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Lara Filipa Gomes Marques

POPULATION PHARMACOKINETIC STUDY INVOLVING DIFFERENT SALBUTAMOL FORMULATIONS

VOLUME 1

Dissertação no âmbito do Mestrado em Investigação Biomédica – Ramo de Especialização Oncobiologia orientada pelo Professor Doutor Nuno Vale e pela Professor Doutora Manuela Grazina e apresentada à Faculdade de Medicina da Universidade de Coimbra.

Junho de 2023

FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

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Aos meus pais e irmã.

Science is not only a disciple of reason but also one of romance and passion.
– Stephen Hawking

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Abstract

Asthma is a complex and heterogeneous respiratory disorder characterized by chronic airway inflammation, reversible airflow obstruction, and bronchial hyperresponsiveness. A wide array of therapeutic options is available to attempt to reduce fatalities and hospitalizations and control the disease by reducing symptoms, preventing exacerbations, and restoring lung function, thereby ensuring a normal standard of living in these patients. Salbutamol is a short-acting β_2-agonist (SABA) used for the relief of classic asthma symptoms such as wheezing, coughing, dyspnea, and chest tightness. Its therapeutic effect is based on its potent smooth muscle relaxant properties, which allow the inhibition of bronchial smooth muscle contractions and subsequent bronchodilation. Due to recent evidence of bronchodilator overuse causing exacerbations, the Global Initiative for Asthma (GINA) has ruled that SABA-only treatment is contraindicated and should be used either in combination with inhaled corticosteroids (ICS). In addition, the high frequency of this chronic condition implies the coexistence of other diseases in many patients. For these reasons, salbutamol is frequently administered with other drugs and drug-drug interactions (DDIs) are critical for a good therapeutic outcome. On the other hand, the use of salbutamol extends to various formulations, namely metered-dose inhaler (MDI), dry powder inhaler (DPI), nebulizer, oral and intravenous (IV) forms, resulting in highly diverse pharmacokinetic (PK) parameters, and, consequently, different efficacy and adverse effects. In light of these considerations, this project aims to develop a PK study of salbutamol through *in silico* approaches. This thesis encompasses two key components: firstly, the development of a predictive model to assess potential CYP-mediated DDIs, and secondly, the development of population PK models capable of accurately describing the PK profile of a specific population undergoing treatment with salbutamol deliver via MDI, DPI, nebulizer, oral, and IV formulations.

Therefore, in Chapter I, through a screening to identify potential interactions with this bronchodilator, fluvoxamine, an antidepressant, was selected for the CYP-mediated DDI study. The physiologically based pharmacokinetic (PBPK) model of

salbutamol was then developed and validated using available clinical PK data and *in silico*-based programs. Salbutamol-fluvoxamine interaction was simulated according to different regimens and patient characteristics (age and physiological status). The results demonstrated that co-administering salbutamol with fluvoxamine enhances salbutamol exposure in certain situations. With this groundbreaking study, the utility of PBPK modeling in predicting CYP-mediated DDIs has been proved and, above all, the importance of supervising the prescription of medicines has been highlighted.

Chapter II focused on investigating the impact of individual patient characteristics on currently available salbutamol formulations. To achieve this, several physiologically based pharmacokinetic (PBPK) models were developed to generate a virtual patient dataset. Subsequently, these data were used to compute the population PK models. The findings revealed significant influences of covariates on the salbutamol's kinetics, depending on its formulation type. Various subpopulations with a potentially heightened risk of experiencing either toxic or subtherapeutic effects were then identified.

Accordingly, the precision medicine era has been revolutionizing the treatment of several diseases, including asthma treatment. Simulation pharmacological research, in particular, has played a pivotal role in expediting progress in this domain. In short, this study contributes to the paradigm shift in asthma treatment, changing from «one size fits all» approach toward a precision medicine model. Integrating patient characteristics offers valuable insights for tailoring treatment strategies and improving therapeutic outcomes and patients' quality of life.

Keywords: Asthma; In Silico Pharmacology; Population Pharmacokinetics; Salbutamol; Therapeutic Regimen; Drug-drug Interactions

Resumo

A asma é uma doença respiratória complexa e heterogénea caracterizada pela inflamação crónica das vias aéreas, obstrução reversível do fluxo de ar e hiperresponsividade brônquica. A sua prevalência tem vindo tendencialmente a ser aumentada, em todo o mundo, e as projeções indicam que este número deverá aumentar nos próximos anos. Atualmente, existe uma ampla variedade de opções terapêuticas disponíveis para tentar reduzir o número de mortes e de hospitalizações, através da redução de sintomas, prevenção de exacerbações e restauração da função pulmonar. No entanto, o controlo da asma enfrenta desafios significativos, como problemas associados à adesão ao tratamento, o uso e dependência excessiva dos medicamentos, bem como preocupações a nível de interações medicamentosas, eficácia e segurança. Estes fatores contribuem para a elevada incidência de efeitos adversos e de episódios agravados que conduzam à hospitalização ou à morte do paciente. O salbutamol é um agonista β_2 de curta duração (SABA) usado para o alívio de sintomas clássicos da asma, como sibilância, tosse, dispneia e aperto no peito. O seu efeito terapêutico baseia-se nas suas propriedades relaxantes do músculo liso, que conduzem à inibição das contrações do músculo liso dos brônquios e, consequentemente, à broncodilatação. Devido a evidências recentes que relacionam o uso excessivo de broncodilatadores e a ocorrência de exacerbações, a Iniciativa Global para a Asma (GINA) determinou que o tratamento único com SABA é contraindicado, devendo ser utilizado em combinação com corticosteroides inalatórios (ICS). Além disso, a elevada prevalência desta condição crónica implica a coexistência de outras doenças em muitos pacientes. Por estes motivos, o salbutamol é frequentemente combinado com outros fármacos, podendo ocorrer interações medicamentosas que comprometem o resultado terapêutico. Por outro lado, o uso de salbutamol estende-se a várias formulações, como inalador de dose medida (MDI), inalador de pó seco (DPI), nebulizador, comprimidos orais e formas intravenosas (IV), resultando em parâmetros farmacocinéticos (PK) altamente diversos e, consequentemente, eficácia e efeitos adversos alterados. Tendo em conta estas evidências, este projeto tem como objetivo desenvolver um estudo PK do salbutamol através de abordagens *in silico*. Esta tese é constituída, assim, por duas partes: em primeiro lugar, o desenvolvimento de um modelo preditivo para avaliar possíveis

interações medicamentosas mediadas pelo citocromo P450 (CYP450) e, em segundo lugar, o desenvolvimento de modelos PK populacionais capazes de descrever o perfil PK de uma população específica, submetida a tratamentos com salbutamol administrado por via inalatória (MDI, DPI), nebulizadora, oral e intravenosa.

Deste modo, no Capítulo I, através de uma triagem de fármacos para identificar potenciais interações com este broncodilatador, a fluvoxamina, um antidepressivo, foi selecionada para o estudo de interações medicamentosas mediadas pelo CYP450. O modelo farmacocinético de base fisiológica (PBPK) do salbutamol foi, assim, desenvolvido e validado utilizando dados clínicos farmacocinéticos disponíveis e através de programas *in silico*. A interação salbutamol-fluvoxamina foi simulada de acordo com diferentes regimes de dosagem e características do paciente (idade e estado fisiológico). Os resultados demonstraram que a coadministração de salbutamol com fluvoxamina aumenta a exposição do salbutamol em determinadas situações. Com este estudo pioneiro, foi demonstrada a utilidade da modelação PBPK na previsão de interações medicamentos e, acima de tudo, foi possível comprovar a importância da supervisão na prescrição dos medicamentos.

O Capítulo II teve como principal foco investigar o impacto das características individuais dos pacientes nas formulações atualmente disponíveis de salbutamol. Para isso, foram desenvolvidos vários modelos PBPK para gerar um conjunto de dados de pacientes virtuais. Posteriormente, estes dados foram aplicados em modelos PK populacionais. Os resultados revelaram influências significativas de covariáveis na cinética do salbutamol, dependendo do tipo de formulação. Desta forma, foram identificadas diversas subpopulações em risco de experienciar efeitos tóxicos ou subterapêuticos.

A era da medicina de precisão tem, de facto, revolucionado o tratamento de várias doenças, e a asma não é exceção. Em particular, estudos de simulação farmacológica desempenham um papel fundamental na aceleração do progresso desta área. Em suma, este estudo contribui para a mudança do paradigma no tratamento da asma, passando de uma abordagem de «*one fits all*» para um modelo de medicina de precisão. A integração das características do paciente oferece informações importantes para adaptar estratégias terapêuticas e melhorar os resultados de um tratamento e qualidade de vida dos pacientes.

Palavras-chave: Asma; Farmacologia In Silico; Farmacocinética Populacional; Salbutamol; Regime Terapêutico; Interações Medicamentosas

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Publications

This dissertation integrates the following published papers:

Marques L, Vale N. Salbutamol in the Management of Asthma: A Review. *International Journal of Molecular Sciences*. 2022; 23(22):14207. https://doi.org/10.3390/ijms232214207

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Marques, L.; Vale, N. Prediction of CYP-Mediated Drug Interaction Using Physiologically Based Pharmacokinetic Modeling: A Case Study of Salbutamol and Fluvoxamine. *Pharmaceutics* **2023**, *15*, 1586. https://doi.org/10.3390/pharmaceutics15061586

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List of Abbreviations

ADME	Absorption, distribution, metabolism, excretion
ADRs	Adverse drug reactions
AIC	Akaike Information criterion
AMP	Adenosine monophosphate
ATPase	Adenosine triphosphatase
AUC	Area under curve
BBB	Blood brain barrier
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CL	Clearance
Cmax	Maximum plasma concentration
Cmax liver	Maximum plasma concentration measured in liver
COPD	Chronic obstructive pulmonary disease
CYP	Cytochrome P450
DDI	Drug-drug interaction
Diff. Coeff.	Diffusion coefficient
DPI	Dry powder inhaler
EC50	Half maximal effective concentration
eGFR	Estimated glomerular filtration rate
EIB	Exercised-induced bronchoconstriction
Fa	Fraction absorbed of a drug
FDp	Fraction of drug concentration in the portal vein
FEV	Forced expiratory volume
FFA	Free fatty acids
GINA	Global Initiative for Asthma
GOF	Goodness-of-fit
HDL	High-density lipoprotein
IC50	Half maximal inhibitory concentration

ICS	Inhaled corticosteroids
IV	Intravenously
IWRES	Individual weighted residuals
Ka	Absorption constant rate
Ki	Inhibitory constant
Km	Michelis-Menten constant
LABA	Long-acting β2-agonist
LAMA	Long-acting muscarinic antagonist
MDI	Metered-dose inhaler
MS	Multiple sclerosis
NCA	Neutrophil chemotactic activity
NEFA	Non-esterified fatty acid
PBPK	Physiological based pharmacokinetic
PD	Pharmacodynamic
PD DDIs	Pharmacodynamic drug-drug interactions
Peff	Human jejunum effective permeability
PK	Pharmacokinetic
PK DDIs	Pharmacokinetic drug-drug interactions
pKa	Ionization constant
q6h	Every 6 hours
RSE	Relative standard errors
SABA	Short-acting β2-agonist
SABINA	SABA Use In Asthma program
SAEM	Stochastic Approximation Expectation-Maximization
SID	Once daily
SSRI	Selective serotonin reuptake inhibitor
Tlag	Lag time
Tmax	Time to peak drug concentration
Vmax	Maximal rate of metabolism
VPC	Visual Predictive Check
WHO	World Health Organization

Introduction

Asthma is a chronic inflammatory disorder of the airways. Demystifying the complexity of this disease has been challenging over the last decades [1,2]. In truth, our concept of asthma is too simplified [3–5]. The pathophysiology of asthma is really rather complex, due to the large number of cells and cellular elements involved. Their phenotypic characteristics – including clinical features of the disease and their underlying mechanisms (endotype) – are complex and represent a variety of host–environment interactions [3].

There is a diverse range of therapeutic options to attempt to reduce fatalities and hospitalizations and control the disease by reducing symptoms, preventing exacerbations, and restoring lung function, ensuring a normal standard of living in these patients [6–8]. Nevertheless, asthma therapy currently faces adherence-related problems, overuse and overreliance, and issues involving drug efficacy and safety, which explain the high number of reported effects, hospitalizations, and deaths [9,10]. Precision medicine therefore becomes indispensable in the management of patients with chronic respiratory diseases. In recent years, the scientific community has observed a boost in innovative therapies and combinations to manage these diseases, namely asthma. With the wide range of therapeutic approaches, it is increasingly important to tailor the best treatment to the patient. Although the path to incorporating personalized medicine into asthma therapy is long, it has been built. Asthma treatment is changing from «one size fits all» therapy to a «precision medicine» model in which patients receive the most appropriate treatment, according to their medical history and individual characteristics, aiming to minimize exacerbations, symptoms, and to provide a better quality of life [11].

Indeed, *in silico* or computer-based simulation software has emerged as an important tool for the advancement of precision medicine, especially when extensive preclinical and clinical data are scarce.

In this section, I will provide a brief overview of asthma disease and delve into the different characteristics of salbutamol, a regularly drug used in asthma treatment.

This information is based on the review article about salbutamol in the management of asthma [12].

1.1 Asthma: definition and pathophysiology

Asthma is defined as a chronic heterogeneous airway disease characterized by inflammation and airway hyperreactivity, leading to a broad-spectrum of symptoms such as wheezing, shortness of breath, chest pain, and cough [13–16], that vary over time and in intensity. The concepts underlying asthma pathogenesis have been unravelling over the last few decades. Bronchoconstriction, airway edema, airway hyperresponsiveness, and airway remodelling are common patterns in the development of this condition [16]. First, bronchoconstriction involves the narrowing of the airways through bronchial smooth muscle contraction, resulting in greater difficulties with airflow. Second, airway edema arises when the poor-controlled disease becomes increasingly persistent. Airway hyperresponsiveness refers to an exaggerated bronchoconstrictor response caused by inflammation, dysfunctional neuroregulation, and smooth muscle structural changes. This physiological event often occurs during anti-asthmatic medication. Finally, permanent airway structural changes may develop, albeit these are usually reversible in most people. This remodelling is linked to progressive loss of lung function and activation of several cells in the lung microenvironment, resulting in a gradually reduced response to therapy [17].

Inflammation is the key role in asthma, where various interactions occur between different cell types and mediators. The discovery and acknowledgement of several cell populations, it is now recognized that lymphocytes, inflammatory mediators such as chemokines and cytokines, mast cells, eosinophils, neutrophils, macrophages, and epithelial cells are some of the central figures in the asthma inflammatory process [16].

The different types of asthma include allergic asthma, non-allergic asthma, adult onset asthma, exercise-induced bronchoconstriction (EIB), occupational asthma, asthma-COPD overlap, and paediatric asthma [18,19]. The most prevalent one, allergic asthma, is triggered by allergens, whereas non-allergic is brought on by stressful situations, viral infections, and extreme weather. Adult-onset asthma is the term used to describe those situations when people only experience their first asthma symptoms as adults. EIB, also known as exercise-induced asthma, occurs when, in asthmatic patients, physical activity causes airways to constrict. Of note, EIB is also experienced in non-asthmatic patients. People who usually work around chemical fumes, dust, or other air irritants may develop occupational asthma. Simultaneous

asthma and chronic obstructive pulmonary disease (COPD) are recognized as asthma-COPD overlap [18,19]. Furthermore, the Global Initiative for Asthma (GINA) [6] distinguishes two additional clinical asthma phenotypes: asthma with persistent airflow limitation and obesity-associated asthma, as obese patients are more predisposed to respiratory problems.

Regarding disease severity, the 2022 GINA guidelines [6] classify asthma into three categories: mild, moderate, and severe (Table 1). This diagnosis, according to GINA, is based on the identification of respiratory symptoms typical of asthma, such as wheezing, shortness of breath, coughing, chest tightness, or a limitation of expiratory airflow (assessed from the bronchodilator reversibility test or from others).

Table 1. The classification of asthma severity according to the 2019 GINA guidelines; Adapted from [6].

Severity level	Clinical characteristics
Mild asthma	Controlled using as-needed reliever medication alone or low-intensity controller therapy such as low-dose inhaled ICSs, leukotriene receptor antagonists, or chromones
Moderate asthma	Controlled with low-dose ICS/LABA
Severe asthma	Requires high-dose ICS/LABA to prevent it from getting out of control, or asthma that is still uncontrolled despite this treatment

ICS, inhaled corticosteroid; LABA, long-acting β_2-agonist

This long-term condition affects all age groups, in particular, the paediatric population [3,20]. The global prevalence has increased, with higher incidence in developed countries than in developing countries [21]. Although still being debated, several theories have been proposed to justify the high incidence of this disease. First, it was assumed that only exposure to environmental factors (air pollutants, indoor allergens) contributed to increases in asthma [22]. Strachan [23] suggested the «hygiene hypothesis», which argues that excessive hygiene in children has a negative impact on the immune system, leading to decreased resistance to these conditions. Later, Rook et al. [24] proposed that lack of exposure to non-pathogenic and commensal microorganisms may also explain the high number of asthma cases.

1.2 Treatment options for Asthma

The treatment of asthma include a variety of drug classes such as short-acting β_2-agonists (SABAs), inhaled corticosteroids (ICS), long-acting β_2-agonists (LABAs), leukotriene antagonists, long-acting muscarinic antagonists (LAMAs),

immunotherapies, and biologic medicines [7,10]. These drugs are prescribed to patients according to their symptomatology and disease severity.

According to the GINA [6], ICS are currently the first-line therapy for asthma. This committee of health experts and patients develop guidelines for the asthma management in light of growing medical knowledge. Therefore, GINA guidelines recommend that all adults, adolescents, and children over 5 years old diagnosed with asthma should be treated with regular or as-needed ICS-containing in the first instance [6,8,25,26]. Alternatively, a SABA reliever with ICS is prescribed, which constitutes a very effective treatment in reducing symptoms.

1.3 Salbutamol in the management of Asthma

Salbutamol, the first selective SABA extensively used in clinical practice, was introduced in 1968 [27]. It is a selective β_2-adrenergic receptor agonist used for symptomatic relief and prevention of acute episodes of bronchospasm caused by asthma as well as other chronic bronchopulmonary disorders [28].

In the past, SABAs were prescribed as first-line therapy in patients with mild asthma [8,29]. Nevertheless, it was shortly demonstrated that prescribing β_2-agonists results in poorly controlled asthma. The rapid symptom relief gives the illusion that asthma is being treated, even though asthma-related airway inflammation is not being addressed [29]. Recent evidence has connected the regular use of SABA with β_2-receptor downregulation, loss of bronchodilator response, increased airway hyperresponsiveness, and increased airway inflammation [30,31], explaining the association of SABA use and mortality [32–34] and risk of exacerbations [35]. According to Gravreau et al. [36], salbutamol in particular has a pro-inflammatory effect when administered regularly. This evidence is further supported by Ritchie et al. [37]. The authors claim that β_2-agonists increase the number of inflammatory mediators, which leads to airway obstruction and hyperresponsiveness, allowing speculation that excessive use of bronchodilators may cause an exacerbation.

Although the World Health Organization (WHO) ranks salbutamol as one of the most effective and safest medicines essential to healthcare systems [38], the subtle deterioration in asthma control over time has led GINA to introduce the most significant change in asthma treatment: SABA-only treatment is no longer recommended [26,39,40]. Therefore, these drugs are currently used either in combination with ICS or as an alternative reliever in specific conditions. The mainstay

of acute asthma therapy, however, remains bronchodilators along with systemic corticosteroid therapy and controlled flow oxygen supplementation [6,41].

Notwithstanding current guidelines, clinical practice continues to be based on the excessive use of SABA relievers. Akker et al. [42] performed a retrospective analysis using medical records of adult asthmatic patients at a health centre in the Netherlands. Of the total individuals under study, 25% overused SABAs and, among these patients, 19% experienced exacerbations. The authors concluded that clinicians still prescribe SABA as they are unaware of this problematic.

Moreover, a recent program – SABA Use IN Asthma (SABINA) – was developed to investigate the overreliance on SABAs worldwide and its impact on clinical outcomes [43]. This program confirmed the large number of asthmatic people who overuse SABA inhalers and confirmed that there is a link between high bronchodilators use and severe risk of exacerbations [35,42].

1.3.1 Chemistry

Salbutamol (Figure 1) is a chiral drug with (R)- and (S)-isomers [44]. Its pharmacological activity is associated to the (R)-enantiomer because it binds to the human β_2-adrenoceptor. The activity of the (S)-enantiomer is controversial [5,44–47]. Although this isomer is assumed to be inert in humans, Patel et al. [44] reported an experimental study that suggested that (S)-isomer may have clinically significant adverse effects. Furthermore, it is believed that (R)-salbutamol in its non-racemic form has beneficial effects. Gumbhir-Shah et al. [48] reported identical pharmacokinetics (PK), pharmacodynamics (PD), and safety of this isomer, provided either as the single enantiomer or racemic mixture by inhalation to subjects with mild to moderate asthma. Controversially, several well-conducted studies reveal that this isomer is not clinically superior to racemic salbutamol.

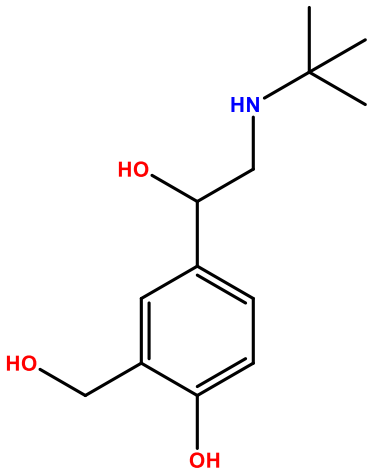

Figure 1. Chemical structure of salbutamol.

1.3.2 Pharmacokinetics and Metabolism

The pharmacokinetics (PK) of salbutamol depends on many variables. The formulations and the delivery mechanism (MDI or DPI) used have an impact on the amount of drug that reaches the airways, absorption, and, consequently, effectiveness and the side effect profile [49]. Following inhalation, the systemic levels of salbutamol are undetectable since it first acts topically on bronchial smooth muscle [28]. After 2–3 h, low plasma concentrations are observed due to the swallowing and oral handling of the inhaled drug. Oral administration is rapidly and well absorbed, with peak plasma salbutamol concentration observed after 2 h. However, the drug undergoes the first-pass effect, related to both strong hepatic and pre-systemic metabolism in the intestinal mucosa, resulting in only 50% of bioavailability [49]. The majority of data on salbutamol blood and urinary concentrations come from studies on healthy non-asthmatic participants who have never taken SABAs [50]. However, as Elers et al. [50] concluded, PK of inhaled and oral salbutamol did not differ between β_2-agonists-naïve non-asthmatic subjects and asthmatic individuals using regular anti-asthmatic medication. Lewis et al. [51] studied 11 acute severe asthmatic patients and all of them presented low or undetectable plasma concentrations of salbutamol after inhalation treatment. There are few PK studies on salbutamol provided intravenously (IV) [52].

Salbutamol is mainly metabolized by sulfate conjugation into the 4'-O-sulphate ester, which possesses negligible pharmacologic activity [28]. This occurs in the liver, where the metabolizer enzyme sulfotransferase is found [53]. The metabolism can also occur in the gastrointestinal tract, due to the swallowing of an inhaled dose, and in the

cytochrome (CYP) P540 enzyme system (minor metabolic route) [28,54,55]. There are also other metabolic pathways of salbutamol, which will be discussed later. As aforementioned, this β_2-agonist is not totally absorbed after inhalation or oral administration, resulting in about 30% of non-metabolized drug. In turn, the portion of non-metabolized salbutamol is approximately 65% when it is administered intravenously. Following metabolization, most of the intake drug is excreted in the urine within 24 h, with a small fraction eliminated in the faeces [28]. The elimination of (R)-salbutamol is substantially faster than that of (S)-salbutamol, since the latter is metabolized up to 10 times slower than (R)-salbutamol [52,56]. Some theories on the subject have been proposed. Both isomers may have different metabolism pathways; however, Ward et al. [57] revealed that there was no differential lung metabolism between (R)- and (S)-salbutamol. The elimination half-life of inhaled or oral salbutamol has been recorded as being between 2.7 and 5 h while after intravenous (IV) administration it has been documented as being approximately 3–4 h [27,28]. Clearance is reported to be 272 ± 38 mL/min after oral administration and 291 ± 70 mL/min after an IV administration [28]. The similarity between oral and inhaled excretion patterns assumes the presupposed theory: a significant portion of an inhaled dose is swallowed [58].

1.3.3 Mechanism of Action

The smooth muscle of the respiratory tract is constituted by a large number of receptors. Their activity is mediated by the production of cyclic adenosine monophosphate (AMP) as a second messenger. Therefore, as an agonist, salbutamol binds reversibly to these receptors, which are believed to be adenyl cyclase, resulting in the conversion of cyclic AMP (Figure 2). Cyclic AMP then triggers a cascade of intracellular events that culminate in the inhibition of the contraction of bronchial smooth muscle, thereby promoting smooth muscle relaxation and bronchodilation – its therapeutic effect. Salbutamol also inhibits the release of immediate hypersensitivity mediators from cells, particularly mast cells. Due to its high selectivity, salbutamol has minimal activity on β_1-adrenergic receptors [59–62].

