus (Simulations Plus, Lancaster, USA). The figures were generated using GraphPad Prism (Version 9.5.1) and Python (Version 3.9).

## Data Collection

In our study, we selected 5-ASA and Ac-5-ASA as model compounds. Relevant physicochemical properties, *in vitro* ADME data, and clinical PK data from healthy volunteers were collected from the literature. The clinical data for Claversal 500 and Pentasa 500 were generated internally at KU Leuven. This study involved the measurement of plasma concentration-time profiles, and total luminal and tissue concentrations of 5-ASA and Ac-5-ASA in the cecum region in healthy subjects. The concentrations in colonic biopsies were measured in ng/mg of dry tissue, and then converted into ng/ml using a tissue density of 1.042 g/ml[42]. These were subsequently compared to the predicted enterocyte concentrations. Moreover, the luminal drug concentrations were measured at a single time point in the clinical study. Detailed information regarding the study can be found in **Section 1 of the Supplementary Materials**.

Clinical data on 5-ASA from 12 trials involving intravenous, oral solution, suspension, uncoated capsule, and controlled release formulations were collected for model development, validation, and application. The summary of the demographic information for subjects in these trials is presented in **Table 1**. Plasma data from clinical studies 1-4 was used in stepwise model building and parameter fitting, while data from studies 5-10 were used for external validation. Clinical studies 11 and 12, which involved Pentasa 500 and Claversal 500, were used for model application where luminal and tissue concentrations of 5-ASA and Ac-5-ASA were predicted in the colon compartment. For each simulation, the trial design was adapted to match the dose, age range, and proportion of females in the reported clinical study under a fasted state. The number of subjects in each trial was set as 100, following the default setting in Simcyp.

**Table 1.** Summary of clinical studies in healthy subjects that were used for model development (1-4), validation (5-10) and application (11-12).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | Formulation | Dose (mg) | No of subjects (Gender) | Age (years) | Weight/BMI | Ref. |
| 1 | IV | 100 | 3 (M), 3 (F) | 19-41 | 56-97 kg | [43] |
| 2 | IV | 250 | 3 (M), 3 (F) | 19-41 | 56-97 kg | [11] |
| 3 | IV | 500 | 6 (F) | 44-58 | - | [44] |
| 4 | Solution | 100 | 13 (M), 7 (F) | 18-51 | 17.8-35.2 kg/m2 | [45] |
| 5 | Suspension | 1000 | 24 (M) | 27.9 | 75.8 kg | [46] |
| 6 | Uncoated capsule | 2400 | 6 (F) | 44-58 | - | [44] |
| 7 | Salofalk | 500 | 18 (M) | 18-36 | 55-77.5 kg | [47] |
| 8 | EM enteric-coated tab | 500 | 18 (M) | 18-36 | 55-77.5 kg | [47] |
| 9 | Apriso | 1125 | 4 (M), 3(F) | 20-51 | 21.3-44.7 kg/m2 | [45] |
| 10 | Pentasa 1000 | 1000 | 7 (M), 3(F) | 22-51 | 17.6-45.2 kg/m2 | [45] |
| 11 | Claversal 500 | 500 | 4 (M), 1 (F) | 22-26 | - | [48] |
| 12 | Pentasa 500 | 500 | 4 (M), 1 (F) | 22-26 | - | [48] |

IV: Intravenous, M: Male, F: Female, BMI: Body mass index

## Model Development and Validation

The 5-ASA and Ac-5-ASA models were developed in Simcyp with data extracted from the literature. The overall summary of input parameters used is given in **Table 2**.

**Table 2.** Model input parameters of 5-ASA and Ac-5-ASA used during simulations.

|  |  |  |
| --- | --- | --- |
| Parameters | 5-ASA | Ac-5-ASA |
| Molecular wt. (g/mol) | 153.14 | 198.19 |
| Log Po:w | 1.2 | 1.6 |
| pKa1 | 2.30 | 2.7 |
| pKa2 | 5.69 | 12.9 |
| B/P | 1.3 | 1 |
| Fraction unbound in plasma | 0.57 | 0.22 |
| Ptrans,0 (cm/s) | 0.145 | - |
| Peff,man (10-4 cm/s) | - | 3.4925 |
| Intrinsic solubility (mg/ml) | 1.19 | 0.0668 |
| Vss (L/kg) | 0.2228 | 0.56937 |
| Clearance | Enzyme kinetics  (See under metabolism) | CLR 30 L/h |

LogPo:w: octanol-water partition coefficient, pka: dissociation constant, B/P: blood to plasma ratio, Ptrans,o: intrinsic passive membrane permeability, Peff,man: passive intestinal permeability, Vss: volume of distribution at steady state, CLR: renal clearance

### Physicochemical data

The physicochemical data of 5-ASA and Ac-5-ASA were collected from the literature[49-53]. For Ac-5-ASA, a blood-to-plasma ratio of 1 was used due to a lack of data.