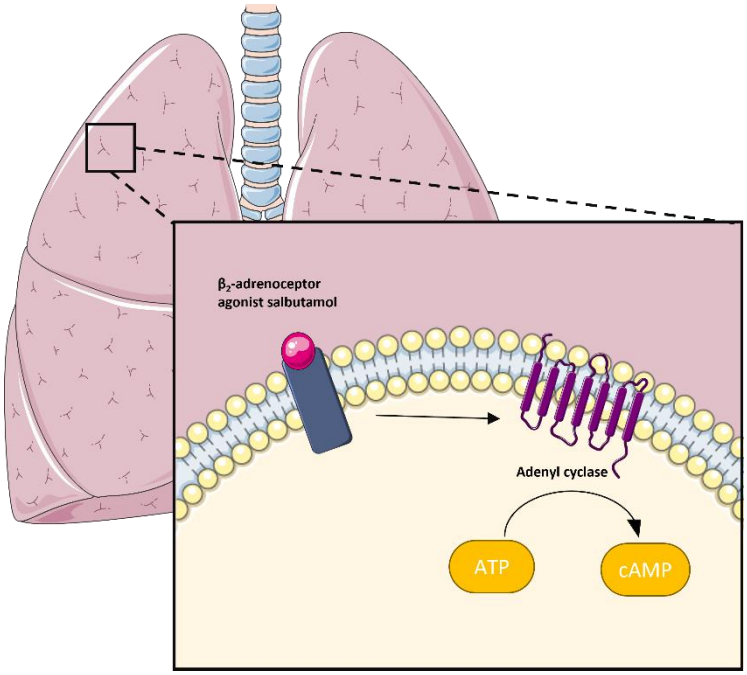

Figure 2. Mechanism of action of salbutamol in the relief and prevention of bronchoconstriction [53]. The β_2-adreneceptor agonist (pink) reversibly binds to the β_2 receptor (dark blue), which activates adenyl cyclase, resulting in the conversion of ATP to cyclic AMP (cAMP). This promotes bronchodilation and relieves symptoms experienced during an acute asthma episode (right). Created with SMART – Servier Medical ART. Available online: https://smart.servier.com (accessed on 16 January 2023). Reproduced by Marques et al. (doi: 10.3390/futurepharmacol3010019).

1.3.4 Pharmacodynamic properties

The major key physiological role of salbutamol is its bronchodilator effect in the lungs [63–65]; however, it also possesses other properties, including cardiovascular, uterine, metabolic, and neurological effects. Usual therapeutic doses of inhaled salbutamol do not significantly affect the cardiovascular system, unlike other formulations [65]. A study in healthy volunteers revealed that IV or nebulized salbutamol induced a dose-related increase in heart rate and systolic blood pressure [66,67]. In asthmatic patients, inhaled and oral salbutamol raised heart rates by 23% and 28%, respectively [68,69]. However, this increase is observed as well in asthmatic patients with cardiovascular disease [62], highlighting the caution with which these patients should be treated when they are prescribed salbutamol. On the other hand, in patients with chronic heart failure, there are beneficial effects when treated with this β_2-agonist [70].

Salbutamol decreases potassium concentration in blood. The mechanism underlying this process is assumed to be related to the stimulation of β_2-adrenoceptors linked to membrane-bound Na/K ATPase on skeletal muscle, which induces an influx of potassium into cells and a subsequent reduction of plasma potassium concentration [71,72]. The lipid effects of this drug are identified as increased blood levels of non-esterified fatty acid (NEFA), insulin, and HDL-cholesterol [73–75].

Wager et al. [73] investigated the cardiovascular and metabolic effects of oral and IV salbutamol in diabetic and non-diabetic pregnant women throughout the third trimester. The findings showed a significant increase in plasma levels of insulin, carbohydrate, and lipid metabolites, revealing that glycogenolysis, lipolysis, and insulin secretion were stimulated. Diabetic women have more pronounced glycogenolytic and lipolytic effects, due to their impaired insulin function. For this reason, salbutamol should be carefully prescribed to diabetics. Rolf Smith and Kendall [72] also stated the association between β_2-receptors and glycogenolysis, and insulin release, with their satisfactory results in increasing plasma glucose and insulin concentrations in healthy volunteers.

Salbutamol is thought to possess antidepressant properties. Although the clinical relevance of these findings is unknown and this topic is still underexplored in the scientific community, it has been proposed that these benefits are mediated through an increase in serotonergic system activity [76].

Pregnancy is not affected by this β_2-agonist drug. A study designed to assess the effect of long-term high-dose oral therapy with salbutamol in previous multiple pregnant women showed no effect of salbutamol on current pregnancy duration nor birth weight [77]. Nevertheless, since it may enter the embryo through the placenta, it is likely to have an impact on the fetus' metabolism, despite the scarcity of human research in this area [63]

Due to its inhibition of mast cell mediator release in asthmatic patients, reducing changes in forced expiratory volume (FEV), plasma histamine, and neutrophil chemotactic activity (NCA), salbutamol has an impact, albeit minimal, in inhibiting allergic responses [63]. This β_2-agonist may be a potent drug for treatment of multiple sclerosis (MS), due to its ability to regulate the expression of several cytokines [27]; however, this topic has not yet been explored. As far as MS-related fatigue is concerned, Almeida et al. [78] questioned the possibility of using salbutamol as an

alternative treatment. In a group of 30 patients with relapsing-remitting MS and fatigue, treatment with this SABA did not improve this condition.

1.3.5 Adverse Effects

β_2-adrenergic receptors, in addition to being found in lung membranes, are found in skeletal vascular, liver, and cell membranes [79]. Thus, salbutamol may have other effects than the bronchodilator effect that has been reported. Since this drug is available in a variety of dosage forms, the side effects are also rather diverse. In fact, IV is the route of administration with the greatest adverse effects identified, followed by oral and nebulized administrations. The inhaled form represents the safest route of administration [62]. The following table summarizes the major adverse effects reported after administration of salbutamol.

Table 2. Adverse effects of salbutamol.

Biological system	Adverse effects	References
Musculoskeletal system	It may cause tremors and myopathy. Systemic salbutamol boosts skeletal muscle strength in young men.	[80,81]
Cardiovascular system	Salbutamol when administered via inhaler may cause tachycardia and peripheral cardiac vasodilation-induced reflex. Arrhythmias and angina are reported events. Asthmatic patients with concomitant cardiovascular disorders should use salbutamol carefully.	[62,82–85]
Respiratory system	Some patients may experience a felling of «thick neck», chest heaviness, erythema, and pulmonary edema. Paradoxical bronchoconstriction may also occur.	[86–89]
Metabolic system	It may cause hypokalemia and insulin, glucose, pyruvate, free fatty acids (FFA), and lactate increases. Salbutamol should be cautiously used in diabetic patients. The risk of hypokalemia is greater when this drug is provided simultaneously with corticosteroids and theophylline.	[90–97]
Nervous system	Hallucinations, tremors, and anxiousness may occur since salbutamol can easily cross the blood-brain barrier (BBB).	[98–100]

SABA overreliance is the main reason for this large number of occurrences. As previously mentioned, when patients experience a worsening of symptoms, they tend to increase their use of SABA, leading to a greater risk of adverse outcomes. Nevertheless, salbutamol is part of the Essential List of Medicines provided by the

WHO and is considered «one of the safest and most effective drugs currently available» [38].

1.3.6 Routes of Administration

Salbutamol can be administered intravenously, intramuscularly, subcutaneously, via inhalation, or orally (Figure 3). The dosages required, efficacy, and adverse effects differ significantly among these routes [27,54].

The first-line route of administration is the inhalation of salbutamol via spacer/inhaler. Indeed, current clinical practice is based on inhaled administration. It is characterized by its rapid onset, low frequency of adverse effects, and convenience of administration [39,101]. Salbutamol is prescribed every 20 min in the initial treatment, at a dose of 0.05 to 0.15 mg/kg. Afterward, the dose can be increased to 0.45 mg/kg, with a maximum dose of 20 mg per hour [102,103]. The recommended dosages for the other salbutamol formulations are shown in Table 2.

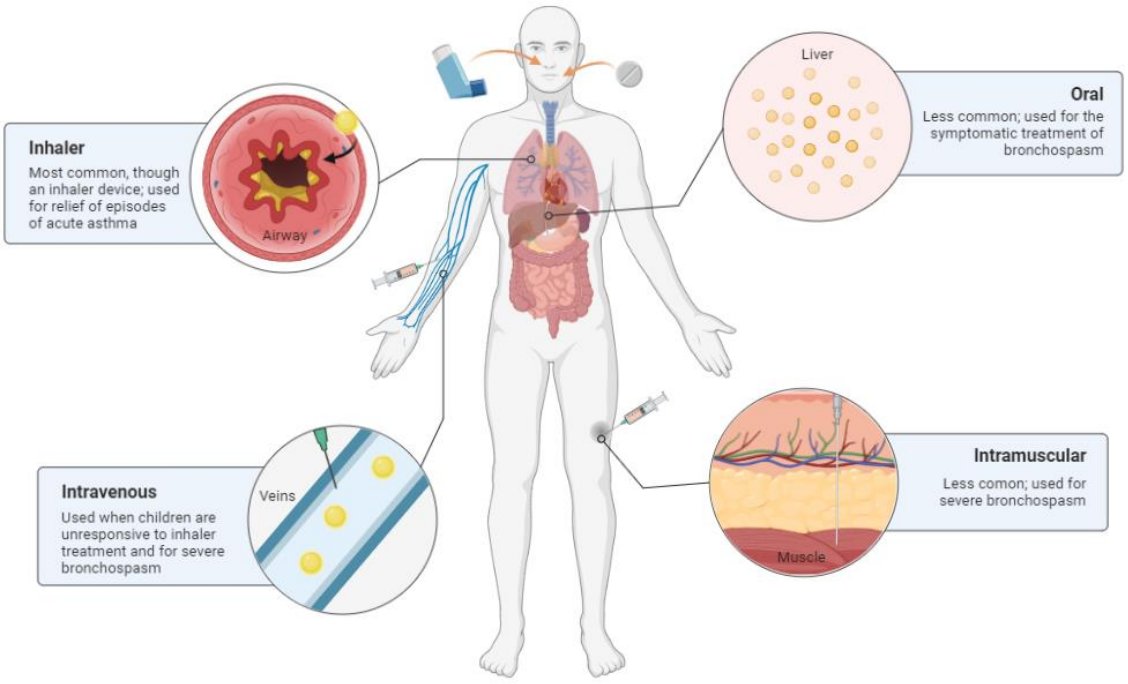

Figure 3. Main routes of administration of salbutamol: inhalation, oral, intravenous, and intramuscular [39,62,104]. Created with BioRender.com. Available online: http://biorender.com/ (accessed on 11 October 2022). Reproduced by Marques et al. (doi: 10.3390/ijms232214207).

Table 3. Recommended dosages for salbutamol formulations.

Clinical Use	Inhaler (100 µg)	Dry Powder Inhaler (200 µg)	Nebulizer (5 mg/mL)	Oral Syrup (2 mg/5 mL)	Oral Tablets (2 or 4 mg)	Intramuscular Subcutaneous	Intravenous
Intermittent asthma attacks or acute bronchospasm	A: 1 to 2 puffs every 4 h up to 4 times a day C: 1 puff every 4 h up to 4 times a day	1 puff up to 4 times per day	AC: 0.5 to 1 mL	A: 5 mL to 20 mL, up to 4 times a day C: 2.5 or 5 mL, 3 or 4 times a day	A: 4 mg, 3 or 4 times a day C: 1 or 2 mg, 3 or 4 times a day	A: 500 µg every 4 h	A: 250 µg injected slowly
Exercise-induced broncho-constriction	A: 2 puffs 15 min before exercise C: 1 puff 15 min before exercise	1 puff 10 to 15 min before exercise	NA	NA	NA	NA	NA
Continuous treatment	NA	NA	1 to 2 mg per hour	NA	NA	NA	NA

A – adults; C – children; NA – not applicable.

Inhaled salbutamol is, in fact, considered the best option for asthma sufferers. However, these patients frequently misuse inhaler devices, which can have a negative impact on clinical outcomes [105].

1.4 Pharmacometrics in Precision Medicine of Asthma

Precision medicine in asthma, in other words described as a method of treating and preventing disease by considering individual variability in patients' genes, environment, and lifestyle, has focused on categorizing asthma endotypes. The identification of clusters of patients with a given phenotype, including clinical pattern, prognosis, and treatment outcome, is the main driver of subclassification of asthma. This respiratory condition, as already mentioned, is a heterogenous and complex disease, so subgrouping patients is a challenging task.

Clinicians aim to prescribe the optimal treatment for each patient according to their phenotype, however, it is still common to base therapy only on the severity of the disease, which does not seem to be the ideal strategy, given the current problems involved with asthma management.

The advancement of biological therapies has recently sparked the introduction of precision medicine in this field. In fact, the GINA guidelines recognize these therapies as an add-on treatment in step 5. For instance, anti-IgE antibodies, by binding themselves to free IgE, prevent free IgE from binding to their receptors on mast cells and basophils. Therefore, due to the inhibition of IgE, one of the main triggers of allergic asthma, this approach is recommended in patients with severe allergic asthma.

Anti-IL5 binds to the IL5 cytokines, preventing the differentiation of eosinophils and the consequent production of their precursors. This therapy, in turn, is targeted for use in patients with eosinophilic asthma.

Therefore, omics actively contribute to the development of these targeted therapies. There are, however, alternative tools that can promote precision medicine in asthma, namely *in silico* methods that allow the development of models capable of predicting the response to a particular treatment while taking individual patient variability into consideration. Thus, pharmacometrics is a very promising field when it comes to tailoring a treatment to the patient.

Pharmacometrics, an emerging revolutionary discipline, is defined as the science that quantifies the interaction between drugs and patients by connecting the biology, physiology, and pharmacology with disease condition through mathematical models. Briefly, pharmacometrics is the analysis of PK and PD data and the development of mathematical models (*in silico* approaches) that can be applied at different drug stages, including accelerating drug development and approval, supporting regulatory decisions, and improving the clinical practice through drug optimization integrating precision medicine. This thesis seeks to broaden knowledge about the currently available salbutamol treatment from the standpoint of pharmacological optimization and, in an ideal scenario, to obtain tools to advance precision medicine in asthma.

1.5 Objectives

The purpose of this thesis is to develop a PK study of salbutamol through *in silico* approaches. First, we will develop, for the first time, a predictive model of the potential CYP P450-mediated interaction between salbutamol and an antidepressant drug, fluvoxamine.

Subsequently, we aim to develop population PK models of salbutamol using virtual patient data, derived from PBPK models, in order to understand which individual characteristics have impact on the therapeutic regimen currently prescribed to asthma patients. Considering the different commercially available salbutamol formulations, a PK population model will be developed for each type of salbutamol formulation.

This thesis is divided into two chapters: *Prediction of salbutamol CYP-mediated drug interaction* and *Population pharmacokinetic study of different salbutamol formulations: metered-dose inhaler, dry powder inhaler, nebulizer, oral syrup, oral tablet and intravenous*, each supported by two original articles.

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Chapter I

Prediction of salbutamol CYP-mediated drug interaction

1. State of art

The process of prescribing a drug significantly impacts the effectiveness and quality of treatment. Clinicians are faced with factors such as dosage, administration route, contraindications, and adverse reactions. An ideal pharmacotherapeutic strategy must evaluate each of these elements, however, it should also consider drug-drug interactions (DDIs), particularly in patients with a wide spectrum of comorbidities. In fact, taking multiple medications simultaneously is the key driver for the increased risk of undesirable DDIs.

A DDI is defined as the interaction that occurs when two or more drugs interact with one other, influencing their effectiveness and/or toxicity, leading to altered drug profiles, raised likelihood of adverse reactions (ADRs), and ultimately, life-threatening outcomes. This interaction can be of a PK nature, resulting in altered absorption, distribution, metabolization, or elimination (ADME) and, as a result, its bioavailability, or it can be PD, which occurs when one drug produces agonistic or antagonistic effects on the other, altering drugs' pharmacological effects [1,2]. Drug-metabolizing enzymes and drug transporters are the epicenters of PK-mediated DDI studies. The monooxygenases metabolizing enzymes, also known as the CYP450 superfamily, are involved in the phase I metabolism of approximately 45% of marketed drugs. These enzymes have numerous isoforms, of which the most relevant for DDI studies are the 1A2, 3A4/5, 2B6, 2C9, 2C19, and 2D6 [3]. PK interactions result from changes in CYP-mediated metabolism through inhibition or induction of their enzyme expression [4,5]. Inhibiting CYP will influence PK parameters, such as maximum concentration (C_{max}) and area under curve (AUC), reflected in increased drug bioavailability [6]. These variations may have positive outcomes, such as greater effectiveness, or negative consequences if toxicity is enhanced. Induction of CYP, on the other hand, promotes the opposite effect.

Remarkably, the management of respiratory disorders usually involves the administration of more than one drug, with different mechanisms of action [7]. In particular, salbutamol monotherapy is currently contraindicated according to the latest GINA guidelines [8]. Thus, this SABA is frequently combined with other medicines, especially ICS and other bronchodilators [9]. In addition, due to the high

prevalence of this chronic disease, there are several disorders that may coexist with asthma [10]. For this reason, asthma sufferers usually undergo polypharmacy (regular use of at least 5 medications).

Therefore, investigating the effect of co-administered drugs on salbutamol is relevant to improving the efficacy and safety of this drug. To do that, having in-depth knowledge of salbutamol's metabolic profile is essential. Salbutamol is metabolized by several pathways (Figure 1) [11]. The main route of metabolism, as previously stated, is sulphate conjugation, where sulfotransferases enzymes are involved. Nevertheless, due to the swallowing effect, and in cases of oral administration, the CYP450 enzyme system plays an active role in the hepatic metabolism of salbutamol [12–14].

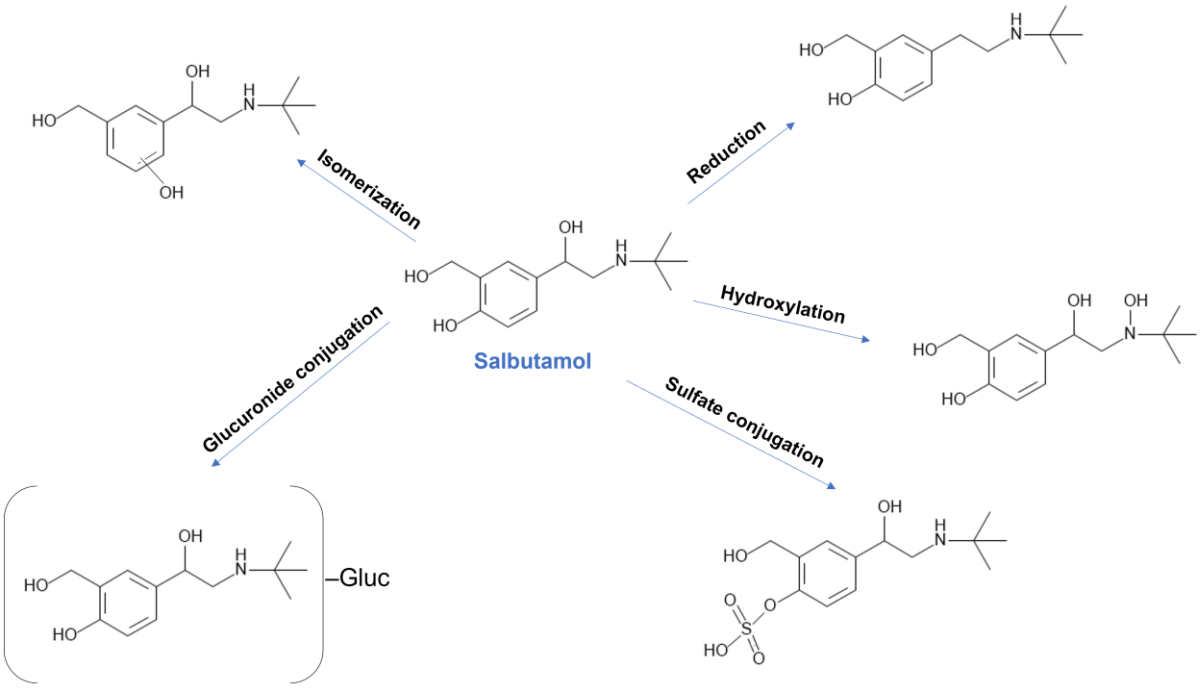


Figure 1. Main metabolic pathways of salbutamol. Created with MedChem Designer. Reproduced by Marques et al. (doi: 10.3390/pharmaceutics15061586).

Despite drug agencies' attempts to explore PK DDIs (driven in part by the inherent difficulty of investigating PD DDIs), this type of research on bronchodilator medicines, namely salbutamol, is quite limited [15]. *In silico* or computer-based simulation software then emerges as an important tool for improving drug characterization when extensive preclinical and clinical data are scarce. In this chapter, the potential CYP-mediated interaction between salbutamol and fluvoxamine will be predicted, through *in silico* approaches. To this end, in a preliminary phase, several potential interactor drugs were screened, with fluvoxamine, an antidepressant, being the eligible candidate. Subsequently, PBPK models that mimic patient characteristics

(age, renal function, pregnancy, weight) were developed for both salbutamol and fluvoxamine and a DDI simulation was conducted.

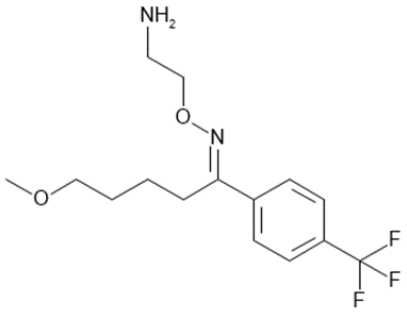


Figure 2. Chemical structure of fluvoxamine. Created with MedChem Designer. Reproduced by Marques et al. (doi: 10.3390/pharmaceutics15061586).

2. Materials and Methods

2.1. Prediction of Pharmacokinetic and Physicochemical Properties of Salbutamol

Salbutamol was characterized according to its physicochemical and PK properties using ADMET Predictor® (Version 10.4; Simulation Plus Inc., Lancaster, CA, USA), a software tool that accurately predicts several features of compounds, including physicochemical and PK properties. The chemical structure of salbutamol was drawn in MedChem Designer (Version 5.5; Simulation Plus Inc., Lancaster, CA, USA) and then imported into ADMET Predictor® in MOL file format. Parameters such as Log P, molecular weight, solubility, human jejunum effective permeability (P_{eff}), diffusion coefficient (Diff. Coeff.), CYP-mediated metabolism and transport, and BBB permeability were estimated in this software tool.

The PKs of salbutamol were simulated with a 4 mg dose given orally over 24 hours, using an ADMET Predictor® functionality (%Fa and %Fb calculator).

2.2. Screening of Potential Drug Interactors with Salbutamol

The screening of the drugs was performed using ADMET Predictor®. A preliminary analysis of drugs frequently combined with salbutamol was conducted. Corticosteroids, anticholinergics, beta-blockers, and others were included in this pre-selection of potential interactors (Table A1, Appendix A). The MOL file of each drug, obtained with MedChem Designer was uploaded into the program. All absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were then predicted, particularly the metabolism mediated by CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4). These determined characteristics were subsequently compared to the predictions derived for salbutamol. The selection of the perpetrator drug was based on the similarity between its metabolic profile and that of salbutamol.

2.3. PBPK Modeling Development

The PBPK models for salbutamol were developed using GastroPlus software (Version 9.8.3; Simulation Plus Inc., Lancaster, CA, USA). The chemical structure of

salbutamol and all the physicochemical and PK parameters previously computed by ADMET Predictor® were imported into this software. Therefore, except for the «Gut Physiology» tab, where we specified the individual characteristics, all sections of the program used predicted values.

The PK parameters of salbutamol were simulated with a 4 mg dose administered orally every 6 hours. Observed values of bioavailability (Fa, fraction absorbed; FDp, fraction of the drug concentration in the portal vein; and F, fraction of the drug concentration in blood), C_{max}, time required to maximum plasma concentration (T_{max}), AUC, and maximum concentration in liver ($C_{maxLiver}$) were derived from ADMET Predictor®. The drug disposition-based parameters were determined in a compartmental PK model in a virtual 30-year-old healthy American male patient. The simulation duration was 24 hours and provided quantitative and visual outputs (plots) of the PK features.

Several characteristics of the subjects, namely age, weight, and health status were modeled for the DDI simulations. Subjects aged 10, 30, and 65 years were included. Weight was established according to the body mass index (BMI) scale, where a BMI of 18.5–24.9 is normal, a BMI of 25–29.9 is overweight, and a BMI ≥ 30 is obese. The health status evaluated in this study was the different severities of renal impairment (mild, moderate, and severe) based on the estimated glomerular filtration rate (eGFR). Healthy was stated as not having any renal or hepatic impairment or weight issues. Additionally, we developed two PBPK models of a healthy woman and a healthy 10-week pregnant woman. Detailed characteristics of these individuals are summarized in Appendix A, Table A2.

2.4. PBPK Model Validation

The PK parameter values obtained from the developed models were compared with literature data. Additionally, a visual inspection of the plots of the plasma concentration profile was performed to establish confidence between the PBPK models and similar studies reported in the literature. The PBPK models were therefore validated.

2.5. DDI Simulation between Salbutamol and Fluvoxamine

After selecting the eligible co-administered drug (fluvoxamine), DDI for salbutamol and fluvoxamine was conducted using the dynamic simulation and the steady-state

mode in the DDI module of GastroPlus. The previously computed dataset was employed as input for the DDI prediction, considering the inhibitory effect of fluvoxamine as a perpetrator on salbutamol (victim). In turn, the inhibition enzyme kinetics constants (Ki, IC50) and induction kinetics constant (EC50) of the perpetrator were already integrated into the software, since the PBPK model of fluvoxamine has been validated by GastroPlus.

The simulations were run according to the previously developed PBPK models. Firstly, the interaction of fluvoxamine on salbutamol was predicted using the PBPK model of a healthy 30-year-old man for 24 hours in both dynamic and steady-state modes. Threedose regimens of fluvoxamine were simulated: 100, 200, and 300 mg, one tablet per day. These doses were obtained from the literature. Subsequently, we used the other PBPK models (different ages and comorbidities) to investigate the DDI of fluvoxamine and salbutamol under different conditions. These predictions were conducted in steady-state mode.

The classification of DDI is based on the AUC ratio in the presence or absence of the perpetrator and is categorized as no interaction, weak, moderate, or strong. With an AUC ratio between 1.25 and 2, the interaction is weak. A moderate interaction is defined with an AUC ratio range between 2 and 5. When the AUC ratio > 5, the interaction is considered to be strong.

3. Results and Discussion

Salbutamol has been combined with several drugs as salbutamol monotherapy is contraindicated [8]. Furthermore, the high prevalence of respiratory diseases worldwide, particularly asthma, leads to the occurrence of comorbidities, or coexisting diseases, requiring the prescription of more than one drug [10]. Many clinical studies have reported the polytherapy-associated increased risk of DDIs. By contrast, little is known about salbutamol PK DDIs. In order to study, for the first time, the PK interaction between salbutamol and fluvoxamine, the physicochemical properties of salbutamol were estimated by ADMET Predictor® (Table 1). The different attributes were compared to values of the main drug databases and with values obtained from other predictive platforms for ADME properties, namely SwissADME and pkCSM (optimized values). As noted, the accuracy of the simulated data is rather considerable, allowing us to proceed with this study.

Table 1. Predicted and optimized physicochemical properties of drug salbutamol.

Physicochemical Properties	Predicted Value	Optimized Value	Reference
Log P	1.6	1.4	[13,14,16–18]
Ionization constant	9.98	10.30	
Molecular Weight (g/mol)	239.32	239.31	
Water Solubility (mg/mL)	15.87	9.53	
Diff. Coeff. ($cm^2/s \cdot 10^5$)	0.804	ND	ND
Peff ($cm/s \cdot 10^4$)	1.331	0.815	Calculated from pkCSM [18]
BBB penetration	Low (97%)	Low	Calculated from SwissADME and pkCSM [17,18]

pKa, Ionization constant; Diff. Coeff, Differential Coefficient; Peff, Effective human jejunal permeability; BBB, blood-brain barrier; ND, not defined.

3.1. Fluvoxamine as the perpetrator drug for salbutamol DDI study

The metabolic profile of salbutamol was examined using ADMET Predictor®. Therefore, the phase I metabolic reactions rely on the participation of the enzymes CYP2C19 and CYP2D6, as outlined in Table 2. Among the 9 CYP superfamily enzymes included in this computer software, salbutamol is a substrate of CYP2C19 and CYP2D6, with an likelihood of 66% and 82%, respectively. This prediction also suggests that this bronchodilator is, with a 49% likelihood, a CYP2D6 inhibitor. In addition to the metabolization sites, data for the enzyme's affinity for the substrate (Km, Michaelis-Menten constant), maximum rate of metabolization (V_{max}), and intrinsic clearance (CL) are also provided.

Table 2. Metabolic profile of salbutamol and fluvoxamine.

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Salbutamol	1A2	No (90%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	No (65%)	NS	NS	NS	NS
	2C8	ND	No (92%)	NS	NS	NS	NS
	2C9	No (99%)	No (98%)	NS	NS	NS	NS
	2C19	ND	Yes (82%)	30.146	157.577	73.179	C7
	2D6	Yes (49%)	Yes (66%)	37.808	2.201	0.466	C17
	2E1	ND	No (91%)	NS	NS	NS	NS
	3A4	No (78%)	No (84%)	NS	NS	NS	NS
Fluvoxamine	1A2	No (51%)	Yes (48%)	1.821	1.500	42.835	C1, C11
	2A6	ND	No (82%)	NS	NS	NS	NS
	2B6	ND	No (83%)	NS	NS	NS	NS
	2C8	ND	No (99%)	NS	NS	NS	NS
	2C9	Yes (41%)	No (78%)	NS	NS	NS	NS
	2C19	No (95%)	Yes (67%)	22.656	250.097	154.542	C1, C3, C11, C12
	2D6	Yes (70%)	Yes (66%)	0.674	3.937	46.721	C1, C3, C11
	2E1	ND	Yes (78%)	ND	ND	ND	C1, C3, C12
	3A4	Yes (80%)	No (54%)	NS	NS	NS	NS

ND, not defined; NS, no substrate

The spectrum of drugs that may be co-administered with salbutamol is extensive, ranging from beta-blockers for heart diseases to antidepressants [15]. The screening of drugs for potential interactions with salbutamol included 17 compounds, whose ADMET properties were predicted. The drug selection for our study was based on the analysis of the CYP metabolizing enzymes of each drug. Since the GastroPlus DDI module is exclusively focused on CYP enzyme-mediated interactions, we have

established the criterion of electing the perpetrator for having at least one salbutamol-metabolizing CYP enzyme. Therefore, fluvoxamine was selected, being metabolized by CYP2C19 and CYP2D6 (Table 2). The respective prediction probabilities point to 67% and 66%. Our screening identified, in addition to fluvoxamine, other equally relevant drugs. Detailed information about these drugs' metabolism is displayed in the Appendix A (Table A3). Nonetheless, due to the lack of clinical data for required software inputs, we chose fluvoxamine for our DDI study, as it is a GastroPlus-verified model.

Fluvoxamine (Figure 2) is a selective serotonin reuptake inhibitor (SSRI) and a sigma-1 receptor agonist, recommended for the treatment of depression and other psychological conditions [19]. Interestingly, this antidepressant received a lot of attention during the pandemic. Several studies have demonstrated benefits of using fluvoxamine in the treatment of patients with COVID-19 [20–22]. Therefore, and because asthmatics constitute a risk group for the COVID-19 infection, the co-administration of salbutamol and fluvoxamine is likely to occur. Our prediction metabolic properties define fluvoxamine as an inhibitor of CYP2D6 and CYP3A4, with a likelihood of 70% and 80%, respectively. In addition to being a substrate for CYP2C19 and CYP2D6, it is also metabolized by CYP1A2 (albeit its prediction likelihood is low) and by CYP2E1. Several studies have reported the interference of fluvoxamine in the metabolism of other drugs via CYP2D6, CYP2C19, and CYP1A2 inhibition [23–26]. Our findings, however, indicate that fluvoxamine is not a CYP2C19 inhibitor with a likelihood of 95% and 51% chance of being a CYP1A2 inhibitor. Undoubtedly, our results contradict the existing literature regarding CYP2D6. *In vitro* studies with liver microsomes should be conducted to support our data. Notwithstanding, since salbutamol is also metabolized by CYP2C19, the interaction between these two compounds may occur through this pathway. However, we should not rule out the influence that fluvoxamine may have on salbutamol in terms of other pathways, namely because both are CYP2D6 substrates and inhibitors.

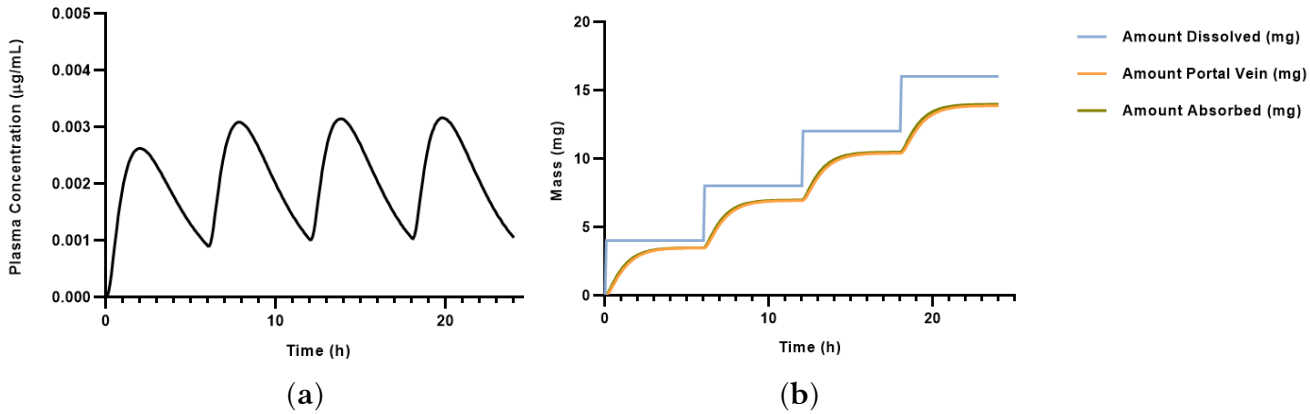
3.2. PBPK model for salbutamol

The PK properties were first estimated by ADMET Predictor® and then transposed to GastroPlus (Table 3). Some of these characteristics (FDp, F, and $C_{max\ liver}$) are not defined owing to ADMET Predictor® limitations. The «Pharmacokinetics» function computed the PK parameters in a healthy 30-year-old American male treated with 4 mg q6h (every 6 hours) oral salbutamol, as detailed in the experimental section. These values were also confirmed according to the literature [13]. Figure 3 illustrates the salbutamol systemic distribution in the defined PBPK model over a 24-hour simulation.

Table 3. Observed (ADMET Predictor®) and estimated (GastroPlus) pharmacokinetic properties of 4 mg salbutamol administered every 6 hours after a 24-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value
Fa (%)	88.82	88.079
FDp (%)	ND	87.486
F (%)	ND	29.447
C_{max} (µg/mL)	0.01013	3.159×10^{-3}
T_{max} (h)	2.73	19.84
AUC_{0-inf} (µg*h/mL)	0.1094	0.05235
AUC_{0-t} (µg*h/mL)	0.1094	0.04929
$C_{max\ liver}$ (µg/mL)	ND	7.57×10^{-3}

ND, not defined



(a) (b)

Figure 3. Pharmacokinetics of 4 mg q6h salbutamol over 24-hour simulation in a 30-year-old American male: (a) evaluation of salbutamol plasma concentration over time, and (b) amount of drug in the portal vein, absorbed, and dissolved over time.

3.3. Effect of different doses of fluvoxamine on salbutamol pharmacokinetics

Aiming to examine the salbutamol-fluvoxamine interaction under different conditions, first, we modelled three different doses of fluvoxamine (100, 200, and 300 mg SID, once daily) on salbutamol kinetics in a healthy 30-year-old American male undergoing fixed-dose salbutamol therapy (4 mg every 6 hours). In this study, we assumed that CYP2C19 and CYP2D6 are the exclusive enzymes of salbutamol metabolism (according to our prediction, about 23% of the drug is metabolized by other enzymes) and therefore we investigated the fluvoxamine's inhibitory effect on these enzymes.

The interaction between fluvoxamine and salbutamol was first simulated in the steady-state mode. Figure 4 depicts the interaction derived-AUC ratio as a function of fluvoxamine dosage. For a fluvoxamine dose of 100 mg, an AUC ratio of 3.630 was recorded, indicating a moderate interaction. When salbutamol is co-administered with 200 mg of fluvoxamine, the AUC ratio increases to 3.973. The highest dose (300 mg) corresponds to an AUC ratio of 4.111. Therefore, a proportional increase in the AUC ratio is observed as the dose of fluvoxamine increases.

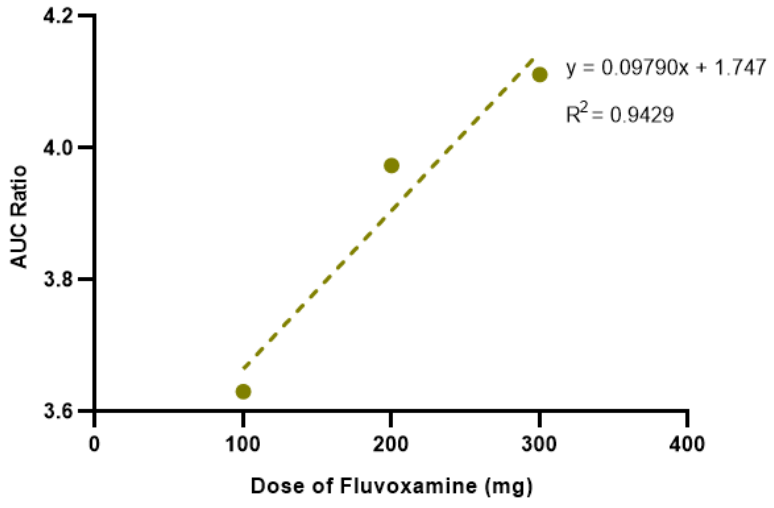


Figure 4. Effect of increasing fluvoxamine dose on the AUC ratio of salbutamol estimated by steady-state prediction in a 30-year-old American male undergoing fixed-dose salbutamol (4 mg q6h) and SID fluvoxamine therapy.

Thereafter, the PK parameters of each combination (salbutamol 4 mg + fluvoxamine 100 mg, salbutamol 4 mg + fluvoxamine 200 mg, and salbutamol 4 mg

+ fluvoxamine 300 mg) were compared to the salbutamol baseline (administered alone), through dynamic simulation (Table 4). The administration of 100 mg fluvoxamine (usual effective dose) with 4 mg salbutamol (recommended oral dose) results, despite the barely noticeable variations, in an increase in all parameters except Fa, FDp, and T_{max}. Fa and FDp refer to the drug bioavailability which, as expected, decreases (not significantly). In turn, the T_{max} of combined therapy is twice as low as the T_{max} of baseline salbutamol. These results support the literature. Fluvoxamine may decrease the clearance of salbutamol, contributing to increased salbutamol serum levels [27]. Hence, if fluvoxamine is effectively a CYP2D6 inhibitor, we can easily hypothesize that this inhibition may reduce the rate of salbutamol metabolism via CYP2D6.

Table 4. Effect of increasing fluvoxamine dose on the pharmacokinetics of salbutamol. Pharmacokinetic parameters were estimated by dynamic simulation for 24 hours in a 30-year-old American male undergoing fixed-dose salbutamol (4 mg q6h) and SID fluvoxamine therapy.

Compound	Fa (%)	FDp (%)	F (%)	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	AUC_{0-inf} (ng.h/mL)
Salbutamol Baseline	88.08	87.49	29.44	0.0032	19.76	52.34	49.65
Salbutamol 4 mg + Fluvoxamine 100 mg	88.06	87.47	38.50	0.0052	7.92	78.04	74.19
Salbutamol 4 mg + Fluvoxamine 200 mg	88.04	87.45	44.76	0.0067	7.92	100.6	95.39
Salbutamol 4 mg + Fluvoxamine 300 mg	88.03	87.43	49.41	0.0080	8.00	120.6	113.9

Co-administration of salbutamol with higher doses of fluvoxamine resulted in a proportional increase in all PK parameters except Fa, FDp, and T_{max}, which remained practically constant. In detail, increasing the fluvoxamine dosage does not influence salbutamol absorption, suggesting that such interaction likely occurs at the metabolism level. The drug fraction measured in the portal vein (FDp), in turn, displays similar drug concentration values when salbutamol is combined with 200 and 300 mg of fluvoxamine, in contrast to the most frequent combination (salbutamol 4 mg + fluvoxamine 100 mg). The portal vein represents the site of drug

entrance into the hepatic systemic where metabolism takes place. As before, this metabolism-unrelated parameter is not changed. We highlight that these parameters may not be accurately predicted, because this software is based on metabolism-mediated interactions. In addition, our findings demonstrate an increase in the proportion of salbutamol in the bloodstream as the dose of fluvoxamine increases. As a CYP2D6 inhibitor, fluvoxamine may impact salbutamol's metabolism rate through enzyme inhibition, leading to higher plasma concentration (Figure 5). As a result of the greater salbutamol non-metabolized fraction, the AUC and C_{max} values are likewise increased. From this standpoint, our results underline the need for monitoring in cases when fluvoxamine ought to be given in higher dosages to asthmatic patients undergoing salbutamol treatment, as the risk of toxicity and ADRs increases.

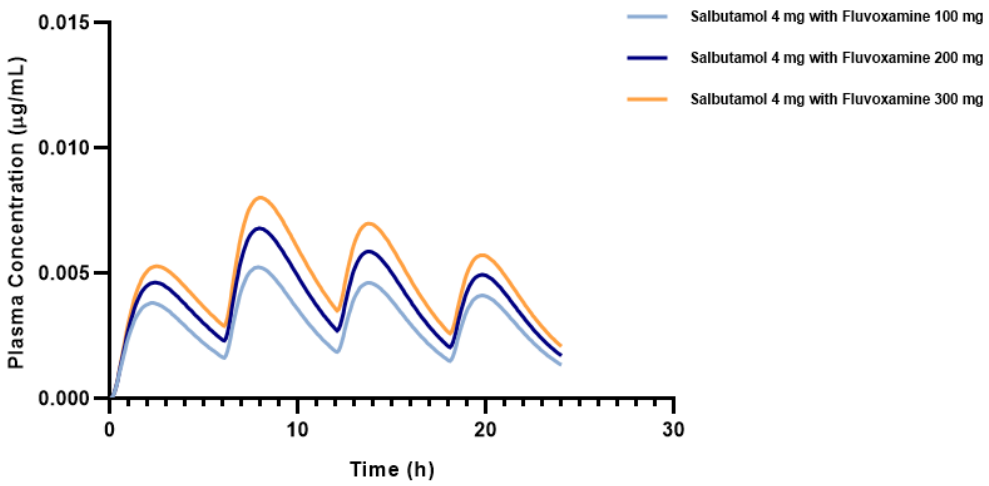


Figure 5. Effect of increasing fluvoxamine dose on the salbutamol plasma concentration estimated by dynamic simulation in a 30-year-old American male undergoing fixed-dose salbutamol (4 mg q6h) and SID fluvoxamine therapy.

3.4. Effect of different ages on salbutamol pharmacokinetics co-administered with fluvoxamine

To investigate whether age had an influence on the co-administration of fluvoxamine and salbutamol, we simulated this therapeutic regimen in virtual male American subjects aged 10, 30, and 65 years (Table 5, Figure 6). In fact, age has been identified as the cornerstone of hepatic clearance alterations, since the rate of drug metabolism in the liver depends on its capacity to remove the drug from the systemic circulation, as well as drug uptake into hepatocytes and enzyme activity,

parameters that change over time [28–30]. For instance, children metabolize medications faster than adults. Although the underlying cause of this phenomenon is unclear, the increased ratio of liver size to body size in children is thought to be the main driver of increased enzyme activity. In addition, CYP450 enzymes have different expression levels depending on age [29–32]. Some are active during pregnancy, while others fully develop days, months, or even years after birth. In the context of our study, CYP2C19 expression reaches adult levels around 10 years of age, whereas CYP2D6 enzyme activity reaches the average adult activity after 5 years of age. At earlier ages, CYP activity exceeds adult levels [32]. Keeping this in mind, we are unable to draw correlations from our results, since the simulated pediatric age was 10 years. Further studies should be conducted to understand whether the administration of more than one drug influences CYP metabolism in younger individuals with enhanced CYP activities. Notwithstanding, our findings reveal that salbutamol concentrations are reduced in 10-year-old children, suggesting that salbutamol-fluvoxamine interaction has no enhanced impact at this age. Of note, AUC ratio of liver unbound, defined as the true inhibitor concentration that determines CYP-mediated DDI, is significantly increased in the PBPK model that mimics pediatric age, compared with adult age.

Table 5. Interaction of different doses (100, 200, and 300 mg) of fluvoxamine on the pharmacokinetics of salbutamol (4 mg q6h) in 10, 30, and 65-year-old virtual subjects.

Dosing Regimen	**Concentration Type**	**AUC ratio**			**DDI Classification**		
Age		**10**	**30**	**65**	**10**	**30**	**65**
Salbutamol with Fluvoxamine 100 mg	C_{max}	3.453	3.630	3.431	M	M	M
	Liver Unbound	2.726	2.021	2.586	M	M	M
Salbutamol with Fluvoxamine 200 mg	C_{max}	3.854	3.973	3.839	M	M	M
	Liver Unbound	3.287	2.558	3.156	M	M	M
Salbutamol with Fluvoxamine 300 mg	C_{max}	4.022	4.111	4.011	M	M	M
	Liver Unbound	3.567	2.903	3.456	M	M	M

M, moderate interaction

In elderly people, drug metabolism may be delayed due to altered CYP enzyme function, reduced liver mass, and blood flow. In fact, several animal studies have documented age-related changes in CYP levels, despite human research failing to

demonstrate such a correlation [28,33,34]. Investigations in human liver microsomes revealed no differences in CYP activities in adult and elderly subjects [35,36]. Our results show declines in the AUC ratio between adulthood and advanced age. This suggests that aging, considering previous studies reporting uncompromised CYP activity, reduces the inhibitory effect of fluvoxamine, as the AUC ratio values decreases (enhanced salbutamol metabolization).

Additionally, we studied whether the previously reported tendency of increased salbutamol kinetics with increasing fluvoxamine dosage in a 30-year-old patient would extend to the other age groups. Our results follow the same pattern (see Figure 5), with greater evidence in the older subject Therefore, the concurrent administration of fluvoxamine and salbutamol should be under close observation in every age group in order to prevent possible adverse outcomes that could result from the increased plasma concentration of salbutamol. Along with this, clinicians may need to readjust the fluvoxamine dosage in patients taking salbutamol on a daily basis. Of note, prescribing more than 100 mg of fluvoxamine is contraindicated in children, thus the other combinations cannot be extrapolated to human clinical trials.

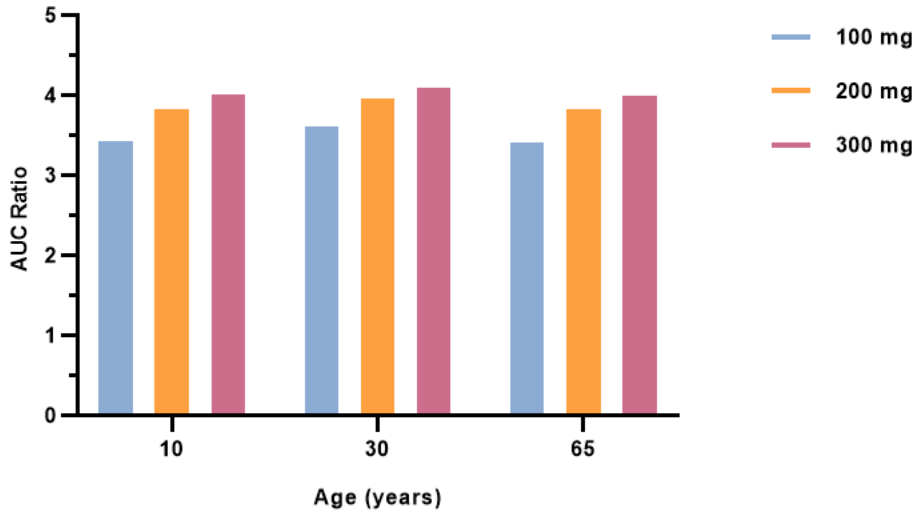


Figure 6. Effect of increasing fluvoxamine dose on the AUC ratio of salbutamol estimated by steady-state prediction in 10, 30, and 65-year-old virtual subjects undergoing fixed-dose salbutamol (4 mg q6h) and SID fluvoxamine therapy.

3.5. Effect of comorbidities on salbutamol pharmacokinetics co-administered with fluvoxamine

Using different PBPK models based on weight and renal function, the interaction between the SABA and antidepressant was further investigated. Table 6 summarizes the systemic and hepatic AUC ratios of salbutamol in patients with different physiological status. In patients with excessive weight, the AUC ratio values are all slightly lower compared to an individual with a normal weight (healthy). These numbers are also reduced in cases of obesity. Therefore, our results go beyond previous studies. Indeed, obesity, as a metabolic disorder, is associated with disturbances in metabolism, leading to an increased risk of ADRs and DDIs. Tamankova et al. [37] have reviewed the effects of obesity on CYP properties. Specifically, the few studies published on the effect of obesity on CYP2D6 expression are contradictory. CYP2C19 activity, in turn, is reported to be higher in obese than in non-obese individuals [38]. Amplified CYP2C19 protein expression may explain our results. Recognizing that CYP2D6 is inhibited and CYP2C19 activity is not impacted by fluvoxamine intake, the increase in CYP2C19 may cover up the fluvoxamine's inhibitory effect, hence, we observed reduced salbutamol AUC ratios in overweight individuals. Thus, we may conclude that increased weight weakens the salbutamol-fluvoxamine interaction.

Table 6. Interaction of different doses (100, 200, and 300 mg) of fluvoxamine on the pharmacokinetics of salbutamol (4 mg q6h) in a 30-year-old American male with different physiological status.