### Distribution

The full PBPK models were selected as the PK model for both substrate and metabolite. The Rodgers and Rowland method (method 2) was used to calculate tissue-specific kp values and volume of distribution[54]. The kp scalar of metabolite was adjusted manually to 0.173 so that predicted kp values for all tissues could be scaled and the predicted Vss could resemble the reported volume of distribution in a clinical PK study with an IV dose, which is 0.569 L/kg[11].

### Metabolism

The 5-ASA is reported to be metabolized by NAT1 into Ac-5-ASA. The majority of the metabolism happens in the enterocytes of the gastrointestinal tract and in the liver[11]. It has been assumed that all absorbed 5-ASA is metabolized by NAT1 only. The generated metabolite is eliminated through renal excretion (CLR) of 30 L/h[11, 43]. The current Simcyp simulator does not have the NAT1 enzyme available. To add the NAT1 in the liver and intestine, the CYP3A4 enzyme option was used. It was assumed that NAT1 is uniformly and homogeneously distributed within a tissue compartment, according to the well-stirred model. The NAT1 absolute abundance in the liver was estimated based on the 100 mg IV clinical data with a constant CLint of 1 µL/min/pmol of protein using parameter estimation. The liver metabolism was further validated with 250 and 500 mg IV clinical data. Similarly, the abundance in the small intestine was estimated based on 100 mg oral solution data and validated against 1000 mg suspension and 2400 mg uncoated capsule assuming absorption happened only in the small intestine. The abundance of NAT1 in the colon compartment was normalized to the surface area while keeping the activity identical to the small intestine based on reported data[55]. The parameter sensitivity analysis (PSA) was performed with small intestine and colon abundance values (See **Section 2 of** **Supplementary materials**). The small intestinal abundance was further divided into duodenum, jejunum, and ileum compartments based on the enzyme activity data and length of the compartment[55]. No other elimination pathway was added to the model. The optimized abundance values for the intestine and liver are given in **Table 3.**

**Table 3.** Relative distribution of NAT1 abundance in the liver, small intestine, and colon compartments.

|  |  |
| --- | --- |
| Region | Optimized NAT1 abundance |
| 1. Liver | 27 pmol/mg of protein |
| 1. Small intestine | 65.4 nmol/small intestine |
| Duodenum | 22.4 % |
| Jejunum 1 | 12.2 % |
| Jejunum 2 | 12.2 % |
| Ileum 1 | 13.3 % |
| Ileum 2 | 13.3 % |
| Ileum 3 | 13.3 % |
| Ileum 4 | 13.3 % |
| 1. Colon | 32.2 nmol/colon |

### Absorption

The Advanced Dissolution, Absorption, and Metabolism (ADAM) model without an unstirred boundary layer (UBL) was used to simulate the absorption of 5-ASA from different formulations[56, 57]. The inclusion of UBL in the model increases the total luminal fluid volume. Specifically, the addition of UBL or a mucus compartment raises the luminal volume in the colon to approximately 143 mL, compared to 13 mL without UBL (see **Section 4 of Supplementary Materials**). However, higher mucus volumes are unrealistic as they are based on rat mucus data[58]. Moreover, excluding the UBL aligned with drug concentration measurements from biopsies conducted without mucus, ensuring consistency between the model and empirical data. Consequently, simulations were conducted without UBL to avoid this increase in luminal fluid volume.

#### Permeability

The permeability of 5-ASA was estimated based on the mechanistic passive regional permeability predictor (MechPeff) model. In the MechPeff model, the prediction of passive intestinal permeability (Peff,man) is achieved by leveraging both regional gut anatomy and specific physicochemical properties of the drug, including molecular weight, compound type, pKa, and Log P. The model scales the drug's intrinsic passive membrane permeability (Ptrans,0) using surface area scalars, which are calculated from the dimensions of the intestinal villi. The Ptrans,0 estimated by log Po:w method 2 was underpredicting the absorption profile of the oral solution, therefore Ptrans,0 was estimated by the top-down approach[59]. The absorption model was further validated against the oral suspension in the fasted state to capture absorption. Finally, 5-ASA Ptrans,0 value of 0.145 cm/s was used for all simulations. Additionally, the permeability for Ac-5-ASA was estimated using the polar surface area model in Simcyp, due to lack of *in-vitro* permeability data.

#### Formulations

The current work involved the use of multiple formulations of 5-ASA. The dissolution from the immediate-release formulations (1000 mg suspension and 2400 mg uncoated capsule) was handled in the simulations using the Diffusion Layer Model[60]. A lag time was added to the uncoated capsule to handle delayed gastric emptying. The controlled/modified release option was selected for Salofalk & EM enteric coated tablet, Apriso, Claversal, and Pentasa formulations. The release profiles of delayed and sustained release formulations were obtained from the literature. The compendial dissolution method for 5-ASA delayed-release tablets (Salofalk, Apriso, Claversal) uses the USP apparatus I/II and is divided into three stages. Moreover, USP apparatus II with 900mL phosphate buffer at pH 7.50 is described for extended-release formulations (Pentasa, Mezavant)[61, 62]. The extracted dissolution profiles are given in **Figure 2**. Additionally, the *in-vitro* release was fitted to the Weibull function in Simcyp before simulations. The “Controlled/modified release” with release profile option was used for all controlled release formulations in Simcyp. Additionally, enteric-coated release with Weibull function was used for the Claversal formulation.