Dosing Regimen	Concentration Type	AUC ratio						DDI Classification					
Physiological Status		**Healthy**	**OW**	**Obese**	**MildRI**	**MRI**	**SRI**	**Healthy**	**OW**	**Obese**	**MildRI**	**MRI**	**SRI**
Salbutamol 4 mg + Fluvoxamine 100 mg	C_{max}	3.630	3.420	3.413	3.423	3.423	3.423	M	M	M	M	M	M
	Liver Unbound	2.021	2.501	2.451	2.511	2.511	2.511	M	M	M	M	M	M
Salbutamol 4 mg + Fluvoxamine 200 mg	C_{max}	3.973	3.831	3.827	3.832	3.832	3.832	M	M	M	M	M	M
	Liver Unbound	2.558	3.075	3.031	3.089	3.089	3.089	M	M	M	M	M	M
Salbutamol 4 mg + Fluvoxamine 300 mg	C_{max}	4.111	4.005	4.001	4.006	4.006	4.006	M	M	M	M	M	M
	Liver Unbound	2.903	3.385	3.341	3.396	3.396	3.396	M	M	M	M	M	M

OW, overweight; MildRI, mild renal impairment; MRI, moderate renal impairment; SRI, severe renal impairment, M, moderate interaction

Likewise, altered renal function, according to our simulations, does not significantly influence the salbutamol-fluvoxamine interaction, since the salbutamol AUC ratio does not vary considerably. Despite this, there is still a moderate interaction between both drugs. Moreover, the severity of renal impairment has no impact on the administration of both drugs, as the AUC ratio are not differentiable. Déri et al. [39] aimed to compare the expression of CYP enzymes in patients with end-stage kidney disease and in healthy individuals. The results indicate a transcription down-regulation of CYP genes in patients with renal impairment, thereby compromising enzymatic activity. Thus, we may correlate kidney function with the transition from extensive CYP-metabolizer to poor CYP-metabolizer. The non-metabolization of the drug at its maximal rate would then explain the increased salbutamol AUC ratio. Additionally, fluvoxamine as a CYP2D6 inhibitor should potentiate the salbutamol plasma concentration when combined with it. In our study, we evidenced otherwise a decrease in the AUC ratio in individuals with renal impairment, compared to the healthy one. Remarkably, the aforementioned proportional increase in the AUC ratio throughout different treatment regimens (100, 200, and 300 mg of fluvoxamine) was observed as well.

The prescription of medication during pregnancy is associated with a high degree of uncertainty, due to the potential risks that some drugs might produce in the fetus and in the woman herself. This critical risk derives from the fact that pregnancy alters the PK profile of several drugs, particularly in terms of hepatic metabolism [40]. The use of salbutamol monotherapy is not contraindicated in pregnancy, and fluvoxamine may be administered under medical supervision [41,42]. With this in mind, we attempted to determine whether the co-administration of these two drugs poses a risk to the pregnant woman and the fetus. Therefore, we used a specific PBPK model for a 30-year-old pregnant American woman and compared its AUC ratio with a non-pregnant 30-year-old American woman (Table 7). Our findings do not demonstrate the aforementioned trend. There are effectively no differences in the AUC ratio between non-pregnant and pregnant women, suggesting that the fluvoxamine-salbutamol interaction is not influenced in this condition. We may therefore conclude that this therapeutic regimen of salbutamol and fluvoxamine is safe in pregnant woman, although there is still a moderate interaction.

Table 7. Interaction of different doses (100, 200, and 300 mg) of fluvoxamine on the PK of salbutamol (4 mg q6h) in a 30-year-old pregnant woman.

Dosing Regimen	Concentration Type	AUC ratio		DDI Classification	
		Female	Pregnant	Female	Pregnant
Salbutamol 4 mg + Fluvoxamine 100 mg	C_{max}	3.426	3.426	M	M
	Liver Unbound	2.530	2.540	M	M
Salbutamol 4 mg + Fluvoxamine 200 mg	C_{max}	3.835	3.835	M	M
	Liver Unbound	3.112	3.117	M	M
Salbutamol 4 mg + Fluvoxamine 300 mg	C_{max}	4.007	4.007	M	M
	Liver Unbound	3.415	3.420	M	M

M, Moderate interaction

Some studies reveal an increment in CYP2A6, CYP2C9, CYP2D6, and CYP3A4 function in pregnant women, whereas those of CYP1A2 and CYP2C19 are decreased [40,43]. Despite the mechanisms of altered CYP-mediated metabolism are not well described, it is believed that gestational hormones play an active role in regulating the expression of these proteins [40]. According to these statements, the prediction of salbutamol metabolism is challenging, since it is metabolized by CYP2D6 and CYP2C19, increased and decreased, respectively, during pregnancy. Given that the metabolic rate is not remarkably affected, we may conclude that pregnancy has little impact on salbutamol regular intake. In fact, as we previously mentioned, salbutamol monotherapy is not contraindicated in pregnancy, suggesting that no ADRs are reported. Nevertheless, salbutamol drug exposure may be altered when co-administered with other medications. Thus, the interaction of fluvoxamine with salbutamol at the CYP2D6 inhibition level, together with reduced CYP2C19 in pregnancy, could potentiate the non-clearance of the parent drug, leading to increased serum levels. As a result, higher AUC ratios are expected in pregnant compared to non-pregnant women. Our results do not corroborate this theo.

Although it was not a primary goal of this study, our results cast a new light on the relevance of gender as a covariate in the interaction of salbutamol with fluvoxamine, since we obtained substantially lower AUC ratios for women than for men. As a matter of fact, being a woman or a man has an impact on drug PKs, mainly due to sex-based differences in metabolism [44–46]. Women, for instance,

exhibit greater CYP2D6 activity than men [47]. Regarding CYP2C19, there have been no reports of significant variations between both genders [46]. Having said that, the metabolic rate of salbutamol is higher due to the greater activity of CYP2D6, leading to a drop in the victim drug's plasma levels. This explains the reduced AUC ratio values in women compared to men. Thus, the influence of fluvoxamine on salbutamol kinetics is not as evident in women as in men.

4. Conclusion

In silico studies of PK interaction between two drugs are scarce. With this study, a predictive model of the CYP-mediated interaction of salbutamol was developed. The main key points to retain are: fluvoxamine given in doses greater than 100 mg, when combined with salbutamol, should be prescribed under medical supervision in order to avoid toxicity and consequent ADRs; renal function, surprisingly, has no impact on the salbutamol-fluvoxamine interaction; overweight has demonstrated a positive effect on this interaction, since it is weakened; pregnangy is not a risk when taking these two drugs concurrently, and there are differences between men and women. Therefore, these are the relevant covariates that should be considered when salbutamol and fluvoxamine are both administered.

We are aware of the limitations of this study, though. Despite we have assumed that salbutamol is exclusively metabolized by CYP enzymes, other metabolization pathways (sulfotransferases) that may influence the salbutamol-fluvoxamine interaction have been identified. Furthermore, we also disregarded the possibility that salbutamol itself may affect drug exposure by CYP2D6 inhibition. *In vitro* assays should be conducted to complement what we present here. More variables, for example, a wider age range, should be included in this study.

Therefore, this study may be extrapolated to other medicines and serves as a pioneer for future PK DDIs studies. As a take-home message, the prescription of various drugs must always be supervised, regardless of the patient's characteristics since this may result in non-accomplished therapeutic effects or undesirable consequences.

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Chapter II

Population pharmacokinetic study of different salbutamol formulations: metered-dose inhaler, dry powder inhaler, nebulizer, oral syrup, oral tablet and intravenous

1. State of art

Effectiveness, defined as the capacity to produce a desired outcome under «real-world» conditions, is determined by a wide range of factors, including PK, which describes how the medication interacts in the human body, and PD, which represents the individual's biological response to the drugs [1–3]. In particular, drugs PK encompasses absorption, distribution, metabolism, and excretion. All these PK parameters depend, among other factors, on the drug's route of administration [4]. Indeed, administration can have a considerable impact on efficacy, hence, depending on the duration and extent of the therapeutic effect, the choice of the route of administration is crucial. In the context of our study, the IV method, which yields faster and complete drug bioavailability [4], will be preferred in severe asthma episodes. On the other hand, in regular asthma therapy, inhaling salbutamol in dry powder form will be the most advantageous from the risk-benefit point of view [5]. Inhaled salbutamol is quickly delivered on the surface area of the airways, leading to rapid relief of bronchospasm-associated symptoms.

Moreover, as we have been arguing throughout this thesis, interindividual variability plays a key role in drug kinetics. Taken together, this will also be relevant in the route of administration selection. Therefore, salbutamol can be administered in several forms: metered-dose inhaler (MDI), dry powder inhaler (DPI), nebulizer, oral (syrup, tablet), and IV [6–8]. In each formulation, the bronchodilator's kinetics are different. Thus, considering the specificity of each patient in the process of choosing a route of administration is a step toward therapeutic success – reduction of adverse effects and exponential therapeutic effect.

Pharmacometrics, through PK and PD modeling, emerges here as a valuable tool in adjusting a treatment for a given patient. In particular, population PK models are used to describe the PK profile of a drug and find sources of variability in patients [9]. In this chapter, population PK models for each currently available formulation of salbutamol will be developed, in order to understand and identify which individual characteristics benefit the therapeutic regimen under investigation. To do

this, a dataset of patients, who will be virtually treated with several salbutamol treatments, will be generated. Afterward, the data will allow the development of population PK models for each formulation, which will incorporate covariates suggestive of influence on PK parameters.

2. Materials and Methods

2.1 Virtual patient data collection

PBPK models developed in GastroPlus software (Version 9.8.3; Simulation Plus Inc., Lancaster, CA, USA) were used to generate virtual patient PK data. All patients in the fasted state were virtually undergoing six types of non-simultaneous treatments, corresponding to six commercially available salbutamol formulations. These treatments are detailed in Table 1.

Table 1. Treatments administered to virtual patients.

Treatment	Salbutamol Metered-dose Inhaler	Salbutamol Dry Powder Inhaler	Salbutamol Nebulizer	Salbutamol Oral Syrup	Salbutamol Oral Tablets	Salbutamol Intravenous
Unit dose	100 µg	200 µg	5 mg/mL	2 mg/5 mL	4 mg	250 µg
Duration	1 puff every 4 hours up to 4 times a day	1 puff up to 4 times a day	NA	5 mL up to 4 times a day	4 times a day	Injected slowly

Salbutamol was characterized according to its physicochemical and PK properties using ADMET Predictor® (Version 10.4; Simulation Plus Inc., Lancaster, CA, USA), as described in Chapter 1. These parameters were subsequently imported into GastroPlus to develop the PBPK models. Therefore, all sections of the program used predicted values derived from ADMET Predictor®.

The PK profile of salbutamol was modeled in different PBPK models with specific individual characteristics, namely age, weight, ethnicity, and gender. In particular, subjects aged 5, 10, 20, 30, and 65 years were included. The BMI scale (outlined in Chapter 1) was employed to determine weight. American, Japanese, and Chinese ethnic groups were examined in this study. Detailed characteristics of these individuals are summarized in Appendix B, Table B1.

The simulation duration was 24 hours, where visual (plots) and quantitative outputs of the drug disposition-based parameters could be drawn. From these

computer models, we categorized the plasma concentrations vs time data by formulation type. All PK parameter values were compared with literature data, and the plasma concentration profile plots were visually inspected to evaluate and validate all PBPK models.

2.2 Population Pharmacokinetic Modeling

Population PK analysis was performed using Monolix Suite 2021R2 (Lixoft, Antony, France). The estimation of population PK parameters was conducted by maximum likelihood using the Stochastic Approximation Expectation-Maximization (SAEM) algorithm.

First, the data set generated for each of the virtual patients treated with each formulation was loaded into Monolix software in .csv text file format. The subject identifier, dose amount information, observations (PK measurements), and patient characteristics (age, gender, BMI, and weight) were recorded. Subsequently, the structural and statistical models were defined. The structural PK models consist of different administration routes (bolus, infusion, oral/extravascular, first-order absorption, or zero-order absorption, with or without lag time) with different compartments (1, 2, 3, or transit compartments) systems with linear or Michaelis-Menten elimination. In turn, the statistical PK models comprise residual error and distribution models and the individual model for the parameters. The covariates tested included age, BMI, gender, ethnicity, and weight.

2.3 Model Evaluation and Validation

After running several population PK models, the Bayesian Information Criterion (BIC), the precision of estimates, and the goodness-of-fit (GOF) allowed the final decision for selecting the best population PK model for each salbutamol formulation. The model with the lowest BIC value was selected. In addition, complementary criteria such as the Akaike information criterion (AIC), to estimate the model quality, and Objective function value (OFV) were used, to find the smaller values which were representative of the best fit. The estimated population parameters' standard errors and the random effects error models' standard errors were computed.

Additionally, several diagnostic plots were used to visually test the model's fit, including individual-predicted salbutamol concentrations and observed versus individual-predicted salbutamol concentrations.

The covariates included in PK models were selected based on the model proposed by the software program. Nevertheless, a more accurate approach was used. Using the ANOVA statistical test for the categorical covariate and the Pearson's correlation test for the continuous covariate, a *p*-value can be calculated. Regardless of their incorporation into the model, the random effect-covariate associations are sorted using the *p*-values. The forward and backward method was employed to select covariates. Until there are no correlation *p*-values over a threshold, the covariate with the smallest correlation *p*-value is included in the model, or the next smallest if the smallest has already been attempted. Until there are no correlation *p*-values below a threshold, the covariate with the highest correlation *p*-value is disregarded, or the next highest if the highest value has already been attempted. Therefore, covariates with a *p*-value less than 0.05 that improved the fit while lowering BIC remained in the model. Shortly, the workflow is schematized in Figure 1.

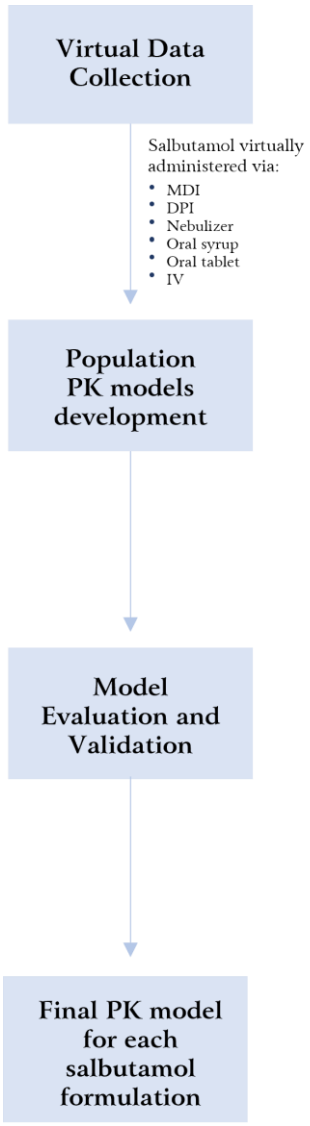

Figure 1. Population pharmacokinetic study workflow.

3. Results and Discussion

3.1 Demographic characteristics

To develop population PK models, a virtual dataset was created using several PBPK models. The employment of virtual populations has emerged as a prominent approach in computational studies, especially when access to clinical data is quite challenging [10,11]. In this case, there are no published clinical studies on salbutamol applicable for this sort of research, where patient demographics and the plasma concentration vs. time profile of the drug are in high demand.

The demographic characteristics are summarized in Table 2. A total of 16 patients, 10 male and 6 female, undergoing the 6 types of salbutamol treatments were included in this study. The mean age and BMI are 27.5 and 22.79, respectively. Furthermore, our virtual population is divided into three groups, according to ethnicity: American represent 75% of total patients, with Chinese and Japanese patients each representing 12.5% of the sample.

Table 2. Demographic characteristics (frequency and mean ± standard deviation) of virtual patients.

Variable	Frequency (n=16)	Mean ± Standard Deviation
Age (years)		
5–20	6	27.5 ± 16.96
21–65	10	
Gender		
Male	10	NA
Female	6	
BMI (kg/m^2)		
16.09–24.70	14	22.79 ± 3.99
24.71–33.73	2	
Weight (kg)		
20–75	14	75 ± 13.12
76–105	2	
Ethnicity		
American	12	NA
Chinese	2	
Japanese	2	

NA: not applicable

3.2 Salbutamol Metered-dose Inhaler

As a first-line route of administration, salbutamol MDI is characterized by its rapid onset and few reported adverse effects. According to the literature, the suggested treatment is a salbutamol inhaler of 100 µg 1 to 2 puffs every 4 hours up to 4 times a day. To investigate the influence of specific patient characteristics, a single dose of 100 µg (1 puff) every 4 hours, not exceeding 4 times a day, was simulated in each of the patients in our study.

3.2.1 Pharmacokinetic Parameters

A preliminary analysis of virtual patient data allowed the gathering of PK parameters for the formulation under discussion. Table 3 displays the main PK parameters: Fa, FDp, F, C_{max}, T_{max}, AUC, and $C_{max\ liver}$. As previously mentioned in Chapter 1, these PK properties were first estimated by ADMET Predictor® (observed values) and then imported into GastroPlus software. Subsequently, data is again computed on the patient in GastroPlus (estimated values). In this case, this

treatment was estimated in 16 different patients. The table shows each parameter' mean and standard deviation (SD).

Table 3. Observed (ADMET Predictor®) and estimated (GastroPlus) pharmacokinetic properties of a single dose of 100 μg salbutamol administered via MDI after a 4-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value (mean ± SD)
Fa (%)	88.82	52.82 ± 0.01
FDp (%)	ND	0.11 ± 0.02
F (%)	71.23	52.59 ± 0.02
C_{max} (μg/mL)	2.50×10^{-4}	$1.05 \times 10^{-3} \pm 0.0005$
T_{max} (h)	2.73	16.08
AUC_{0-inf} (μg*h/mL)	2.74×10^{-3}	$4.19 \times 10^{-3} \pm 0.001$
AUC_{0-t} (μg*h/mL)	2.74×10^{-3}	$3.94 \times 10^{-3} \pm 0.001$
$C_{max\ liver}$ (μg/mL)	ND	$3.84 \times 10^{-3} \pm 0.002$

The values recorded after simulating this therapy deviate considerably from the predicted values. We might have a few justifications for this. First, while calculating PK properties, ADMET Predictor® does not include the «inhaler» form of administration, which might have an impact on these predicted values. Second, ADMET Predictor® bases its results only on the compound's chemical structure, and GastroPlus uses PBPK models to mimic the drug intake. Thus, bioavailability, represented by Fa, FDp, and F, as well as maximum blood concentration, are significantly reduced in the simulation of patient dosing, while T_{max} and AUC have higher values than expected ones.

Figure 2 illustrates the salbutamol plasmatic concentration in the defined PBPK models over a 4-hour simulation. According to the interindividual variability of the virtual patients, the data were stratified according to population subgroups: age, BMI, and ethnicity.

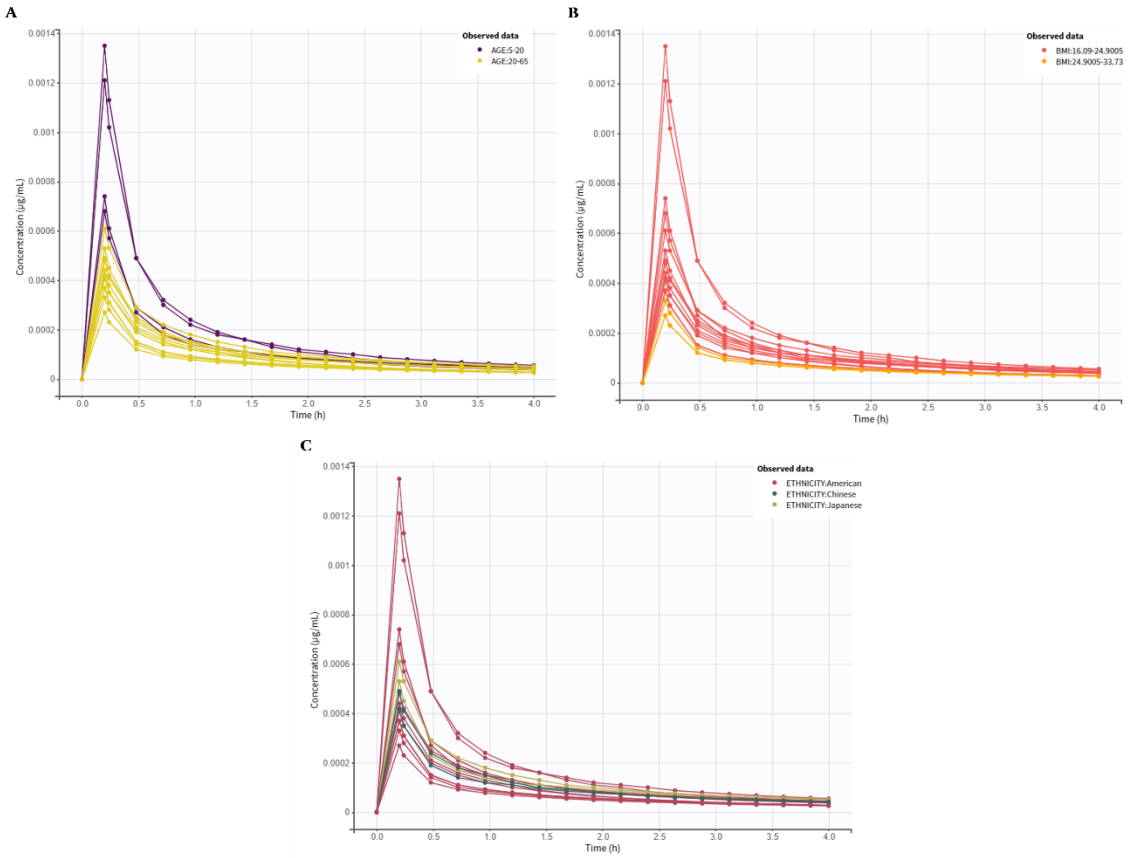

Figure 2. Observed data of plasma salbutamol concentration (µg.mL^{-1}) through time (h), stratified by age (A), BMI (B), and ethnicity (C).

Based on these first results, age and BMI are determining factors in the effect of salbutamol on individuals. In fact, the observed C_{max} in the youngest (5–20 years) is much greater than the values observed in adulthood (20–65 years), suggesting that some toxicity may be reached in this age range. Children often represent a group of particular concern when prescribing a drug, and lower dosages are usually recommended. In fact, the exposure of drugs in children varies from that in adults due to the PK and PD differences between children and adults [12,13]. In the context of our study, the dose administered to virtual patients is equivalent to the dosage recommended for children (a single dose of 100 µg of salbutamol). As a result, we may conclude that age is a factor that directly impacts the plasma concentration of salbutamol, and indirectly affects the effectiveness and safety of the drug due to the increased risk of toxicity.

Although information regarding the influence of obesity on drug PK is sparse, this condition has been found to interfere with drug distribution (ADME). Obesity-related changes in normal physiology such as changes in lipid content, metabolizing enzymes, and drug transporters, are reported [14–16]. In particular, obese patients may experience an alteration in the penetration of the drug into the tissue (increased or decreased), with a noticeable impact on drug absorption. Our findings reveal that patients with increased body weight (BMI ≥ 24.90) demonstrated lower peak plasma levels, suggesting that weight gain has an impact on salbutamol absorption (since drug plasma concentration increases with the extent of absorption). This might be related to lower tissue permeability and hence the greater difficulty of salbutamol entering the bloodstream. Therefore, it could indicate that a higher dosage is required to achieve effective concentrations.

Ethnic disparities in drug response are linked to environment-gene interaction. Thus, this effect is more evident in drug metabolism, resulting from the uneven distribution of polymorphisms in the metabolizing enzyme encoding genes [17,18]. To our knowledge, there are no studies addressing ethnic differences in drug absorption. Similarly, no conclusions can be drawn about salbutamol absorption in Americans, Japanese, and Chinese.

3.2.2 Model Building Process

The basic model that best described the population PK profile, as determined by the smallest BIC value, is a three-compartment model with first-order absorption, no delay, and linear elimination (Figure 3). A combined error model and normal parameter distribution were used to further develop this model. The covariate analysis demonstrated an effect of age on Vd and an effect of weight on Cl, on Vd in the three compartments, and on intercompartmental clearance (Q). Adding other covariates did not significantly improve the model. BIC values for the computed models are included in Appendix B (Table B2).

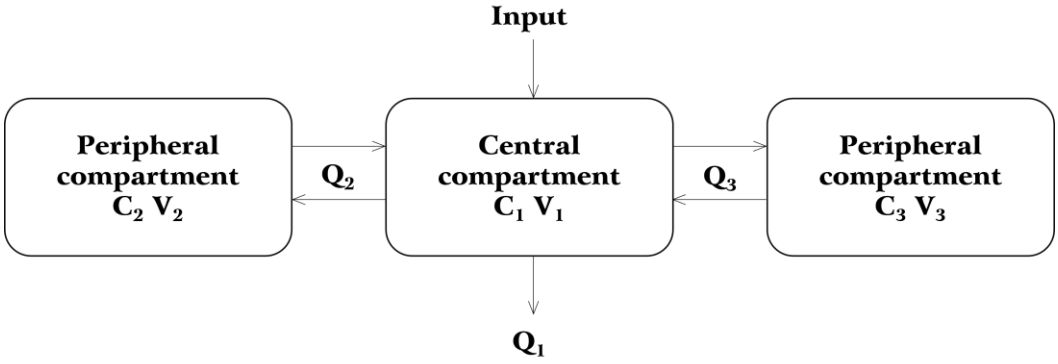

Figure 3. Population pharmacokinetic model used to describe salbutamol plasma concentration-time profiles. C_1, C_2, and C_3 are the compartments, V_1, V_2, and V_3 represent the Vd of each compartment, and Q_1, Q_2, and Q_3 are the intercompartmental clearance.

The parameter estimates of the final model are summarized in Table 4. The relative standard errors (RSE), calculated via Fisher Information Matrix, represent the uncertainty of the estimated population parameters [19]. The software was, however, unable to compute the RSE of some parameters, as verified below. In short, the obtained RSEs are slightly increased.

Table 4. Estimates of the population pharmacokinetic parameters of the final model for salbutamol MDI formulation.

Parameter	Estimate	RSE (%)
K_a pop	ND	ND
Cl pop	114.11	ND
V1 pop	64.67	17.20
Q2 pop	108.94	56.20
V2 pop	172.51	ND
Q3 pop	730.48	ND
V3 pop	98.39	55.20
Error model parameters		
b	0.05	3.86

RSE: Relative standard error; K_a – Absorption constant rate; Cl – Clearance; V1 and V2 – The volume of distribution of the compartments one (central) and two (peripheral); Q – intercompartmental clearance; ND – not defined.