**Figure 2.** Mean *in-vitro* release profiles of 5-ASA formulations (Salofalk& EM enteric coated tablets, Apriso, Pentasa and Claversal) used for simulations[47, 61, 63, 64].

##### Salofalk and EM enteric-coated tablet

Salofalk 250mg (SM) manufactured by Dr. Falk Pharma GmbH, Germany and its generic version (EM) are gastric-resistant (Eudragit L coated) tablets, offering prolonged drug release from a matrix core centered on the pH-independent polymer[65]. The *in-vitro* release profiles for SM and EM enteric-coated tablets were extracted from the literature[47]. Briefly, a three-stage dissolution test was performed for EM and SM enteric-coated tablets according to Dissolution Test 1 in the USP40-NF35 monograph “Mesalamine Delayed-Release Tablets” with pH 1, pH 6.0 and pH 7.2 buffers[47].

##### Apriso

Apriso (Salix Pharmaceuticals, Inc., Raleigh, NC, USA) is a controlled-release formulation of 5-ASA. A total of 1125 mg (3 ×375mg) dose was given to healthy volunteers and the same was modelled in Simcyp[45]. The *in-vitro* release profile for the formulation was extracted from Abinusawa *et al,* 2015[63]. The dissolution experiment was carried out in three stages with initial acid (2 h at pH 1.0) followed by two buffer stages (1 h at pH 6.0 followed by 8 h at pH 6.8) using USP I apparatus.

##### Pentasa 500 and 1000

Pentasa 500 mg tablets (Ferring Pharmaceuticals Ltd., UK) are made of compressed ethylcellulose coated granules, where drug release from granules is mediated by diffusion through the insoluble polymer coat[65]. The *in-vitro* dissolution data with 900 mL of a phosphate buffer at pH 7.50 in the USP II (Erweka DT 700, Heusenstamm, Germany) at 100 rpm were extracted from Andreas *et al*, 2015[61]. Pentasa 1000 consists of two Pentasa 500 tablets; therefore, the dissolution profile of Pentasa 500 was used to represent that of Pentasa 1000. Furthermore, release data from USP IV with biorelevant media were also used to compare the predictive performance of USP II with buffer[61].

##### Claversal 500

Claversal 500 mg (Recordati Pharma GmbH, Ulm, Germany) is an example of a pH-dependent mesalazine formulation with Eudragit-L coating disintegrating at pH≥6, targeting the mid to distal ileum and colon as the sites of delivery[66]. The *in-vitro* release data for Claversal 500 mg tablets during a simulated fasted GI passage with a continuous SI passage in the Paddle apparatus in bicarbonate-based intestinal media were taken from Karkossa *et al.,* 2018[64].

## Model Evaluation and Applications

The models were evaluated by comparing the predicted and observed pharmacokinetic parameters Cmax, AUC0-t, and Tmax. A two-fold criterion was used for model evaluation, where predictions were considered accurate if the predicted to observed ratio ranged from 0.5 to 2. Predictions falling outside this range were categorized as poorly predicted. Two-fold criteria were selected for model evaluation based on reference data used for model development from multiple independent studies involving different populations. For qualitative analysis, the mean predicted concentration-time profile, along with the 5th and 95th confidence intervals, was compared against the observed clinical data.

In this investigation, Claversal 500 and Pentasa 500 models were selected to predict concentrations of 5-ASA and Ac-5-ASA in the luminal and local tissue of the colon. These models were selected based on the ability to accurately predict drug plasma concentrations. For Claversal extended release, the oral absorption model was used in conjunction with the USP II *in vitro* release profile of Claversal 500. Accuracy in predicting plasma concentrations of 5-ASA and Ac-5-ASA was assessed by comparing predictions with clinical data. Additionally, estimated luminal and tissue concentrations in the colon at specific times were compared to reference data. To compare the colonic tissue concentrations, we calculated key PK parameters such as Cmax, AUC4.25-5.75, and Tmax based on both observed and predicted concentration within the 4.25-5.75h range. This approach was adopted due to the limited number of sampling points in colonic tissue. Similarly, the extended-release formulation model for Pentasa 500 was developed using USP II *in vitro* dissolution data. To further assess predictive performance, different dissolution methods (USP II and USP IV) were applied to both Pentasa 500 and Pentasa 1000 formulations. The objective was to enhance prediction accuracy and ensure alignment with clinical data, thus validating the models and the choice of *in vitro* dissolution inputs.

# Results

## Model Development and Validation

The PBBMs of 5-ASA and Ac-5-ASA were successfully developed using the Simcyp simulator. The intravenous disposition model accurately predicted the plasma concentration-time profiles for 100 mg, 250 mg, and 500 mg doses of 5-ASA and Ac-5-ASA within 2-fold criteria (**Figure 8S** and **Table 4**), demonstrating reliability across multiple doses and the enzymatic conversion of 5-ASA to Ac-5-ASA in the liver. Similarly, the models effectively captured the oral absorption of various formulations, including solutions, suspensions, and immediate-release capsules, across multiple doses (**Figure 9S** and **Table 4**). This underscores the model's capability in terms of dissolution, intestinal absorption, and gut wall metabolism by the NAT1 enzyme.

Additionally, the plasma concentration-time profiles along with PK parameters of extended-release formulation models for Salofalk, EM enteric-coated tablets, and Apriso were predicted accurately within the 2-fold criteria, indicating the models' ability in predicting multiple formulations with variable *in vitro* release profiles and colon absorption, as depicted in **Figures 10S** and **3**. This comprehensive model development confirms the predictive accuracy of the models under various formulation conditions, validating their applicability in different scenarios. The overall predicted versus observed pharmacokinetic parameters, including Cmax, AUC0-t, and Tmax, for all formulations, are presented in **Table 4** and illustrated in **Figure 3**. Figures related to intravenous, oral immediate-release, and extended-release formulations can be found in **Section 5 of the Supplementary Materials**.



**Figure 3.** Comparison of observed versus predicted Cmax, AUC0-t and Tmax for formulations used in model development and validation. 5-ASA (red) and Ac-5-ASA (green) are shown as dots, with dotted lines indicating the 2-fold criteria.

**Table 4.** Predicted versus observed pharmacokinetic parameters of 5-ASA and Ac-5-ASA for formulations used in model development and validation.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Formulation | Study | Dose (mg) | 5-ASA | | | | | | | | | Ac-5-ASA | | | | | | | | |
| **Cmax (ng/ml)** | | | **AUC0-t (ng.h/ml)** | | | **Tmax  (h)** | | | **Cmax (ng/ml)** | | | **AUC0-t (ng.h/ml)** | | | **Tmax  (h)** | | |
| **P** | **O** | **P/O** | **P** | **O** | **P/O** | **P** | **O** | **P/O** | **P** | **O** | **P/O** | **P** | **O** | **P/O** | **P** | **O** | **P/O** |
| IV | Bondsen et al. 1991[43] | 100 | 7503 | 6541 | 1.15 | 3245 | 2780 | 1.17 | 0.04 | 0.07 | 0.57 | 1894 | 1548 | 1.22 | 4094 | 3586 | 1.14 | 0.36 | 0.47 | 0.77 |
| IV | Vree et al. 2000[11] | 250 | 11905 | 15743 | 0.76 | 7320 | 8188 | 0.89 | 0.12 | 0.09 | 1.33 | 4736 | 5091 | 0.93 | 10234 | 10990 | 0.93 | 0.36 | 0.48 | 0.75 |
| IV | Myers et al, 1987[44] | 500 | 40730 | 50587 | 0.81 | 17378 | 23020 | 0.75 | 0.08 | 0.08 | 0.96 | 9462 | 10796 | 0.88 | 20331 | 26390 | 0.77 | 0.40 | 0.76 | 0.53 |
| Solution | Yu et al, 2017[45] | 100 | 1250 | 1410 | 0.89 | 2171 | 1295 | 1.68 | 0.84 | 0.53 | 1.58 | 1338 | 1422 | 0.94 | 4059 | 4012 | 1.01 | 1.08 | 1.02 | 1.06 |
| Suspension | Yu et al, 1990[46] | 1000 | 12498 | 13220 | 0.95 | 21713 | 29290 | 0.74 | 0.84 | 1.01 | 0.83 | 13378 | 10550 | 1.27 | 40589 | 40730 | 1.00 | 1.08 | 0.95 | 1.14 |
| Uncoated capsule | Myers et al, 1987[44] | 2400 | 33849 | 36105 | 0.94 | 53141 | 84560 | 0.63 | 1.88 | 2.00 | 0.94 | 33972 | 34338 | 0.99 | 94333 | 118700 | 0.79 | 2.16 | 2.51 | 0.86 |
| Salofalk | Zhang et al, 2022[47] | 500 | 2088 | 2020 | 1.03 | 7695 | 8662 | 0.89 | 4.68 | 6.07 | 0.77 | 3113 | 3816 | 0.82 | 16196 | 23580 | 0.69 | 5.04 | 6.01 | 0.84 |
| EM enteric-coated tablet | Zhang et al, 2022[47] | 500 | 1988 | 1560 | 1.27 | 7564 | 7432 | 1.02 | 4.68 | 6.07 | 0.77 | 2951 | 2779 | 1.06 | 15913 | 23870 | 0.67 | 5.40 | 6.90 | 0.78 |
| Apriso | Yu et al, 2017[45] | 1125 | 1911 | 1179 | 1.62 | 15013 | 7520 | 2.00 | 5.04 | 6.02 | 0.84 | 3408 | 2914 | 1.17 | 31795 | 28770 | 1.11 | 7.20 | 7.01 | 1.03 |

IV: Intravenous, P: Predicted value, O: Observed value, P/O: Predicted to observed ratio

## Model Applications

The plasma-validated PBBMs accurately predicted the plasma concentration-time profile of 5-ASA and Ac-5-ASA within the two-fold criteria for Claversal but not for Pentasa when using USP II dissolution input. Additionally, the models poorly predicted the luminal and tissue concentrations for both Claversal and Pentasa formulations. However, applying USP IV dissolution input to the models for Pentasa 500 and Pentasa 1000 formulations improved plasma predictions, although it did not affect colonic luminal and tissue concentrations. Detailed results for each formulation are provided below.