3.2.3 Final Population Pharmacokinetic Model

After running the best model that fits our dataset, a correlation between observed vs. individual and population-predicted concentrations was established (Figure 4). Both plots demonstrate no misspecifications in the model since there are not a large proportion of outliers (defined as points that lie outside the 90% prediction interval). Figure 5 represents the scatter plots of individual weighted residuals (IWRES) and normalized predictive distribution errors (NPDE), revealing a random distribution centered on zero [20]. In addition, these plots allow misspecifications in the structural and statistical models to be detected by approximating the residuals to the horizontal zero line.

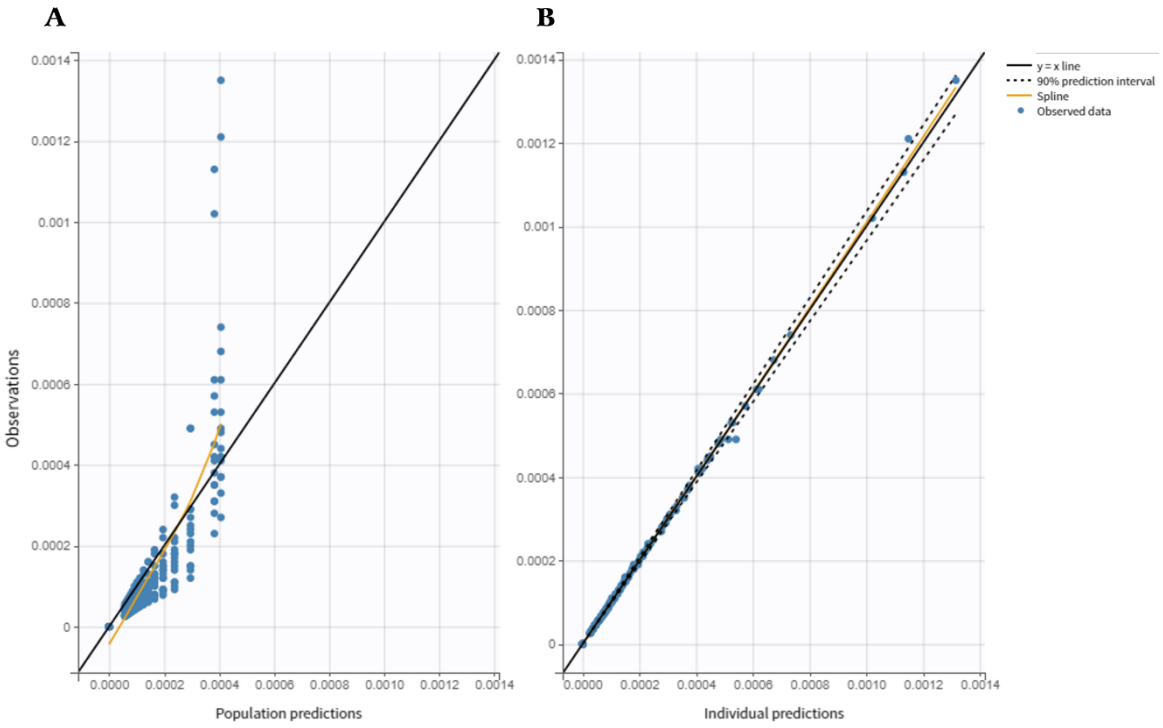

Figure 4. Final salbutamol MDI covariate pharmacokinetic model: (A) observation vs. population predictions, and (B) observations vs. individual predictions of salbutamol concentration ($\mu g.mL^{-1}$).

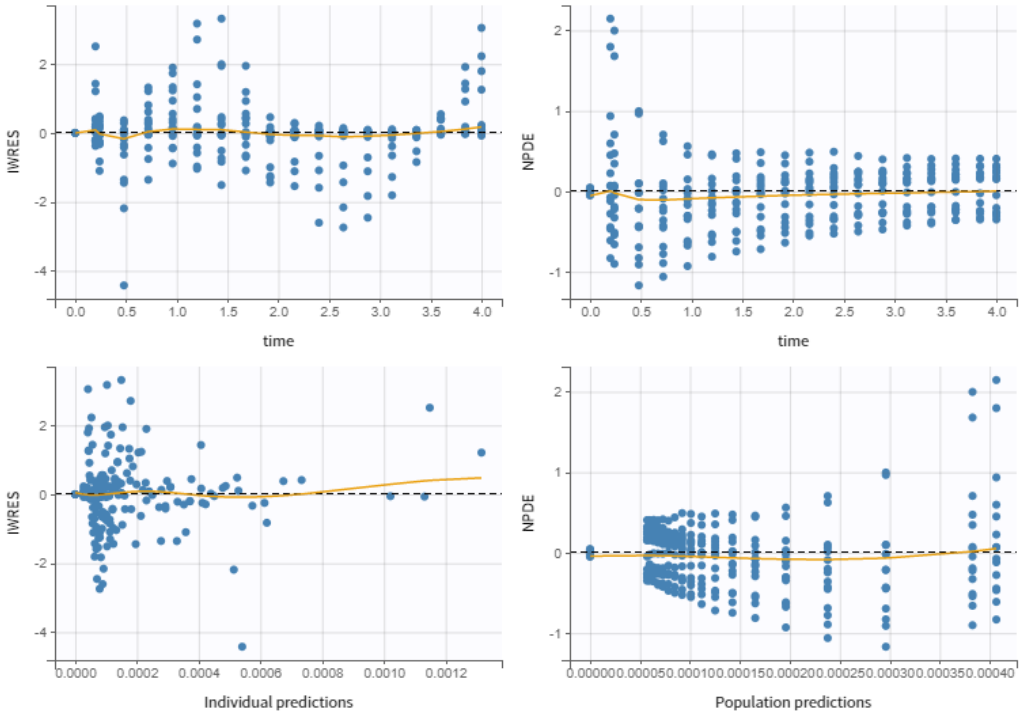

Figure 5. IWRES and NPDE versus time, individual, and population predictions for the salbutamol MDI pharmacokinetic model. The yellow line represents the spline of the data, and the theoretical mean is shown as a dotted line.

The visual predictive check (VPC), through multiple simulations with the model and the design structure of the observed data grouped in bins over successive time intervals with optimized binning criteria, determines the ability of the model to incorporate the data variability [20]. Therefore, the VPC plot of this salbutamol inhaler model (Figure 6) demonstrates the fit of the simulated observations within the 90% prediction interval, which indicates a good agreement between the observed and simulated salbutamol concentration values.

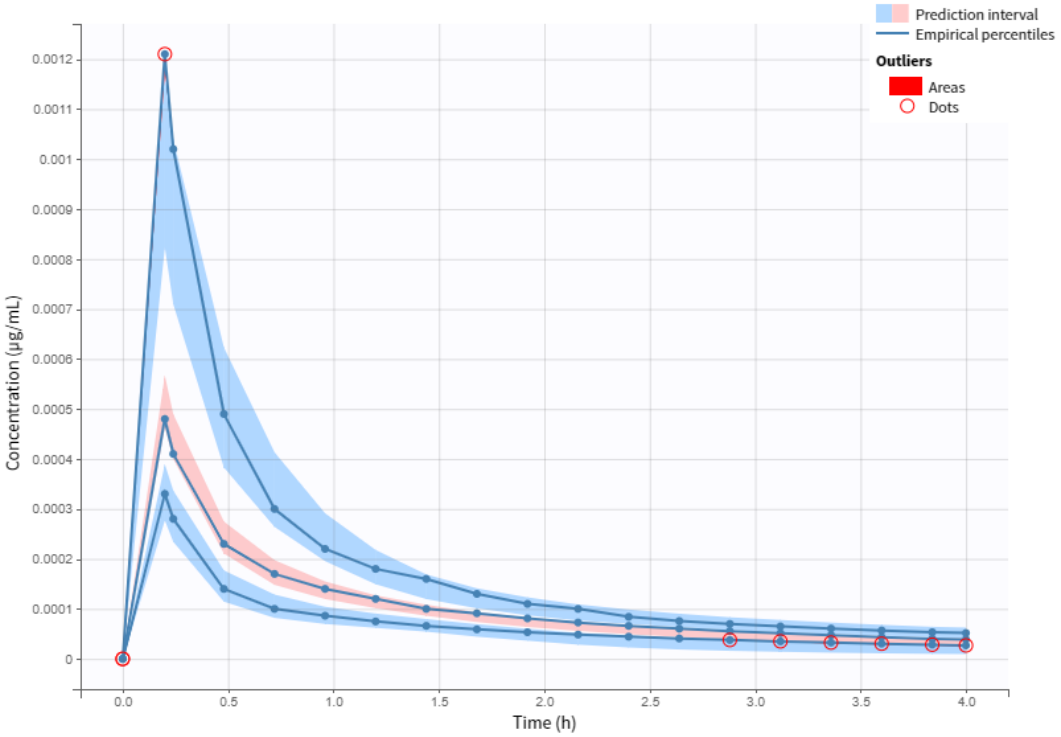

Figure 6. VPC plot versus time using the final salbutamol MDI covariate pharmacokinetic model. Prediction intervals for the 10th, 50th, and 90th percentiles (from bottom to top, respectively) are displayed. Outliers are visualized as red dots and areas.

Therefore, the time course of salbutamol plasma concentration was modeled by three compartments, with first-order absorption, no delay, and linear elimination. However, the final model is not consistent with the one developed by Courlet et al. [21]. Furthermore, we have included age and weight as covariates that improve the developed population PK model. We may thus assume that these variables affect the pharmacological profile of salbutamol. This is in accordance with our prior discussion of the salbutamol serum levels evolution (Figure 1). Age has a significant impact on Vd, a PK parameter defined as an estimate of distribution in extracellular fluid [22]. A higher Vd means that salbutamol is more likely to be found in the tissues, whereas a decreased Vd translates to a higher probability of the drug being found in the circulatory system [23]. Considering that salbutamol's therapeutic effect is based on its action on smooth muscle receptors, we infer that salbutamol Vd is relatively high, indicating a distribution throughout lung tissue to act on the intracellular target. Hence, a high value of Vd corresponds to a low value of salbutamol plasma concentration. Rescuing the inferred conclusions about the effect

of age on serum levels (Figure 1), where young individuals have increased values compared to adults, the inverse relationship between both parameters predicts a lower Vd in this age range. As previously stated, the Vd value is expected to be higher in order to reach the intracellular target. We might then speculate that the effectiveness of salbutamol in young patients may be compromised, as the Vd parameter is reduced. On the other hand, high serum concentrations are associated with a greater risk of toxicity, as already noted. Therefore, it is important to consider age when prescribing salbutamol in inhaler form.

The patients included in this study range in age from 5 to 65 years. Notwithstanding, it would also be interesting to evaluate the PK profile of this bronchodilator in young infants. According to Eidelman et al. [13], the high amount of water stored in newborns (80% of the total weight) leads to hydrophilic drugs, such as salbutamol, to be widely distributed in the tissues, translating into lower systemic distribution, compared to adults.

Weight, in turn, has an influence on Cl, Vd, and Q parameters. In fact, multiple studies have found differences in these PK parameters in obese people [14–16]. Clearance, referring to the rate at which a drug is removed from the body, usually occurs in the liver, and thus several obesity-related hepatic pathological conditions, such as fat accumulation in the liver altering blood flow and decreased cardiac output, directly impacts this PK parameter [24,25]. Likewise, several studies document an increased Vd in obese individuals compared to normal-weight people. Our model efficiently demonstrates that the pharmacological profile of MDI salbutamol form varies with subject weight. Again, these findings confirm what has been previously discussed.

3.3 Salbutamol Dry Powder

Due to the inherent difficulty of using conventional inhalers, new inhalers generation has been developed. DPI provides a convenient, easier, and more effective way to deliver the medication directly to the lungs [26–29]. Therefore, salbutamol DPI consists of a fine dry powder contained in a device specifically designed for inhalation. This type of formulation is associated with multiple advantages, including portability (patients can easily use this device on-the-go), reliable dose delivery, and environmental friendliness as it does not rely on propellants [30,31].

Thus, the PK profile of salbutamol DPI was assessed by administering a single dose of 200 µg in patients over 6 hours.

3.3.1 Pharmacokinetic Parameters

Following simulated administration of salbutamol in dry powder form in accordance with the recommended therapeutic regimen, the PK parameters (mean ± SD) were obtained (Table 5). With the exception of AUC values, all other PK parameters differ considerably from the predicted values. This might be related to ADMET Predictor®'s limitations in terms of routes of administration. Similar conclusions concerning the distribution of salbutamol inhaler serum levels can be drawn for this type of formulation (Figure 7). This is due to the fact that salbutamol DPI is administered via inhalation. Thus, we may already state that age and BMI are factors that should be considered when prescribing a treatment with salbutamol DPI.

Table 5. Observed and estimated pharmacokinetic properties of a single dose of 200 µg salbutamol administered via DPI after a 6-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value (mean ± SD)
Fa (%)	88.82	52.81 ± 0.01
FDp (%)	ND	0.11 ± 0.02
F (%)	71.23	52.59 ± 0.02
C_{max} (µg/mL)	5.10×10^{-4}	$2.30 \times 10^{-3} \pm 0.002$
T_{max} (h)	2.73	18.10
AUC_{0-inf} (µg*h/mL)	5.47×10^{-3}	$5.22 \times 10^{-3} \pm 0.002$
AUC_{0-t} (µg*h/mL)	5.47×10^{-3}	$4.97 \times 10^{-3} \pm 0.002$
$C_{max\ liver}$ (µg/mL)	ND	$5.21 \times 10^{-3} \pm 0.003$

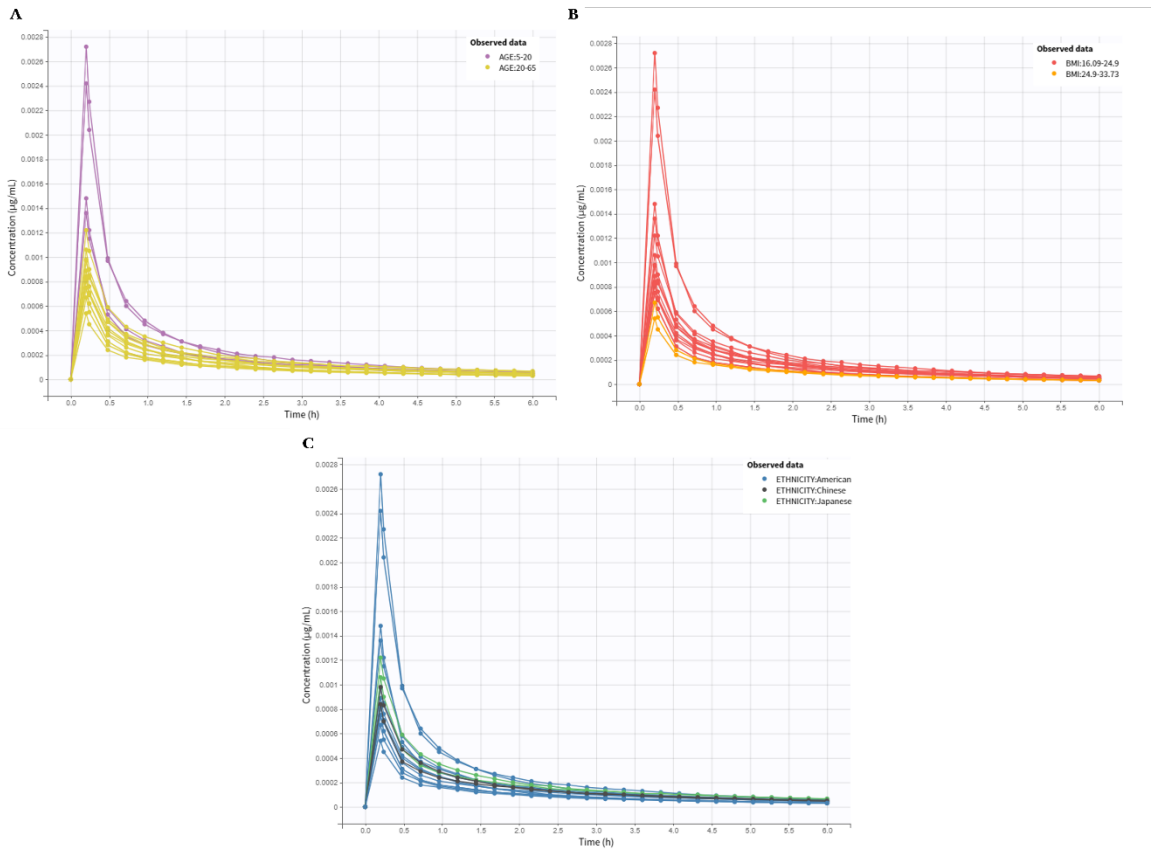

Figure 7. Observed data of plasma salbutamol concentration ($\mu g.mL^{-1}$) through time (h), stratified by age (A), BMI (B), and ethnicity (C).

3.3.2 Model Building Process

The administration of a single dose of 200 µg of salbutamol dry powder in 16 virtual patients, with distinct characteristics, was best described by a two-compartment model with first-order absorption, no delay, and linear elimination (Figure 8). This model, as previously acknowledged, does not correspond to the PK models documented in the literature. A proportional error model and normal parameter distribution were employed. Age is included as a covariate on Cl, BMI on Cl and V2, ethnicity on Cl, and gender on Cl and Q. Other models were also tested (Appendix B, Table B3). The estimated values have expected associated uncertainties, except the K_a parameter (Table 6). From a critical point of view, we consider that both the estimated value of K_a and the RSE of this parameter are rather high. We believe that the model's development may have run into an unidentified issue.

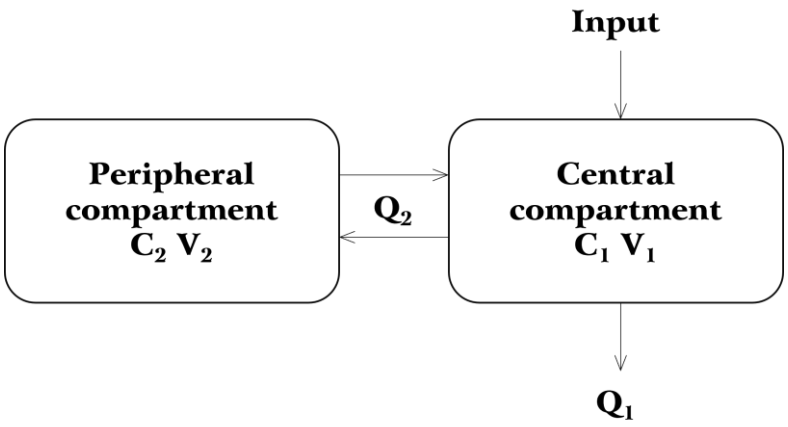

Figure 8. Population pharmacokinetic model used to describe salbutamol DPI plasma concentration-time profiles. C_1, and C_2 are the compartments, V_1, and V_2 represent the Vd of each compartment, and Q_1, and Q_2, are the intercompartmental clearance.

Table 6. Estimates of the population pharmacokinetic parameters of the final model for salbutamol DPI formulation.

Parameter	Estimate	RSE (%)
K_a pop	81069.09	464488.50
Cl pop	147.97	15.50
V1 pop	40.45	16.90
Q pop	39.06	11.30
V2 pop	143.94	ND
Error model parameters		
b	0.05	3.86

RSE: Relative standard error; K_a – Absorption constant rate; Cl – Clearance; V1 and V2 – The volume of distribution of the compartments one (central) and two (peripheral); Q – intercompartmental clearance; ND – not defined.

3.3.3 Final Population Pharmacokinetic Model

The model was validated by visual inspection of the plots extracted from the software. First, observations versus individual and population predictions indicate that the model is accurate (Figure 9). The majority of the observations fit within the 90% prediction range, so there are no misspecifications. On the other hand, the residual scatter plots demonstrate dispersed points, which was not expected (Figure 10). Finally, the VPC plot (Figure 11) confirms that it is a suitable model for the population under study, revealing only small red areas, corresponding to outliers.

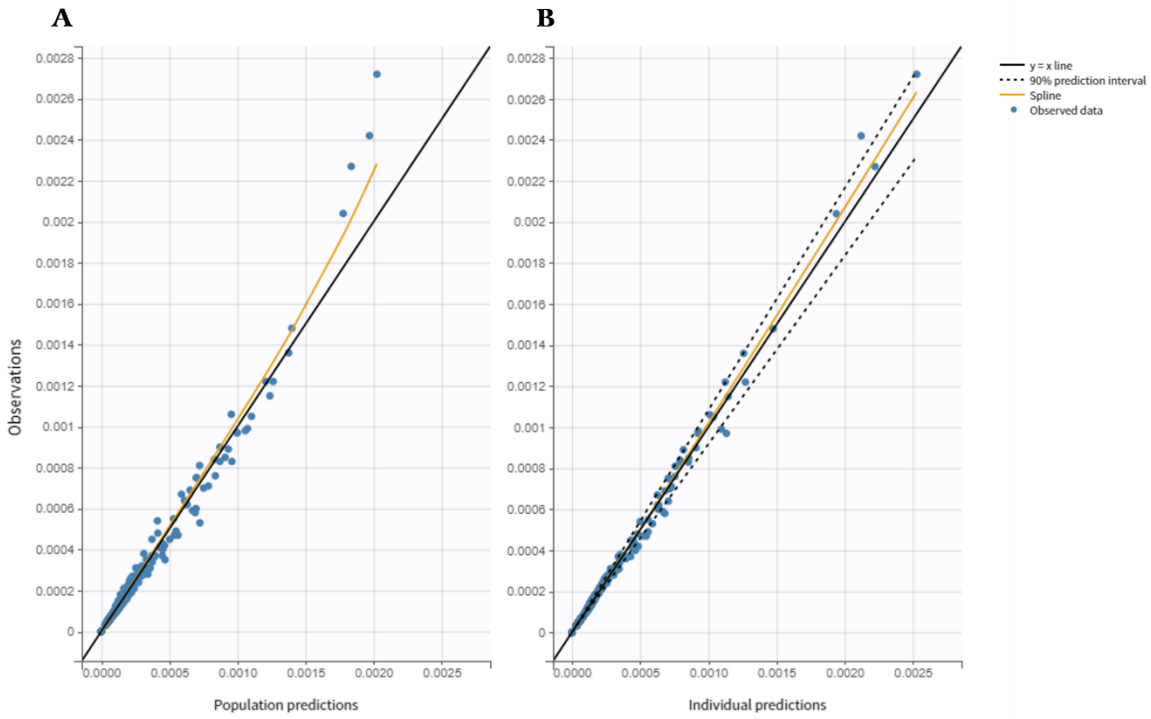

Figure 9. Final salbutamol DPI covariate pharmacokinetic model: (A) observation vs. population predictions, and (B) observations vs. individual predictions of salbutamol concentration (μg.mL^{-1}).

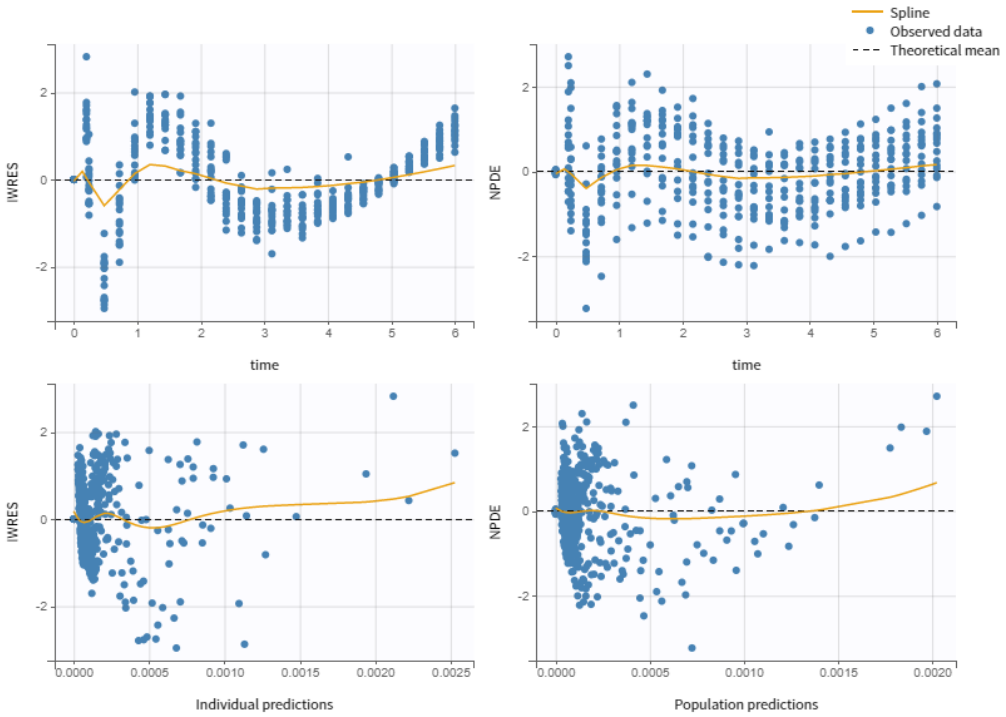

Figure 10. IWRES and NPDE versus time, individual, and population predictions for the salbutamol DPI pharmacokinetic model. The yellow line represents the spline of the data, and the theoretical mean is shown as a dotted line.