### Plasma, colonic luminal and tissue drug concentrations

#### Claversal 500 model

The oral absorption model, combined with the USP II *in vitro* release profile of Claversal 500, was used to develop the Claversal ER model. This ER formulation model accurately predicted the mean plasma concentrations of 5-ASA and Ac-5-ASA (**Figure 4**). The predicted and observed Cmax, AUC0-t and Tmax are given in **Table 5**.



**Figure 4.** Observed (dots) and predicted (line) plasma concentration-time profiles of 5-ASA (red) and Ac-5-ASA (green) for Claversal 500. The dots include error bars representing the standard deviation. The solid line represents mean predictions while dotted lines represent the 5th and 95th percentiles.

**Table 5.** Predicted versus Observed pharmacokinetics parameters, colonic luminal and tissue concentrations of 5-ASA and Ac-5-ASA for Claversal 500formulation.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Claversal 500** | **Parameter** | **5-ASA** | | | **Ac-5-ASA** | | |
| **Predicted** | **Observed** | **P/O** | **Predicted** | **Observed** | **P/O** |
| Plasma | Cmax (ng/ml) | 1222 | 846 | 1.44 | 1705 | 1574 | 1.08 |
| AUC0-t (ng.h/ml) | 7232 | 5889 | 1.23 | 12930 | 16380 | 0.79 |
| Tmax (h) | 7.32 | 8.00 | 0.92 | 8.28 | 9.00 | 0.92 |
| Colonic lumen | C5.75h (mg/ml) | 20.1 | 3.4 | 5.86 | 0 | 0.03 | 0.00 |
| Colonic tissue (observed) or enterocyte (predicted) | Cmax (ng/ml) | 2497 | 1264652 | 0.002 | 521 | 28481 | 0.018 |
| AUC4.25-5.75 (ng.h/ml) | 879 | 3505100 | 0.0003 | 189 | 66270 | 0.003 |
| Tmax (h) | 5.76 | 4.25 | 1.36 | 5.76 | 5.50 | 1.05 |

P/O: Predicted to observed ratio.

The luminal drug concentrations in the colon compartment at 5.75 h after administration of the Claversal 500 formulation (C5.75h), estimated from the same model, were found to be overpredicted for 5-ASA by a factor of 5.86, while underpredicted for Ac-5-ASA (**Figure 5** and **Table 5**). The model predicted no luminal presence of Ac-5-ASA. Moreover, the tissue concentrations in the colon compartment were underpredicted by factors of 506 and 55 at Cmax for 5-ASA and Ac-5-ASA, respectively (**Figure 6** and **Table 5**).

**Figure** **5.** Observed and predicted luminal drug concentrations of 5-ASA and Ac-5-ASA in colon at 5.75 h after administration of the Claversal 500 formulation.



**Figure 6.** Observed colonic tissue (dots) and predicted colonic enterocyte (lines) concentrations of 5-ASA and Ac-5-ASA for Claversal 500 formulation. The dots include error bars representing the standard deviation. The solid line represents mean predictions while dotted lines represent the 5th and 95th percentiles.

#### Pentasa500 model

The extended-release formulation model of Pentasa 500, using USP II *in vitro* dissolution data, poorly predicted the mean plasma concentrations of 5-ASA and Ac-5-ASA (**Figure 7**). The predicted and observed values for Cmax, AUC0-t and Tmax are presented in **Table 6**.



**Figure 7.** Observed (dots) and predicted (line) plasma concentration-time profiles of 5-ASA (red) and Ac-5-ASA (green) for Pentasa 500. The dots include error bars representing the standard deviation. The solid line represents mean predictions while dotted lines represent the 5th and 95th percentiles.

**Table 6.** Predicted versus observed pharmacokinetics parameters, colonic luminal and tissue concentrations of 5-ASA and Ac-5-ASA for Pentasa 500 formulation with USP II dissolution input.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Pentasa 500** | **Parameter** | **5-ASA** | | | **Ac-5-ASA** | | |
| **Predicted** | **Observed** | **P/O** | **Predicted** | **Observed** | **P/O** |
| Plasma | Cmax (ng/ml) | 1314 | 441 | 2.98 | 1860 | 745 | 2.50 |
| AUC0-t (ng.h/ml) | 7145 | 1649 | 4.33 | 14170 | 7929 | 1.79 |
| Tmax (h) | 2.04 | 4.50 | 0.45 | 3.12 | 5.00 | 0.62 |
| Colonic lumen | C5.75h (mg/ml) | 10.45 | 0.51 | 20.33 | 0.00 | 0.34 | 0.00 |
| Colonic tissue (observed) or enterocyte (predicted) | Cmax (ng/ml) | 1634 | 213636 | 0.0076 | 717 | 264122 | 0.003 |
| AUC4.25-5.75 (ng.h/ml) | 3820 | 574400 | 0.0067 | 1680 | 758400 | 0.002 |
| Tmax (h) | 7.08 | 4.25 | 1.67 | 7.20 | 4.25 | 1.69 |

Similar to Claversal 500, the luminal drug concentrations after intake of Pentasa 500 were overpredicted for 5-ASA by a factor of 20, while the model predicted no luminal concentration of Ac-5-ASA (**Figure 8** and **Table 6**). The tissue concentrations in the colon compartment were significantly underpredicted by the model, with fold differences of 131 and 368 at Cmax for 5-ASA and Ac-5-ASA, respectively (**Figure 9** and **Table 6**).

**Figure 8.** Observed and predicted luminal drug concentrations of 5-ASA and Ac-5-ASA in colon at 5.75 h after administration of the Pentasa 500 formulation with USP II dissolution input.



**Figure 9.** Observed colonic tissue (dots) and predicted colonic enterocyte (lines) concentrations of 5-ASA and Ac-5-ASA for Pentasa 500 formulation. The dots include error bars representing the standard deviation. The solid line represents mean predictions while dotted lines represent the 5th and 95th percentiles.

### USP II versus USP IV *in-vitro* dissolution input

The Pentasa 500 and Pentasa 1000 models with USP IV dissolution inputs were developed and compared with those using USP II inputs. Figures and tables referenced in this section are provided in **Section 6 of the supplementary materials**. The plasma concentration-time profiles for the Pentasa 500 formulation are presented in **Figure 11S**, while the corresponding pharmacokinetic parameters, including Cmax, AUC0-t, and Tmax, are provided in **Table 3S**.

The predicted plasma concentration-time profile for Pentasa 500 was poorly predicted using the USP II method, but it improved with the USP IV dissolution input. Additionally, we evaluated the luminal and tissue concentrations in the colon for the Pentasa 500 formulation using both USP II and USP IV dissolution inputs. Our findings indicated that these concentrations were similar regardless of the dissolution method employed (**Figures 12S** and **13S**), suggesting that the choice of dissolution input method does not significantly impact the predicted concentrations in these compartments.

Similar results were observed for Pentasa 1000 in terms of the plasma drug concentration-time profile (**Figure 14S**), with the observed concentrations derived from a reported study. The results indicated that the predictions for Pentasa 500 and Pentasa 1000 were improved with USP IV dissolution inputs compared to USP II (**Table 3S**).

# Discussions

The developed PBBMs for 5-ASA and Ac-5-ASA accurately predicted (within a 2-fold prediction error) the plasma drug concentrations for intravenous, oral IR, and ER formulations. The model effectively predicted the conversion of 5-ASA to Ac-5-ASA by the NAT1 enzyme. However, due to the absence of NAT1 in the current Simcyp human physiology models, an alternative method was employed to approximate the enzyme abundance. The CYP3A4 enzyme option was selected to represent the NAT1 abundance levels in the gut wall and liver. Additionally, we used a constant intrinsic clearance value instead of NAT1-specific Vmax and Km values due to the unavailability of *in vitro* enzyme kinetics data. This approach is not ideal and presents several drawbacks, such as the inability to reflect enzyme saturation or follow Michaelis-Menten kinetics, due to the use of a constant NAT1 clearance value[67]. Despite these limitations, it would be beneficial to evaluate the model performance using precise absolute abundance data for NAT1. This underscores the importance of generating definitive NAT1 abundance data and establishing a robust *in-vitro in-vivo* extrapolation (IVIVE) process, which could enhance future modeling efforts[68].

A recent study focuses on developing biopredictive dissolution media, using Salofalk as the reference formulation, to predict systemic pharmacokinetics and bioequivalence for 5-ASA formulations. This research, utilizing GastroPlus software, primarily optimizes dissolution to enhance systemic bioavailability predictions. It also employs a PBBM to simulate 5-ASA concentrations in the colonic lumen and enterocytes, noting comparable local concentrations for test and reference formulations[69]. However, the model lacks validation against clinical data specific to colonic tissue levels and suggests that the predicted luminal concentrations are lower than those reported in literature, indicating potential limitations in the dissolution model or assumptions used. While this approach offers valuable insights for systemic efficacy, our study advances understanding of local dynamic behaviour by incorporating the role of the NAT1 enzyme and validating predictions with clinical data related to colonic tissue concentrations.