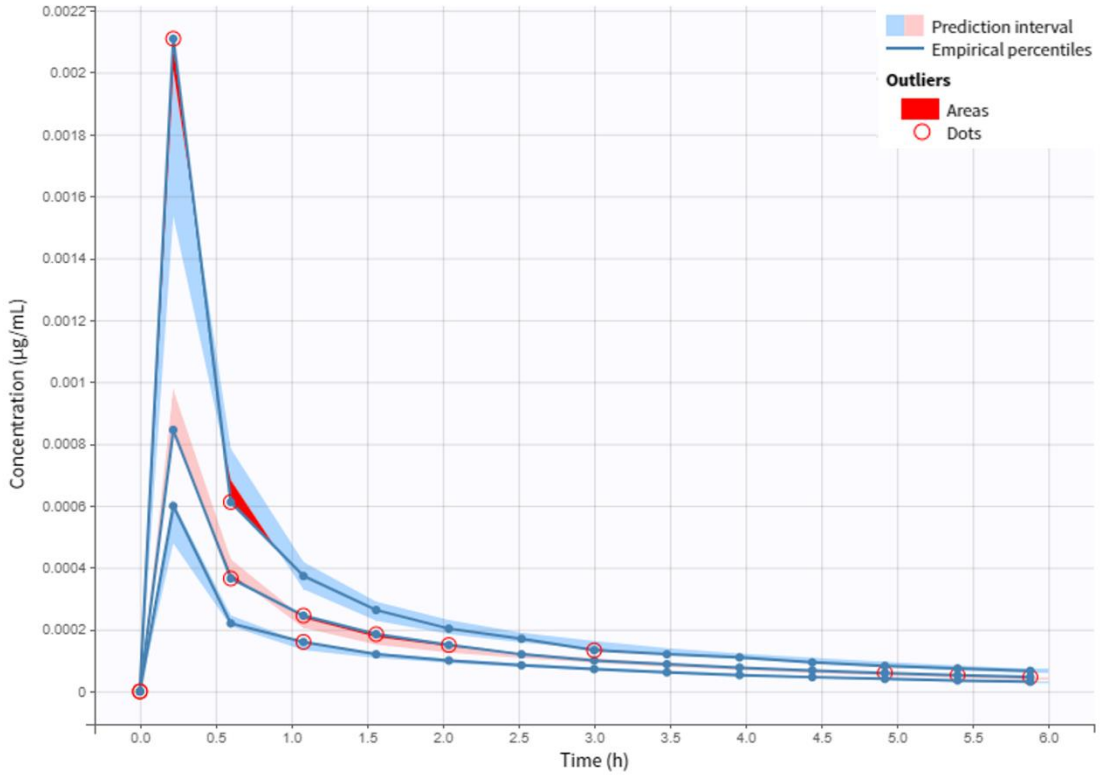

Figure 11. VPC plot versus time using the final salbutamol DPI covariate pharmacokinetic model. Prediction intervals for the 10th, 50th, and 90th percentiles (from bottom to top, respectively) are displayed. Outliers are visualized as red dots and areas.

Our results reveal that all individual characteristics play an active role in salbutamol's pharmacological profile, particularly in Cl. Thereby, the influence of BMI on Cl has already been explored in Section 3.2.3. Age, in turn, is known to be associated with progressive loss of hepatic function, where most drugs are removed [32]. There are some inconsistencies in the literature regarding aging-associated PK alterations. Some studies have reported reduced clearance of many drugs metabolized by phase I pathways in the liver [33,34]. As explored in Chapter I, salbutamol is not only metabolized by sulfotransferases (Phase II reactions), but also by Phase I enzymes. Contrary to these findings, other investigations have shown a maintenance CYP activity throughout the lifespan [35]. Notwithstanding, older people should always be monitored during a given treatment, and our study highlights the importance of this clinical practice in DPI salbutamol therapy.

Furthermore, ethnicity is included as a covariate. As previously discussed, polymorphism differences between ethnic groups are the key driver for the reported

clearance changes. Indeed, several polymorphisms have been reported in CYP450 enzymes. Therefore, recognizing that salbutamol is metabolized by CYP2D6 and CYPC19, we may assume that including ethnicity as a covariate in this PK model is reasonable.

The PK differences between men and women have been thoroughly investigated in the literature [36]. In the previous chapter, we have confirmed that enzyme activity of salbutamol phase I metabolizing enzymes is not equivalent. We have also found studies claiming that lower glomerular filtration in women impacts the level of renal clearance. These observations align with our results. For this reason, female sex is more likely to experience ADRs.

Comparing this population PK model with the population PK model developed for the inhaler formulation, we found some discrepancies. The administration of salbutamol DPI is characterized by a two-compartment model, while the administration of salbutamol inhaler is described by a three-compartment model. Since the models followed the same administration method, we hypothesize that the number of compartments should be identical. Therefore, based on our knowledge, the two-compartment model would better fit our study, considering that salbutamol, in addition to acting on the lungs via inhalation, a part of the drug is also swallowed (gastrointestinal).

3.4 Salbutamol Nebulizer

Salbutamol nebulizer is a delivery method that converts liquid medication into a fine mist or aerosol, which is then deposited directly into the lungs [37]. This section presents the results regarding the simulation of the administration of a 5 mg/mL salbutamol nebulizer in virtual patients.

3.4.1 Pharmacokinetic Parameters

Table 7 highlights the PK characteristics of the study population, whereas Figure 12 depicts the distribution of salbutamol plasma concentration in patients over a 24-hour simulation. Significant differences between the predicted and estimated values are again observed.

The administration of 5 mg/mL salbutamol via nebulizer to all 16 participants displayed a pharmacological profile remarkably comparable to prior modalities of drug delivery, in the stratified groups. Hence, the same rationale may be applied:

subjects of different ages and BMI have distinct time courses, and salbutamol intake in these patients should be observed in order to minimize potential side effects and ensure the intended therapeutic effect. It should also be noted that this dosing regimen is recommended for both adults and children, so the increased plasma concentration values observed in younger people are not related to overdose.

Table 7. Observed and estimated pharmacokinetic properties of a single dose of 5 mg/mL salbutamol administered via nebulizer after a 24-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value (mean ± SD)
Fa (%)	88.82	ND
FDp (%)	ND	ND
F (%)	71.23	ND
C_{max} (µg/mL)	1.27×10^{-2}	32.52 ± 18.89
T_{max} (h)	2.73	0.24
AUC_{0-inf} (µg*h/mL)	0.14	37.95 ± 20.90
AUC_{0-t} (µg*h/mL)	0.14	37.84 ± 20.85
$C_{max\ liver}$ (µg/mL)	ND	230.05 ± 170.53

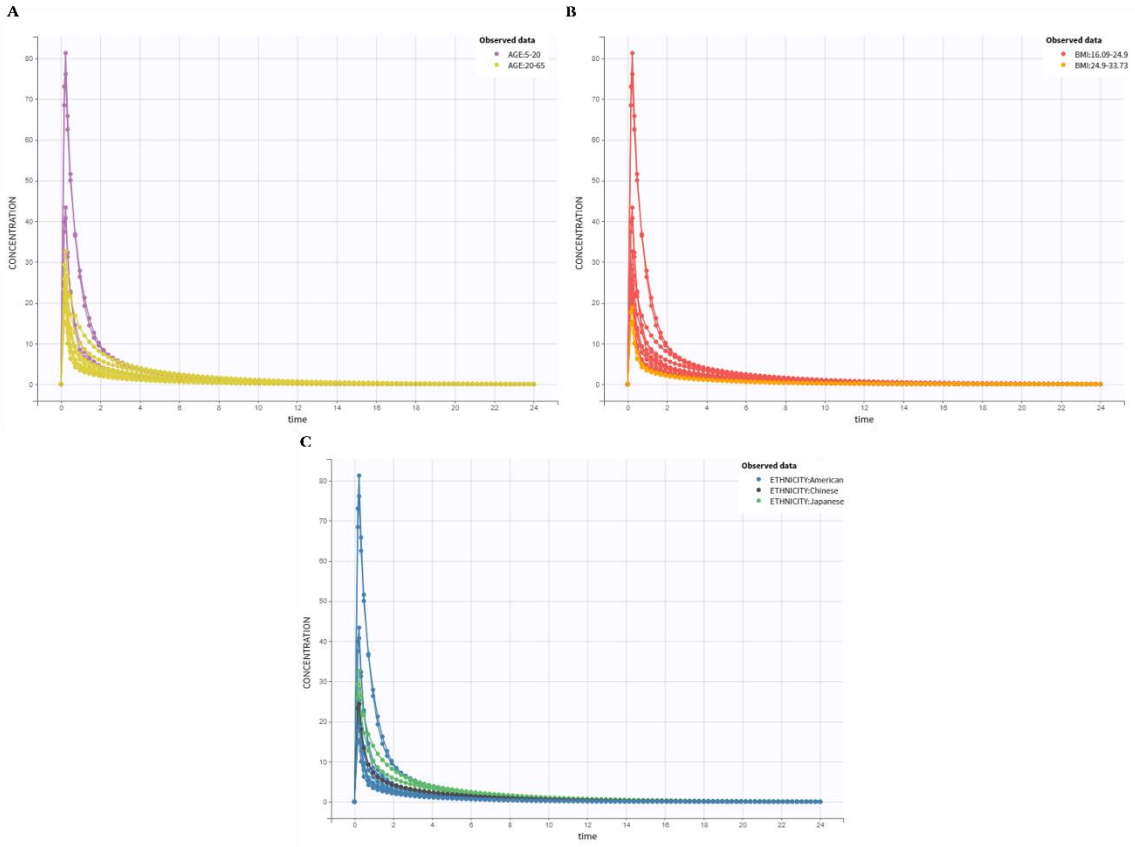

Figure 12. Observed data of plasma salbutamol concentration (µg.mL^{-1}) through time (h), stratified by age (A), BMI (B), and ethnicity (C).

3.4.2 Model Building Process

A two-compartment model with first-order absorption, no delay, and linear elimination best fitted the population PK profile (as presented in Figure 8). We have implemented a proportional error model and a normal parameter distribution. According to the software model proposal, age has a significant effect on Cl, and BMI is a covariate on Cl and V2. Other models were also examined (Appendix B, Table B4) however, they all obtained BIC values higher than the selected model. As a result, the inclusion of these covariates, as we have observed in previous models, suggests that they are crucial features in how the drug is removed from the body and distributed in the tissues. We have proved, with the development of this PK model, that clinical practice should progressively implement precision medicine, and tailor treatment to the patient's individual variability. The parameter estimates for the final model are collected in Table 8. The associated RSE values are, in turn, significantly high (above 50%).

Table 8. Estimates of the population PK parameters of the final model for salbutamol nebulizer formulation.

Parameter	Estimate	RSE (%)
Tlag pop	0.017	90.30
K_a pop	1963.10	250.00
Cl pop	0.047	61.00
V1 pop	0.047	ND
Q pop	0.066	91.30
V2 pop	0.14	68.70
Error model parameters		
b	0.031	ND

RSE: Relative standard error; Tlag – Lag time; K_a – Absorption constant rate; Cl – Clearance; V1 and V2 – The volume of distribution of the compartments one (central) and two (peripheral); Q – intercompartmental clearance; ND – not defined.

3.4.3 Final Pharmacokinetic Population Model

Similarly to the previous models, we validate this model as not having misspecification despite the presence of some outliers (Figure 13). Visual inspection of the scatter plots of IWRES and NPDE, however, exposes some inaccuracies, as the points do not line up on the horizontal line 0, as expected (Figure 14). Finally, the fit of the simulated data within the 90% prediction interval is further exhibited in

the VPC plot (Figure 15), indicating a good agreement between the observed and simulated salbutamol concentration levels.

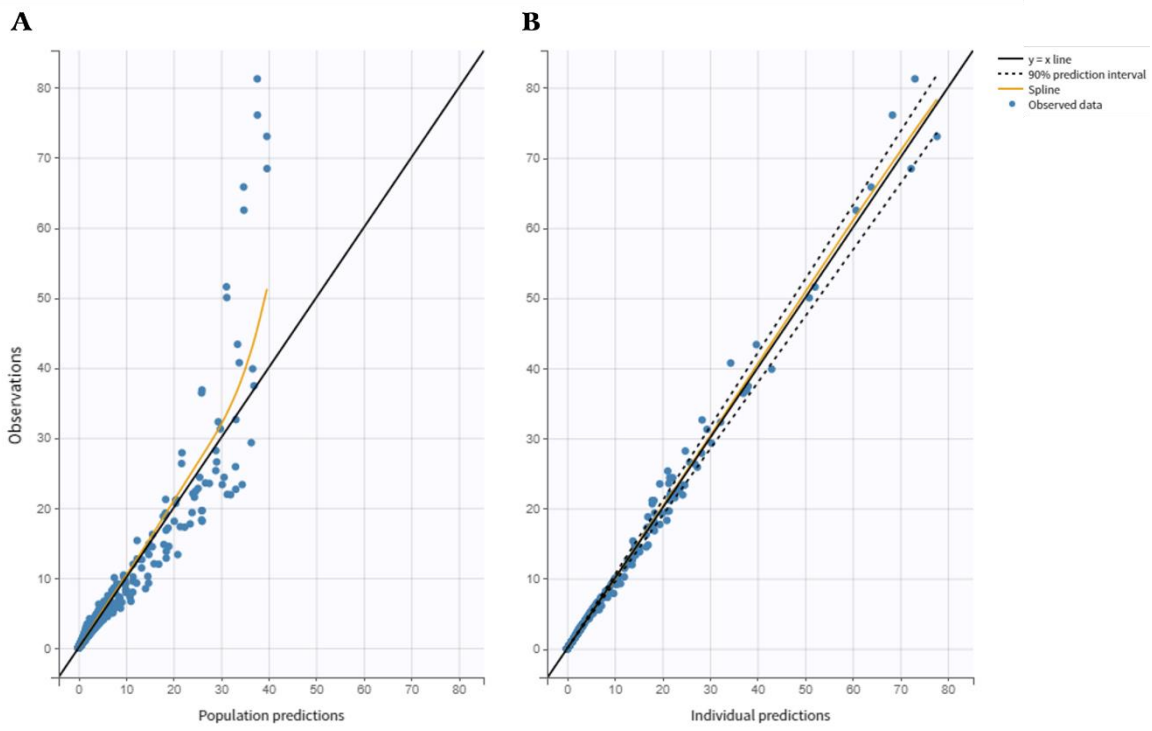

Figure 13. Final salbutamol nebulizer covariate pharmacokinetic model: (A) observation vs. population predictions, and (B) observations vs. individual predictions of salbutamol concentration ($\mu g.mL^{-1}$).

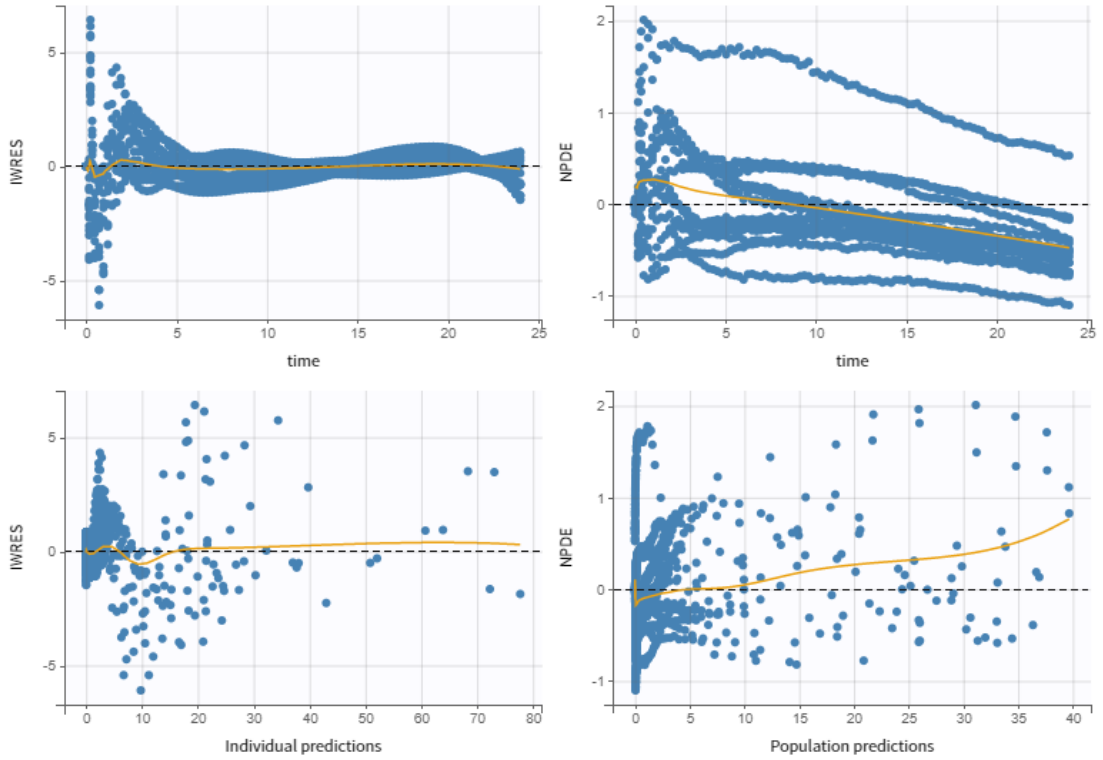

Figure 14. IWRES and NPDE versus time, individual, and population predictions for the salbutamol nebulizer pharmacokinetic model. The yellow line represents the spline of the data, and the theoretical mean is shown as a dotted line.

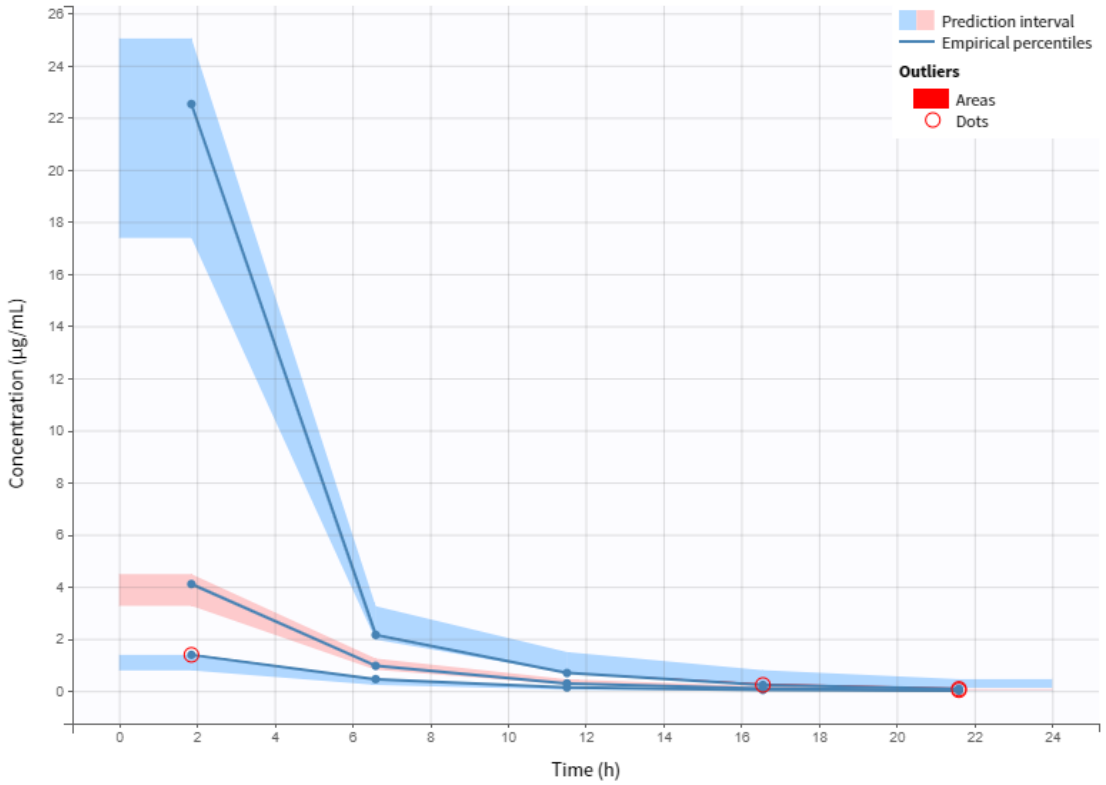

Figure 15. VPC plot versus time using the final salbutamol nebulizer covariate pharmacokinetic model. Prediction intervals for the 10th, 50th, and 90th percentiles (from bottom to top, respectively) are displayed. Outliers are visualized as red dots and areas.

3.5 Salbutamol Oral Syrup

In addition to the inhaled route of administration, salbutamol can also be taken orally. This drug in syrup form is generally prescribed for individuals who have difficulty swallowing tablets or capsules, such as children and elderly people. Nevertheless, oral salbutamol intake is discouraged in most developed countries, as there is no evidence of benefit on asthma symptoms [38]. In fact, an increased likelihood of adverse effects is reported. For these reasons, oral salbutamol is no longer included in the WHO Essential Medicines List for both children and adults [39,40]. Although it not currently recommended, our goal was to analyze all salbutamol formulations, hence, this formulation (2 mg/5 mL) was examined in patients during a 6-hour simulation.

3.5.1 Pharmacokinetic Parameters

All patients received 2 mg/mL salbutamol syrup treatment. The obtained PK characteristics are shown below (Table 9). Serum salbutamol levels were measured

during a 6-hour simulation (Figure 16). Compared to the previously explored forms of administration, we may witness considerable variations in drug distribution over time. For instance, when delivered orally, the maximum peak is not reached as quickly and the drug concentration after C_{max} is not lowered as drastically, implying that the drug is removed more slowly. This is in accordance with recent evidence about its slower onset of action [38].

Table 9. Observed and estimated pharmacokinetic properties of 2 mg/5 mL of salbutamol administered via oral syrup after a 6-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value (mean ± SD)
Fa (%)	88.82	91.28 ± 2.69
FDp (%)	ND	82.02 ± 2.95
F (%)	71.23	16.20 ± 7.58
C_{max} (µg/mL)	5.06	2.18×10^{-3} ± 0.001
T_{max} (h)	2.73	18.56
AUC_{0-inf} (µg*h/mL)	5.47×10^{-3}	0.022 ± 0.01
AUC_{0-t} (µg*h/mL)	5.47×10^{-3}	0.021 ± 0.01
$C_{max\ liver}$ (µg/mL)	ND	0.043 ± 0.03

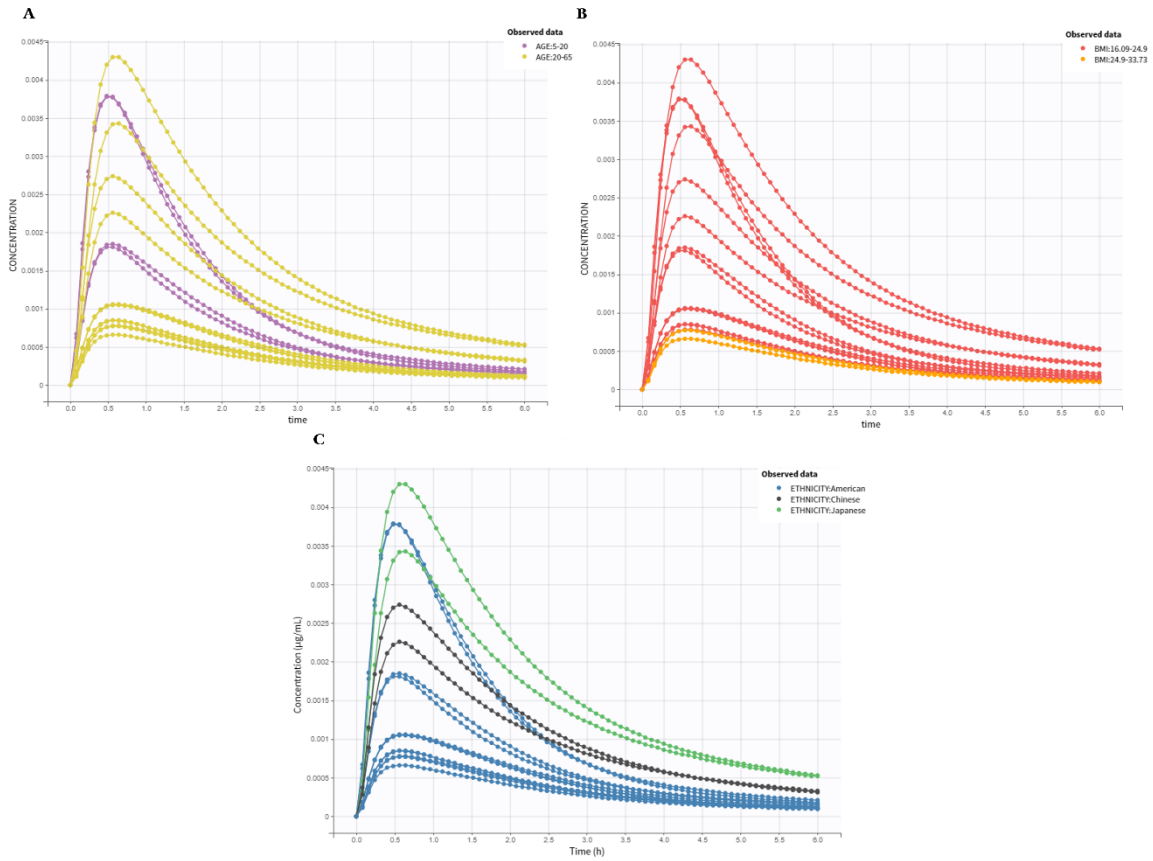

Figure 16. Observed data of plasma salbutamol concentration (µg.mL^{-1}) through time (h), stratified by age (A), BMI (B), and ethnicity (C).

These results go beyond our previous findings. Age does not appear to be a determining factor in the PK profile of orally administered salbutamol syrup. In turn, overweight people (BMI above 24.90) show low plasma concentration values relative to normal-weight people. On the other hand, the distribution of this compound varies according to ethnic groups: the Japanese exhibit higher concentrations, followed by the Chinese and the Americans. Therefore, we might preliminarily infer that ethnicity and BMI have a significant impact on the kinetics of this particular salbutamol formulation.