The systemically validated ER formulation model was used for Claversal and Pentasa formulations. The model accurately predicted the plasma drug concentrations of 5-ASA and Ac-5-ASA for Claversal 500. However, it did not successfully predict drug concentrations within the luminal space and colonic tissue. This discrepancy may stem from limitations in clinical data collection related to feasibility and in the modeling approach (**Section 7 of supplementary materials**). For instance, measurements of luminal and biopsy drug concentrations were taken from the cecum, which most likely does not accurately represent concentrations throughout the entire colon. For instance, the ADAM model considers 13 mL luminal fluid volume for whole colon. This volume may not fully capture the unique fluid dynamics and volumes present in the caecum, potentially affecting drug concentration predictions. Luminal drug concentrations are significantly influenced by physiological processes such as ileocecal transfer and water reabsorption. For instance, the reabsorption of water can result in increased drug concentrations within the lumen. However, accurately determining the occurrence and extent of these processes poses a challenge, as *in-vivo* measurements, such as aspirations, do not readily provide this information. This limitation complicates the precise modeling and prediction of drug behavior in the gastrointestinal tract. Additionally, luminal drug concentrations were recorded at a single time point, potentially missing the dynamic behavior of the drug in the gastrointestinal tract (GIT). The study also relied on assumptions to translate biopsy data into specific enterocyte concentrations, which is problematic given that biopsies may contain other tissues in addition to enterocytes and exhibit different drug-binding affinities. Additionally, a tissue density of 1.042 g/mL was used to convert biopsy concentrations from ng/mg of tissue to ng/mL, which may introduce errors due to variability in tissue composition. Despite these challenges, the biopsy concentrations observed in the present study were comparable to those reported in another research study[70]. Clinically, both 5-ASA and its metabolite demonstrated accumulation in biopsy tissues, a phenomenon also noted for celecoxib in previous studies[16, 48].

The inability of the current ADAM model to adequately simulate drug accumulation in enterocytes, leading to potentially poor predictions, might be attributed to several factors. The model often employs simplified assumptions regarding drug distribution and kinetics within enterocytes, failing to capture detailed mechanistic processes such as drug uptake, binding, and efflux. Clinical studies have shown that drugs can accumulate in enterocytes or biopsy tissues, reaching higher concentrations compared to luminal and plasma drug levels. However, the ADAM model primarily operates on the principle of concentration gradients, moving from higher to lower concentrations through passive transport, which does not adequately represent this observed accumulation. Certain limitations in parameter estimation further affected model predictions. For example, the permeability of Ac-5-ASA was estimated using its polar surface area, while absolute NAT1 abundance data were unavailable as described earlier. Similarly, the Ptrans,0 value for 5-ASA was optimized based on oral solution data. Importantly, the current model also lacked the ability to simulate the active secretion of Ac-5-ASA from enterocytes to the lumen, as it does not incorporate an efflux mechanism[66, 71]. The fraction of drug unbound in the gut (fugut) plays a crucial role in Simcyp absorption models, including the First Order, ADAM, and Multi-compartment Gut Wall ADAM (M-ADAM) models[72]. fugut represents the unbound fraction of the drug within enterocytes which can be either user-inputted or predicted using physico-chemical properties, blood binding, and tissue composition data. fugut values are essential for understanding drug metabolism within enterocytes because they influence the fraction of the drug available for metabolism. The variability of fugut values may significantly impact both the total and unbound drug concentrations in enterocytes, as they determine the extent to which a drug is accessible for metabolic processes and transport. However, PSA showed only minimal impact on the total enterocyte concentrations of mesalamine for an fugut range of 0.001 to 1. In this study, we focused on the total drug available in enterocytes with fugut of 1.

Another significant limitation of the ADAM model is its inadequate simulation of drug absorption dynamics between systemic circulation and enterocytes or luminal fluid, whether drugs are administered intravenously or orally. These gaps can be addressed by the more advanced M-ADAM model, which incorporates detailed mechanistic processes, including passive diffusion, transcellular and paracellular permeability, active transport, and lymphatic transport. With the M-ADAM model, drugs absorbed via the paracellular pathway bypass intestinal enzymes and efflux transporters, while those entering systemic circulation via the lymphatic pathway avoid hepatic first-pass metabolism. Moreover, the M-ADAM model allows drug access to enterocyte cytoplasm through both apical and basolateral membranes, unlike the ADAM model, which limits access to the apical membrane only. This comprehensive framework provides a better understanding of drug absorption and metabolism in the gut.

Using plasma data to estimate colonic luminal and colonic tissue concentrations has its limitations. While multiple studies have identified either direct or indirect relationships between predicted colonic concentrations and plasma levels, none have verified these predictions against actual clinical data[15, 17, 73]. This is particularly true for compounds where a significant fraction reaches the colonic lumen, such as extended-release formulations or those with low solubility but high doses. For these compounds, accumulation in the colon may be driven by gut processes rather than systemic circulation, as previously illustrated for celecoxib and sulindac[16, 74]. Without clinical validation, establishing a linear or nonlinear correlation between predicted colonic concentrations and plasma data remains unsubstantiated and potentially misleading.

The dissolution input plays a crucial role in determining luminal drug concentrations and, subsequently, tissue concentrations. However, when it comes to extended-release formulations, there is a lack of specific guidance regarding the choice of dissolution method input for modeling. Additionally, mechanistic models for estimating *in-vivo* release remain elusive. In most modeling studies, the quality control USP II or I method is preferred as dissolution input, either with or without biorelevant dissolution media. However, specific guidance exists for 5-ASA formulations, where dissolution at multiple pH levels is recommended[75]. Notably, some studies have reported advantages associated with the USP IV dissolution method over USP II, particularly in terms of media volume and hydrodynamics[61, 64]. It is essential to recognize that current models assume *in-vitro* release is identical to *in-vivo* release for controlled release formulations and that physiological changes do not significantly impact *in-vivo* dissolution[76]. To address this limitation, we employed a semi-mechanistic option with an initial release by the Weibull function followed by dissolution with the diffusion layer model in Simcyp[60, 77]. These models aim to achieve physiological sensitivity by ensuring that *in-vivo* drug release will vary based on the population's physiological parameters. Despite our efforts, the developed Pentasa models still exhibited poor predictions for plasma, luminal, and tissue drug concentrations using the USP II method. However, slight improvements were observed in plasma concentration predictions when employing the USP IV method. Additionally, both USP II and IV dissolution methods overestimated the luminal drug concentration compared to the actual measured percentage dissolved. The overprediction of Pentasa 500 and 1000 mg plasma and luminal drug concentrations could be attributed to the use of non-biopredictive dissolution profiles, often associated with compendial setups that prioritize quality control over biorelevance. Specifically, the USP II setup appears to release the drug too quickly, as indicated by the predicted pharmacokinetic profile, which does not exhibit the typical characteristics of a sustained release formulation. Our in-house dissolution data for Pentasa 500 mg, obtained using a non-compendial setup with lower volumes, suggests a slower release rate of approximately 20% after 3.5 hours[48, 66]. This slower release is more consistent with the expected behavior of a sustained release formulation, indicating that the compendial dissolution conditions used in the study may not accurately reflect the *in-vivo* release characteristics of Pentasa. Our study utilized PBBM to predict local 5-ASA and Ac-5-ASA concentrations, without detailed focus on formulation-specific impacts. Recognizing limitations in assuming identical *in-vivo* to *in-vitro* release inherent in ER dissolution models, we acknowledge the importance of incorporating critical material attributes (CMA) like particle size and solubility for developing biopredictive dissolution methods[69, 78]. Future research will aim to integrate CMAs into PBBMs, enhancing their predictive accuracy for drug dissolution and absorption, and offering deeper insights into formulation performance. In summary, while dissolution input remains critical, further research is needed to enhance our understanding of *in-vivo* release mechanisms and improve predictive models for extended-release formulations.

Our study primarily focused on constructing a 5-ASA model using data from healthy populations. However, it is important to recognize that this model has the potential to be extrapolated to disease states such as UC. Similar to budesonide, which has been extensively studied in both healthy and diseased individuals, our 5-ASA model holds potential clinical relevance beyond healthy subjects[79]. Advancements in technology and the availability of population data facilitate the development of disease-specific physiological models[79-83]. By incorporating disease-related parameters and accounting for relevant pathophysiological changes, we can enhance the accuracy of drug concentration predictions in patients with UC or CD. Future work should prioritize generating definitive NAT1 abundance data with an *in-vitro* to *in-vivo* extrapolation link, enhancing models to predict local drug concentrations in the gastrointestinal tract, and addressing gaps in luminal and tissue concentration predictions, especially for GI-locally acting compounds. These advancements are essential for improving the applicability of PBBMs in simulating formulation changes and supporting drug development and regulatory decision-making. Additionally, research should explore disease-specific applications to refine clinical predictions. While this study demonstrated that the 5-ASA and Ac-5-ASA models are sufficiently accurate for predicting plasma drug concentrations, further investigation is needed to assess the adequacy and challenges of extrapolating these models to diseased states.

# Conclusion

This study successfully developed and validated PBBMs for 5-ASA and its metabolite, Ac-5-ASA, demonstrating their ability to accurately predict plasma concentrations for various formulations. The models also captured the intestinal and hepatic metabolism of 5-ASA to Ac-5-ASA. However, significant discrepancies were observed in predicting luminal and tissue concentrations in the colon, highlighting the complexities of modeling locally acting oral drugs. Despite these challenges, the study underscores the potential benefits of PBBMs in improving drug development processes. Future research should focus on generating definitive NAT1 abundance data with an *in-vitro* to *in-vivo* extrapolation link, refining models to enhance predictions of local drug concentrations in the gastrointestinal tract and addressing the current limitations in predicting luminal and tissue concentrations in the colon. These advancements are crucial for enhancing the applicability of PBBMs in simulating formulation changes, supporting drug development and regulatory decision-making.

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# Author contributions: CRediT

**Harshad Jadhav:** Conceptualization, Methodology, Software, Writing original draft, Visualization. **Arno Van Camp**: Investigation, Data Curation. **Christer Tannergren:** Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **Glenn Lemmens**: Investigation, Data Curation. **Joachim Brouwers**: Investigation, Data Curation, Formal analysis. **Tim Vanuytsel**:Writing - Review & Editing, Supervision. **Sebastian Steigert:** Writing - Review & Editing. **Patrick Augustijns:** Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

# Conflict of interest

The authors have no competing interests to declare. AstraZeneca has ongoing license agreements for Simcyp Simulator.

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