3.5.2 Model Building Process

The PK profile of the population in this study undergoing treatment with this type of salbutamol formulation is best described by a two-compartment model, with lag time, first-order absorption, and linear elimination (as presented in Figure 8). A combined error model and a normal parameter distribution were used. In addition, we have introduced BMI as a covariate on V1, ethnicity as a covariate on Tlag and

Cl, and gender on Tlag as well. Other models were also investigated (Appendix B, Table B5). Table 10 comprises the parameter estimates for the final model.

The final PK model has BMI, ethnicity, and gender as covariates, supporting our earlier speculations. BMI has a substantial effect on boosting drug distribution if we use the preceding reasoning about the inverse relationship between Vd and Cp (plasma concentration). In other words, overweight patients showed reduced Cp, indicating that the drug is detected in low concentrations in blood, and as a consequence in high concentrations in the tissues. This means that the intracellular target is reached. Thereby, we might likely observe the full therapeutic effect of salbutamol. Some studies corroborate this hypothesis. Vd is often higher in obese patients than in normal-weight individuals [14].

As mentioned above, the Japanese, Chinese, and Americans revealed distinct PK profiles of salbutamol oral syrup. These effects are particularly noticeable in Tlag, or the period between salbutamol intake and its occurrence in the bloodstream, as well as in its Cl. The influence of ethnicity on Cl has already been addressed, and the main reason we point out is the inherent polymorphism differences between ethnic groups caused by epigenetic interference. The observed impact on Tlag may be extended in the same way.

Lastly, gender is also included as a relevant variable in PK parameters, in particular, in Tlag. Again, extrapolation is possible based on the ideas previously provided to explain this phenomenon.

Table 10. Estimates of the population pharmacokinetic parameters of the final model for salbutamol oral syrup formulation.

Parameter	Estimate	RSE (%)
Tlag pop	0.045	1.81
K_a pop	0.70	6.43
C*l* pop	223.1	9.17
V1 pop	26.76	43.4
Q pop	198.93	12.1
V2 pop	968.36	37.5
Error model parameters		
b	0.019	ND

RSE: Relative standard error; Tlag – Lag time; K_a – Absorption constant rate; Cl – Clearance; V1 and V2 – The volume of distribution of the compartments one (central) and two (peripheral); Q – intercompartmental clearance; ND – not defined.

3.5.3 Final Pharmacokinetic Population Model

After selecting the best PK model, the most relevant graphs were extracted (Figure 17, 18, and 19). Based on these analyses, we are able to validate the PK model since there are minimal misspecifications. Comparing the accuracy of the models developed for salbutamol inhaler, DPI, and nebulizer, we have noticed that this model has a greater proportion of outliers. This suggests that the predictions provided by this model are not associated with such a high degree of confidence.

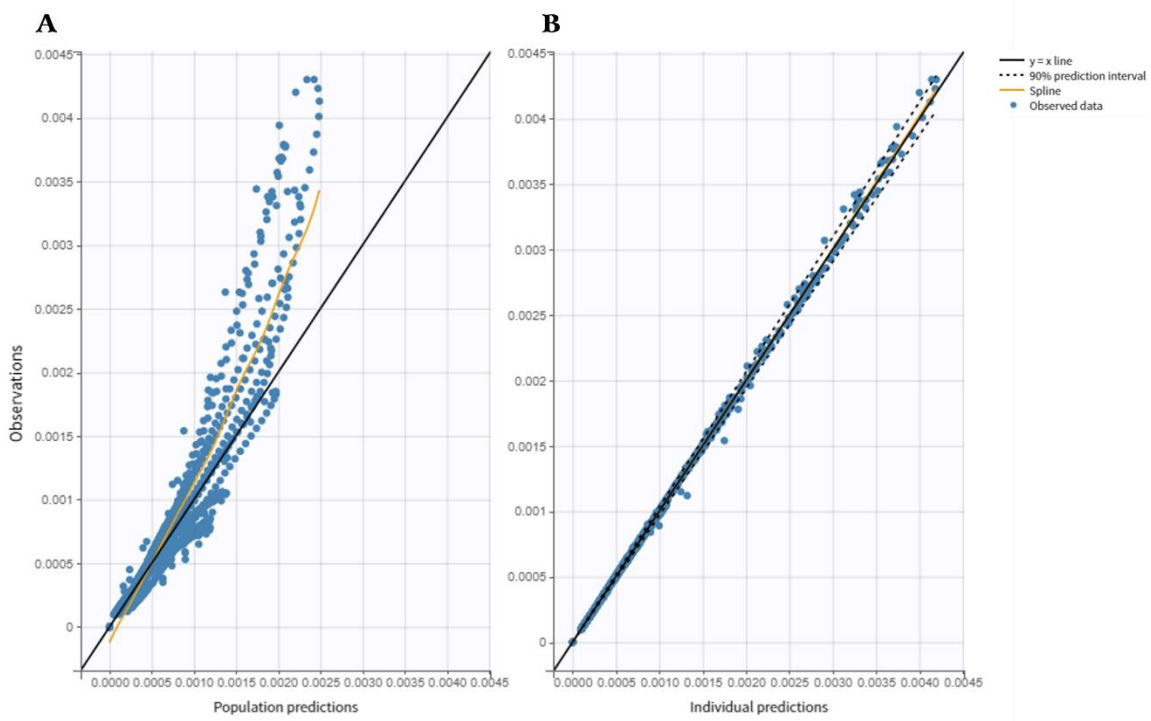

Figure 17. Final salbutamol oral syrup covariate pharmacokinetic model: (A) observation vs. population predictions, and (B) observations vs. individual predictions of salbutamol concentration ($\mu g.mL^{-1}$).

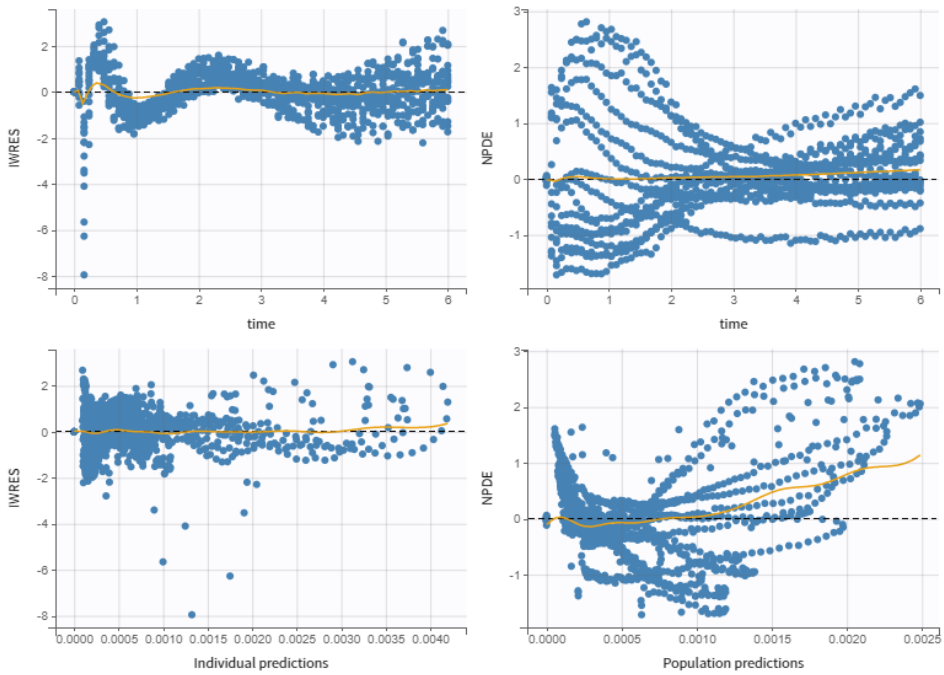

Figure 18. IWRES and NPDE versus time, individual, and population predictions for the oral syrup pharmacokinetic model. The yellow line represents the spline of the data, and the theoretical mean is shown as a dotted line.

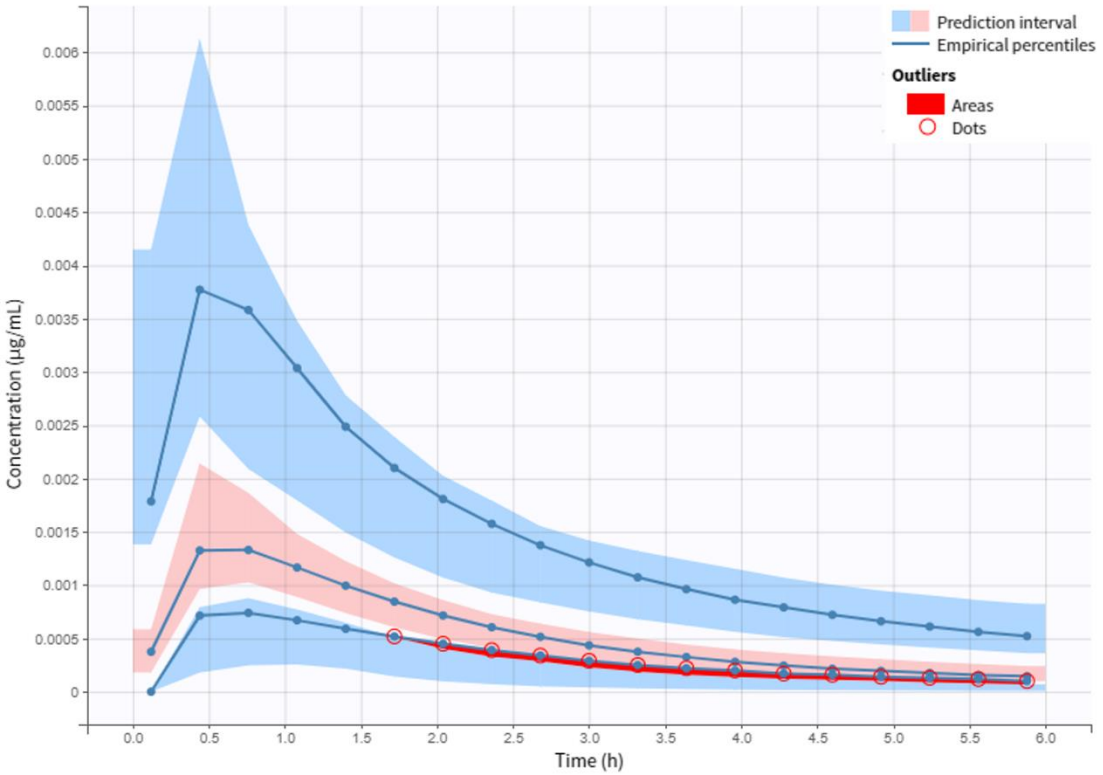

Figure 19. VPC plot versus time using the final oral syrup covariate pharmacokinetic model. Prediction intervals for the 10th, 50th, and 90th percentiles (from bottom to top, respectively) are displayed. Outliers are visualized as red dots and areas.

3.6 Salbutamol Oral Tablet

The oral forms of salbutamol are recognized as having a slower onset of action and less efficacy, compared to the inhaled versions [38]. In this part of the study, the 16 participants underwent a single-dose treatment of 4 mg salbutamol administered orally.

3.6.1 Pharmacokinetic Parameters

Drug disposition characteristics of the virtual subjects over 6 hours are discussed further below (Table 11, Figure 20). Likewise salbutamol syrup, age has no effect on the PK profile (we have highly diverse drug distribution in the stratified groups), while BMI and ethnicity seem to be important covariates to be included in the PK model. It should also be mentioned that this therapeutic regimen is not the recommended dosage for children, but rather a 2 mg dose.

Table 11. Observed and estimated pharmacokinetic properties of 4 mg of salbutamol administered via oral tablet after a 6-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value (mean ± SD)
Fa (%)	88.82	91.22 ± 2.72
FDp (%)	ND	83.08 ± 3.30
F (%)	71.23	20.48 ± 17.05
C_{max} (µg/mL)	0.010	3.90×10^{-3} ± 0.002
T_{max} (h)	2.73	18.80
AUC_{0-inf} (µg*h/mL)	102.11×10^{-3}	0.043 ± 0.03
AUC_{0-t} (µg*h/mL)	109.40×10^{-3}	0.041 ± 0.02
$C_{max\ liver}$ (µg/mL)	ND	0.061 ± 0.04

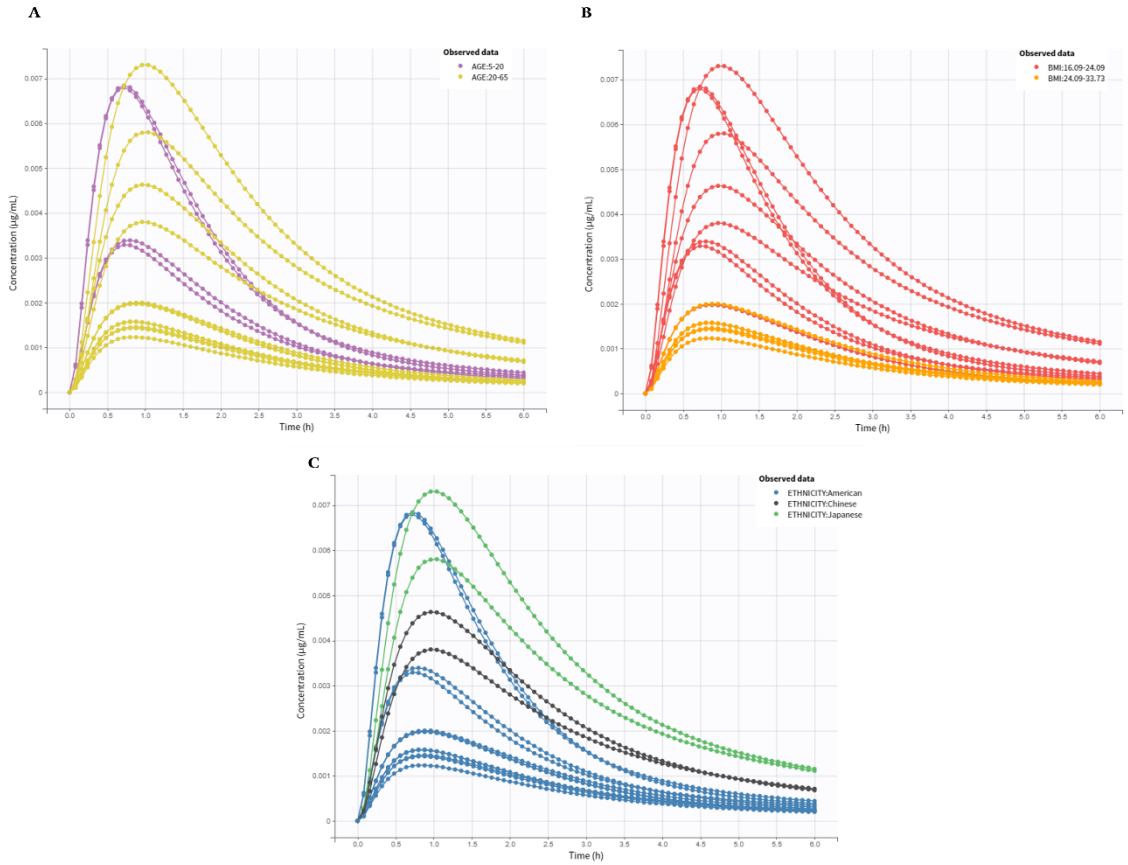

Figure 20. Observed data of plasma salbutamol concentration (µg.mL^{-1}) through time (h), stratified by age (A), BMI (B), and ethnicity (C).

3.6.2 Model Building Process

The PK profile of the study population receiving treatment with this particular salbutamol formulation is most accurately characterized by a one-compartment model, with lag time, first-order absorption, and linear elimination (Figure 21). This final outcome is supported by the literature [21]. To account for individual

variability, a combined error model with a normal parameter distribution was applied. We incorporated covariates in the model as well: BMI has a significant effect on Cl, ethnicity on Tlag and Cl, and gender on Cl. Other models were also tested (Appendix B, Table B6). Table 12 highlights the parameter estimates for this model.

As aforementioned, the inclusion of these covariates was expected. Since the route of administration is the same, the final PK population model ought to be comparable to that derived for the oral syrup.

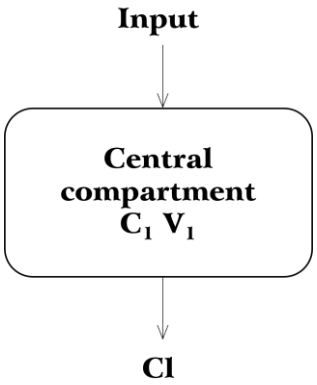

Figure 21. Population pharmacokinetic model used to describe salbutamol oral tablet plasma concentration-time profiles. C_1 is the compartment, V_1 represents the Vd of each compartment, and Q_1 is the elimination phase.

Table 12. Estimates of the population pharmacokinetic parameters of the final model for salbutamol oral syrup formulation.

Parameter	Estimate	RSE (%)
Tlag pop	0.065	1.06
K_a pop	0.46	3.94
Cl pop	103.70	23.60
V pop	170.66	15.40
Error model parameters		
b	0.051	3.97

RSE: Relative standard error; Tlag – Lag time; K_a – Absorption constant rate; Cl – Clearance; V – The volume of distribution of the compartment; ND – not defined.

3.6.3 Final Pharmacokinetic Population Model

Subsequently, the most pertinent plots from the optimal PK model were extracted (Figures 22, 23, and 24). Despite the presence of some outliers, this was the best model found to reflect the PK profile of the 16 individuals who were given a 4 mg salbutamol tablet.

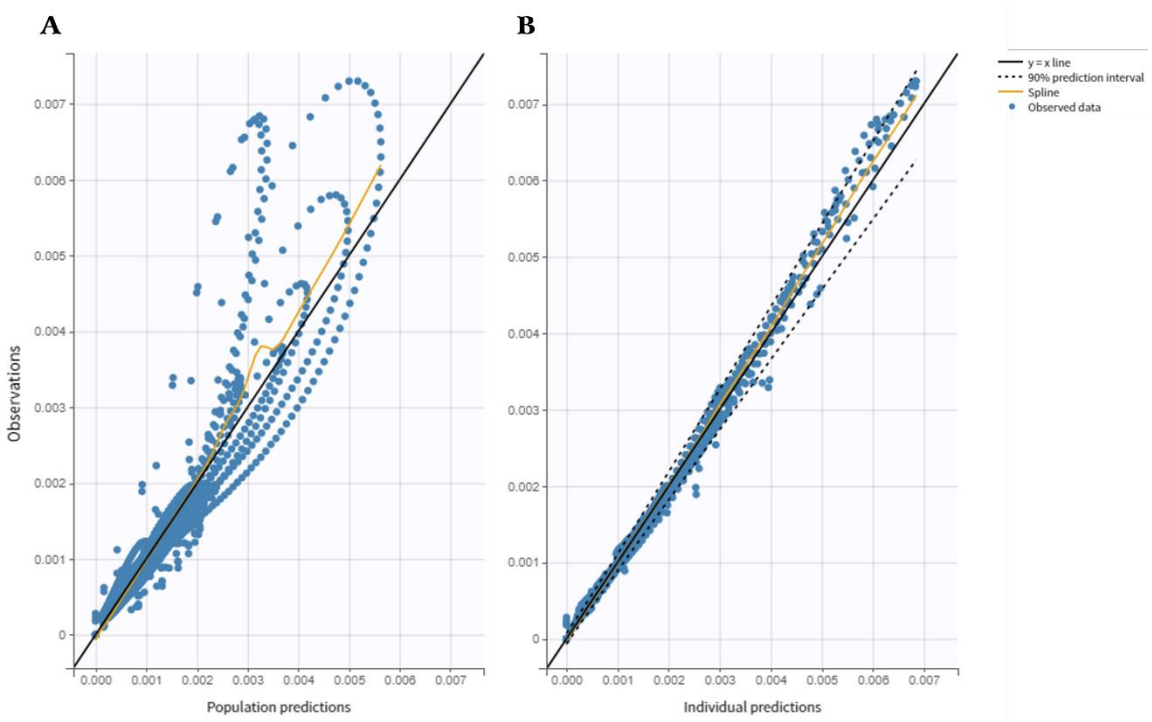

Figure 22. Final salbutamol oral tablet covariate pharmacokinetic model: (A) observation vs. population predictions, and (B) observations vs. individual predictions of salbutamol concentration ($\mu g.mL^{-1}$).

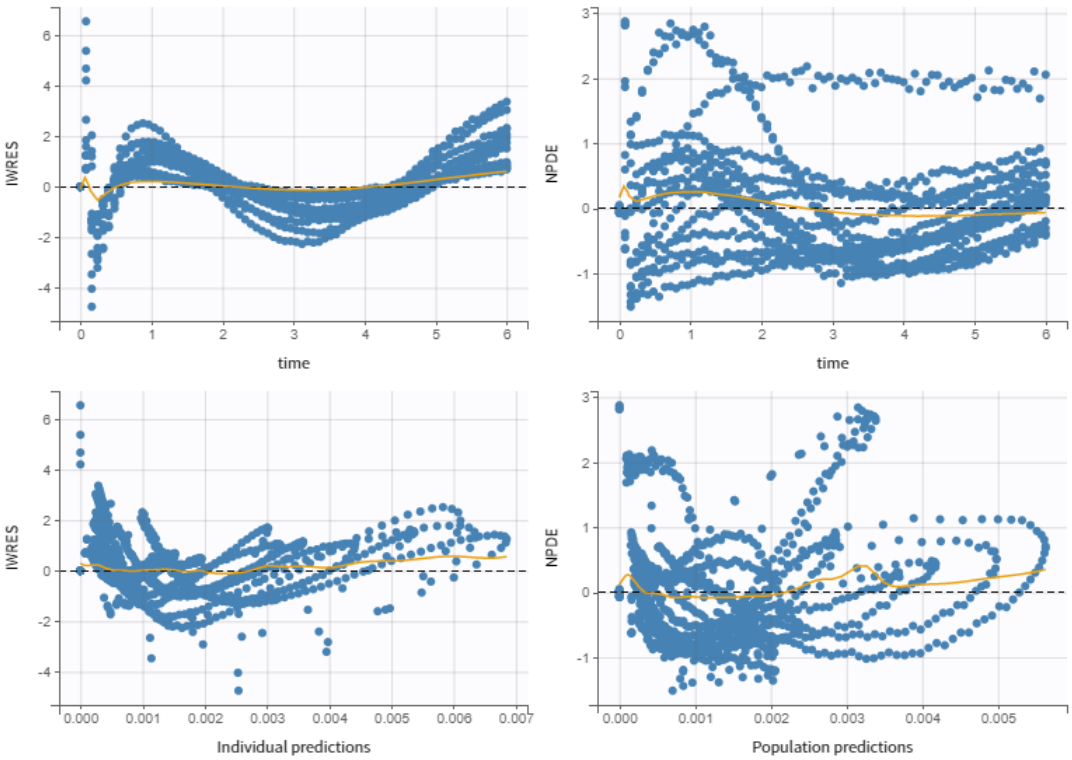

Figure 23. IWRES and NPDE versus time, individual, and population predictions for the salbutamol oral tablet pharmacokinetic model. The yellow line represents the spline of the data, and the theoretical mean is shown as a dotted line.

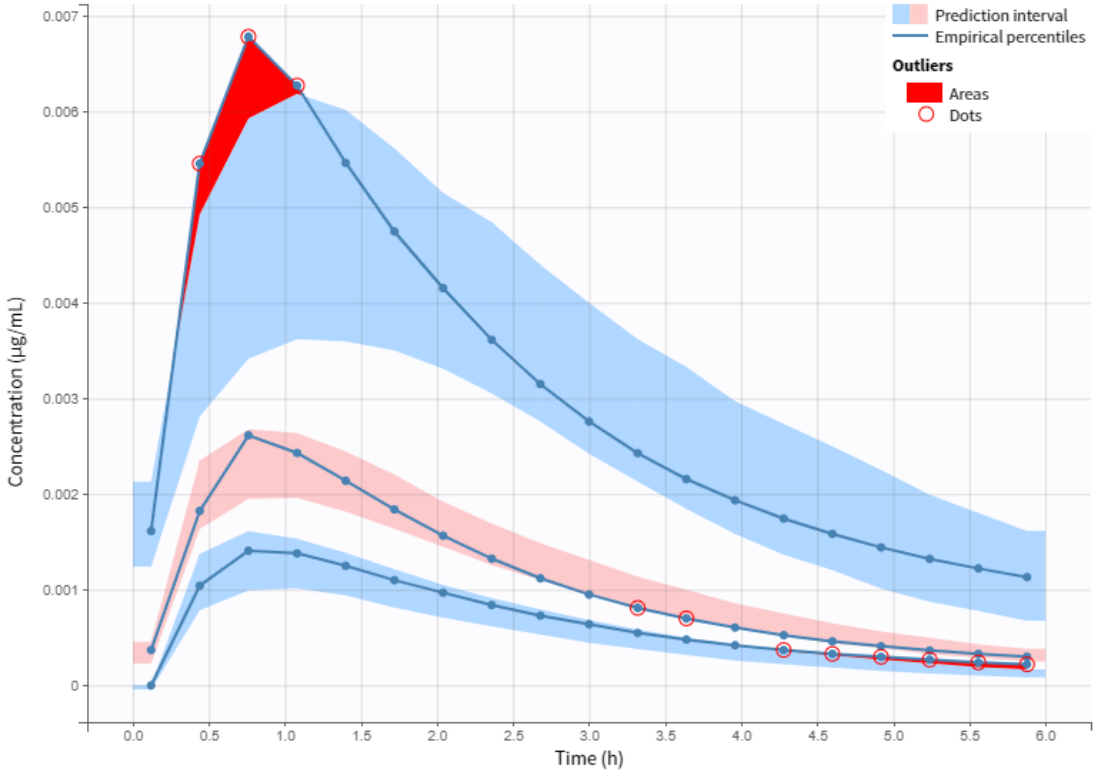

Figure 24. VPC plot versus time using the final salbutamol oral tablet covariate pharmacokinetic model. Prediction intervals for the 10th, 50th, and 90th percentiles (from bottom to top, respectively) are displayed. Outliers are visualized as red dots and areas.

3.7 Salbutamol Intravenous

Salbutamol IV is a valuable medication used in emergency situations when immediate relief of bronchospasm is required. Usually, this type of therapy is recommended for those with severe asthma who frequently experience acute exacerbations [41]. Therefore, it is administered by medical professionals, and the patient must be monitored. This formulation, at the recommended dose, was also assessed in the virtual study population.

3.7.1 Pharmacokinetic Parameters

The administration of 250 µg salbutamol via IV to all 16 participants demonstrated a typical pharmacological profile of an IV drug (an immediate concentration peak corresponding to the drug's entrance into the bloodstream). Table 13 gathers the observed and estimated PK properties. As noted, bioavailability indicators (Fa and F) do not differ between observed and estimated values. Other parameters show slight differences.

Table 13. Observed and estimated pharmacokinetic properties of 250 µg of salbutamol administered via oral tablet after a 24-hour simulation.

Pharmacokinetic Parameters	Observed Value	Estimated Value (mean ± SD)
Fa (%)	99.60	99.88 ± 0.01
FDp (%)	ND	99.68 ± 0.05
F (%)	99.60	99.93 ± 0.04
C_{max} (µg/mL)	1.21×10^{-3}	$9.11 \times 10^{-2} \pm 0.05$
T_{max} (h)	2.73	ND
AUC_{0-inf} (µg*h/mL)	9.54×10^{-3}	$4.04 \times 10^{-3} \pm 0.001$
AUC_{0-t} (µg*h/mL)	9.06×10^{-3}	$4.08 \times 10^{-3} \pm 0.001$
$C_{max\ liver}$ (µg/mL)	ND	0.014 ± 0.007

Figure 25 depicts the concentration versus time profiles according to population subgroups. The conclusions that can be drawn are related to the influence of age and weight on maximal drug absorption. Indeed, plasma peaks in individuals under the age of 20 are almost twice as high as in adults. Overweight patients, in turn, do not achieve C_{max} values as high as normal-weight individuals, which may compromise the therapeutic effect. Data on salbutamol plasma levels stratified by ethnicity cannot predict its effect on salbutamol IV kinetics.

It should also be noted that this route of administration is not frequently used in children, thus the recorded C_{max} values, indicative of eventual toxicity, do not represent a concern in asthma management.

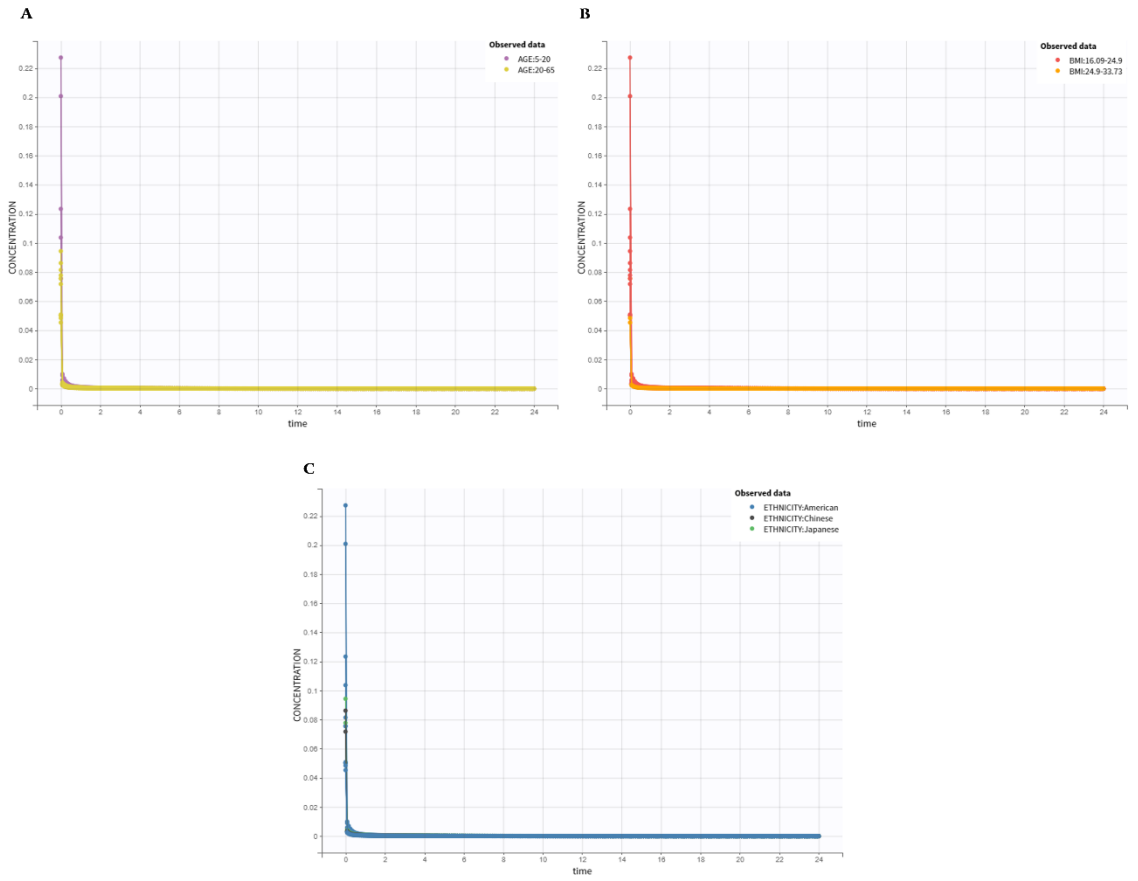

Figure 25. Observed data of plasma salbutamol concentration (μg.mL^{-1}) through time (h), stratified by age (A), BMI (B), and ethnicity (C).

3.7.2 Model Building Process

The PK profile of the study population receiving treatment with this particular salbutamol formulation is most accurately characterized by a three-compartment model, with no delay, first-order absorption, and linear elimination (as schematized in Figure 3). According to the literature, although there are other types of documented models, a 3-compartment model is commonly used to describe the PK of intravenously administered drugs [42]. To account for individual variability, a proportional error model with a normal parameter distribution was applied. Incorporating covariates did not significantly improve the model. Other models were also analysed (Appendix B, Table B7). Therefore, this formulation does not require critical consideration of the individual characteristics of a given patient. In fact, the administered dose depends only on the severity of the respiratory disease, which must be diagnosed by the clinician. Table 14 highlights the parameter estimates for this model.

Table 14. Estimates of the population pharmacokinetic parameters of the final model for salbutamol oral syrup formulation.

Parameter	Estimate	RSE (%)
Cl pop	34.31	30.80
V1 pop	0.91	10.50
Q2 pop	1327.29	ND
V2 pop	22.14	60.40
Q3 pop	14.48	69.80
V3 pop	41.52	83.60
Error model parameters		
b	0.057	ND

RSE: Relative standard error; Cl – Clearance; V1, V2, and V3 – The volume of distribution of the compartments one (central), two, and three (peripheral); Q – intercompartmental clearance; ND – not defined.

3.7.3 Final Pharmacokinetic Population Model

Figure 26 demonstrates the observed concentrations versus individual and population predictions. The scattered plots of IWRES and NPDE are displayed in Figure 27. Additionally, the VPC plot of this salbutamol DPI model (Figure 28) demonstrates the fit of the simulated observations within the 90% prediction interval, which indicates a good agreement between the observed and simulated salbutamol concentration values.

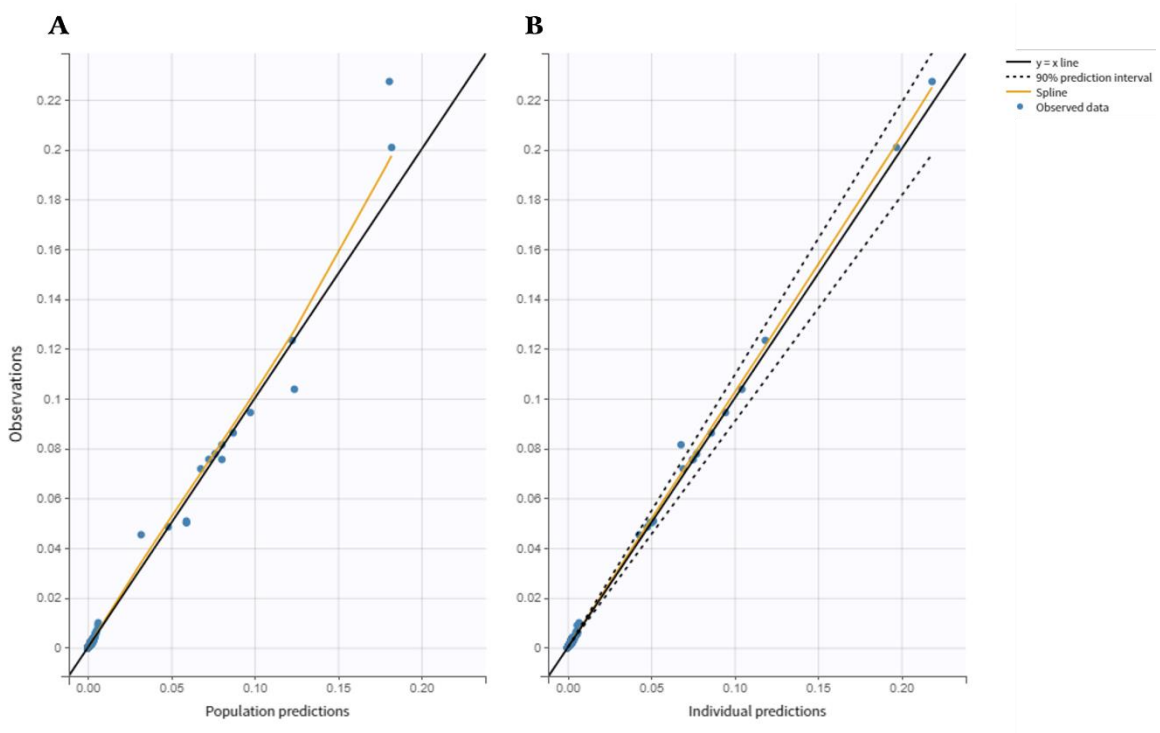

Figure 26. Final salbutamol IV covariate pharmacokinetic model: (A) observation vs. population predictions, and (B) observations vs. individual predictions of salbutamol concentration ($\mu g.mL^{-1}$).

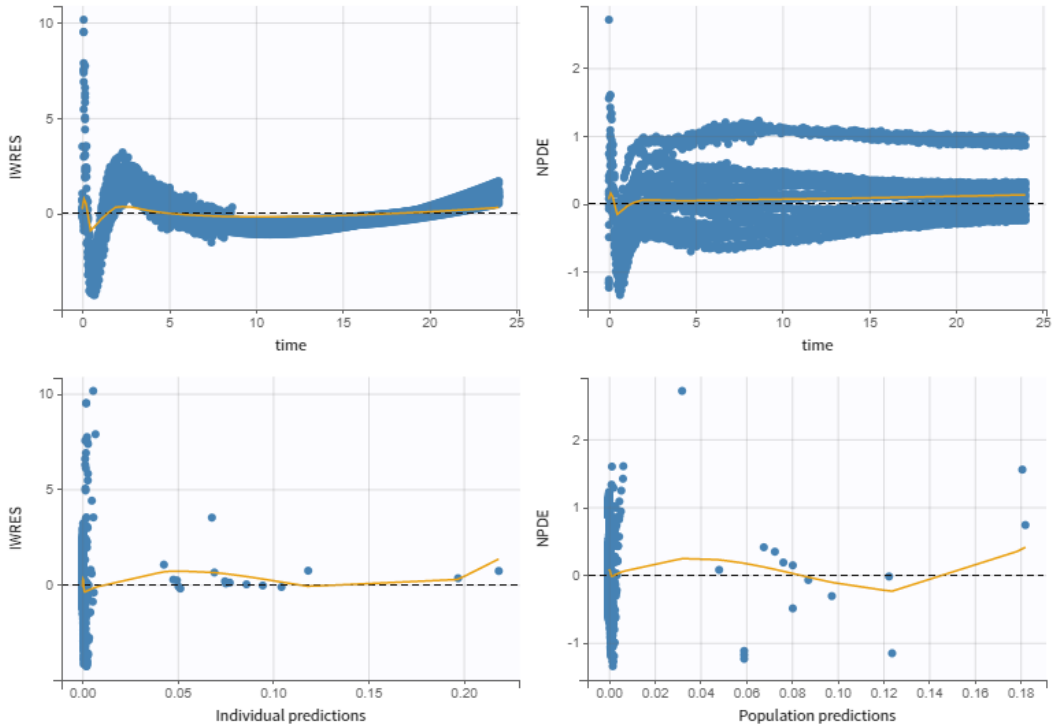

Figure 27. IWRES and NPDE versus time, individual, and population predictions for the salbutamol IV pharmacokinetic model. The yellow line represents the spline of the data, and the theoretical mean is shown as a dotted line.

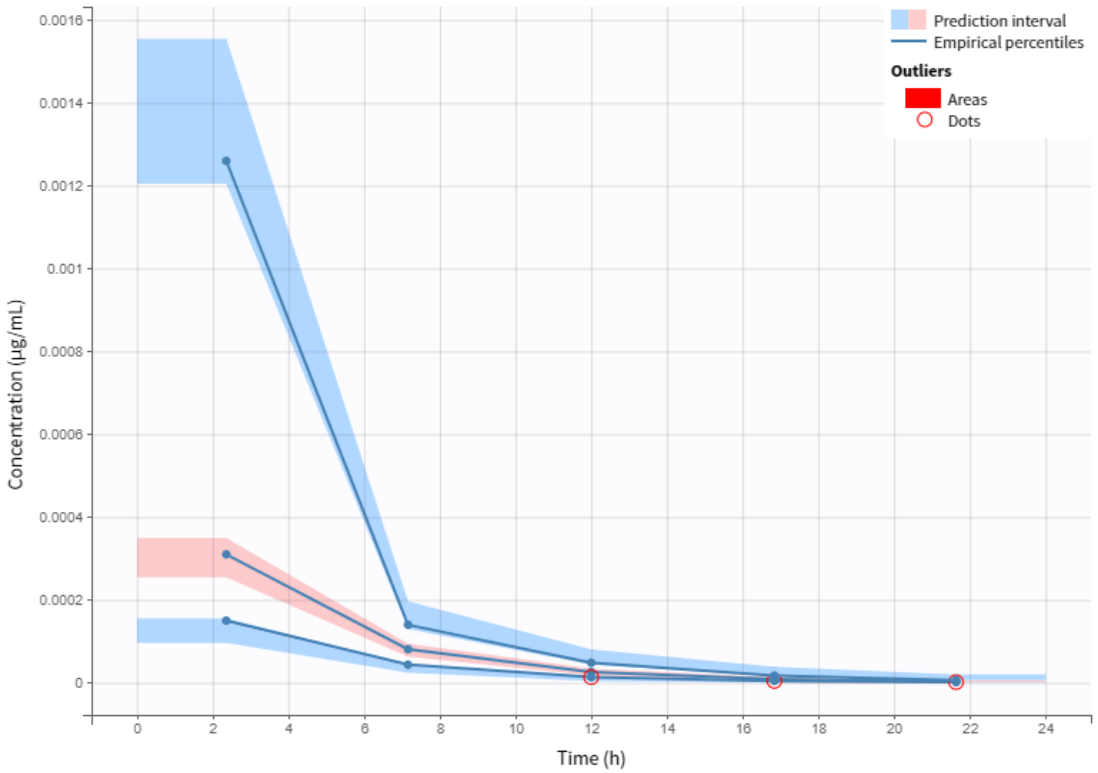

Figure 28. VPC plot versus time using the final salbutamol IV covariate pharmacokinetic model. Prediction intervals for the 10th, 50th, and 90th percentiles (from bottom to top, respectively) are displayed. Outliers are visualized as red dots and areas.

4. Conclusion

This study provides evidence of the impact of individual characteristics on the PK parameters of various salbutamol formulations. In summary, BMI, or weight, should be considered in the overall prescription of salbutamol; age is exclusively a determining factor in the inhaled route of administration (MDI, DPI, and nebulizer); ethnicity and gender both influence the oral administration of salbutamol and its DPI form. Therefore, with the development of population PK models, we have proved that clinical practice should progressively implement precision medicine in the management of respiratory diseases such as asthma. Tailoring treatment to interindividual variability, in addition to medical history and disease diagnosis, is one step closer to achieving maximum effectiveness, minimal risk of ADRs and, consequently, the best quality of life for asthma sufferers.

Nonetheless, some drawbacks in this study can be identified. First, using virtual data to develop PK models, although it is an increasingly popular approach, has a certain level of inaccuracy. These models should be further validated with real-world data. Second, the study focused only on single doses of substance. Salbutamol DPI, for instance, can be administered up to 2 puffs at a time; our study did not evaluate this. Finally, the confirmation of the PK parameters was very difficult. Despite repeated attempts to corroborate the obtained PK parameters, literature data for salbutamol formulations are either scarce or non-uniform across several studies.

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Future Perspectives

Pharmacological simulation studies have been revolutionizing the medical field. The development of virtual models that take into account human physiology and the interactions of drugs in the human body has accelerated the search for answers such as «what is the effectiveness of the drug in a given scenario?» or «is it possible to adjust this particular dose?».

Nevertheless, as these technologies are relatively new, there is still a long way to go in optimizing these studies. Several constraints exist concerning data accuracy and confidence in the developed models. In the context of our study, the models were not externally verified, that is, no additional tools were employed. It was rather performed a prior comparison of PK data with the documented literature. Additionally, the limited access to comprehensive real-world data greatly restricts the scope of this type of research. Throughout this thesis, the challenges associated with clinical data access have been highlighted, emphasizing the need for better considerations by relevant entities.

Therefore, in a future perspective, it would be valuable to validate these population PK models using new models that incorporate real-world data. In fact, there are already completed clinical trials that could be relevant for furthering this study. Moreover, to validate the results of our DDI study, in vitro studies should be conducted. Once this predictive model has been validated, it would be interesting to evaluate the interaction of salbutamol with other drugs, in order to create a database that clinicians can use when prescribing medications. To conduct more comprehensive in silico studies, we could include a more diverse dataset (wider age range, weight, comorbidities, ethnicities).

By addressing these aspects, future pharmacological simulation studies can be a useful tool for providing accurate and valuable insights in medical field, in particular, in precision medicine.

Appendix

Chapter I and II

Appendix A: Chapter I

Table A1. List of potential salbutamol-interacting drugs, belonging to different drug classes, from corticosteroids to antidepressants.

Drug	Drug Class
Atenolol	β-blocker
Beclomethasone	Corticosteroid
Doxepin	Tricyclic antidepressant
Fluticasone Propionate	Corticosteroid
Fluticasone	Corticosteroid
Formoterol	Long-acting β-agonist
Furosemide	Diuretic
Isocarboxazid	Monoamine oxidase inhibitor
Ketotifen	Antihistamine
Methylprednisolone	Corticosteroid
Prednisolone	Corticosteroid
Prednisone	Corticosteroid
Propranolol	β-blocker
Rosiglitazone	Thiazolidinedione
Salmeterol	Long-acting β-agonist
Theophylline	Xanthine
Fluvoxamine	Selective serotonin reuptake inhibitor

Table A2. PBPK models and respective individuals' characteristics.

PBPK Model	Population	Gender	Age (years)	Health Status	Height (cm)	Weight (kg)	BMI (kg/m^2)	Body Fat (%)
1	American	Male	30	Healthy	176.43	75.0	24.0944	23.41
2	American	Male	10	Healthy	145.23	38.9	18.4432	17.18
3	American	Male	65	Healthy	173.76	75.0	24.8406	24.08
4	American	Male	30	Mild Renal Impairment	176.43	75.0	24.0944	23.41
5	American	Male	30	Moderate Renal Impairment	176.43	75.0	24.0944	23.41
6	American	Male	30	Severe Renal Impairment	176.43	75.0	24.0944	23.41
7	American	Male	30	Healthy (Overweight)	176.43	85.53	27.4773	26.34
8	American	Male	30	Healthy (Obese)	176.43	105.0	33.7322	31.80
9	American	Female	30	Healthy	162.20	65.0	24.71	33.28
10	American	Female	30	Healthy (Pregnant)	162.20	65.0	24.71	33.28

BMI, Body Mass Index

Table A3. Metabolic profile of the 17 potential salbutamol-interacting drugs, estimated by ADMET Predictor ®.

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Atenolol	1A2	No (63%)	No (90%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	No (98%)	NS	NS	NS	NS
	2C8	ND	No (92%)	NS	NS	NS	NS
	2C9	No (95%)	Yes (98%)	NS	NS	NS	NS
	2C19	No (99%)	Yes (39%)	74.179	422.087	79.662	C17, C7
	2D6	Yes (46%)	Yes (66%)	0.778	1.291	13.282	C17, C7
	2E1	ND	No (98%)	NS	NS	NS	NS
	3A4	No (78%)	No (84%)	NS	NS	NS	NS
Beclomethasone	1A2	No (82%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	Yes (71%)	ND	ND	ND	C28, C34 e C27
	2C8	ND	No (63%)	NS	NS	NS	NS
	2C9	No (99%)	No (98%)	NS	NS	NS	NS
	2C19	No (95%)	No (88%)	74.179	422.087	79.662	C17, C7
	2D6	No (95%)	No (95%)	0.778	1.291	13.282	C17, C7
	2E1	ND	No (84%)	NS	NS	NS	NS
	3A4	Yes (80%)	Yes (89%)	40.448	16.885	46.338	C7, C33 e C27
Doxepin	1A2	No (97%)	Yes (91%)	23.920	10.658	23.171	C20 e C21
	2A6	ND	No (70%)	NS	NS	NS	NS
	2B6	ND	Yes (89%)	ND	ND	ND	C20, C21 e C11
	2C8	ND	Yes (91%)	ND	ND	ND	C20, C21
	2C9	No (90%)	Yes (66%)	5.823	0.149	1.867	C20, C21
	2C19	Yes (31%)	Yes (82%)	69.614	15.082	3.033	C20, C21
	2D6	Yes (51%)	Yes (87%)	3.417	7.545	17.667	C20, C21, C13 e C11
	2E1	ND	No (69%)	NS	NS	NS	NS
	3A4	No (90%)	Yes (74%)	74.034	4.391	6.584	C20, C21

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Fluticasone Propionate	1A2	No (76%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	Yes (44%)	ND	ND	ND	S28
	2C8	ND	No (86%)	NS	NS	NS	NS
	2C9	No (99%)	No (88%)	NS	NS	NS	NS
	2C19	No (89%)	No (88%)	NS	NS	NS	NS
	2D6	No (95%)	No (85%)	NS	NS	NS	NS
	2E1	ND	No (76%)	NS	NS	NS	NS
	3A4	Yes (80%)	Yes (98%)	19.997	6.993	39.033	S28, C7, C33 e C29
Fluticasone	1A2	No (90%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	Yes (61%)	ND	ND	ND	S28
	2C8	ND	Yes (58%)	ND	ND	ND	C29, S28, C16, C24, C19 e C7
	2C9	No (99%)	No (96%)	NS	NS	NS	NS
	2C19	No (94%)	No (88%)	NS	NS	NS	NS
	2D6	No (95%)	No (95%)	NS	NS	NS	NS
	2E1	ND	No (71%)	NS	NS	NS	NS
	3A4	Yes (38%)	Yes (98%)	40.068	1.515	4.197	S28, C7, C29
Formoterol	1A2	Yes (58%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (94%)	NS	NS	NS	NS
	2B6	ND	No (98%)	NS	NS	NS	NS
	2C8	ND	No (92%)	NS	NS	NS	NS
	2C9	No (95%)	No (98%)	NS	NS	NS	NS
	2C19	No (96%)	No (88%)	NS	NS	NS	NS
	2D6	Yes (70%)	Yes (87%)	5.614	8.237	11.739	C19, C23
	2E1	ND	No (98%)	NS	NS	NS	NS
	3A4	No (46%)	No (48%)	NS	NS	NS	NS

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Furosemide	1A2	No (86%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (70%)	NS	NS	NS	NS
	2B6	ND	No (98%)	NS	NS	NS	NS
	2C8	ND	No (77%)	NS	NS	NS	NS
	2C9	Yes (49%)	Yes (45%)	5.540	3.661	48.237	C2 e C6
	2C19	Yes (99%)	Yes (82%)	1813.378	3637.938	38.086	C6, C2 e C1
	2D6	No (55%)	No (68%)	NS	NS	NS	NS
	2E1	ND	Yes (82%)	ND	ND	ND	C2
	3A4	No (80%)	No (38%)	NS	NS	NS	NS
Isocarboxazid	1A2	Yes (95%)	Yes (74%)	524.501	15.319	1.519	C7, C17 e C3
	2A6	ND	Yes (82%)	ND	ND	ND	C7, C17, N13 e C3
	2B6	ND	No (89%)	NS	NS	NS	NS
	2C8	ND	Yes (58%)	ND	ND	ND	C7, C17 e C3
	2C9	Yes (41%)	No (88%)	NS	NS	NS	NS
	2C19	No (78%)	Yes (61%)	57.052	132.576	32.533	C17 e C7
	2D6	No (95%)	No (68%)	NS	NS	NS	NS
	2E1	ND	Yes (40%)	ND	ND	ND	C7, C3 e N13
	3A4	Yes (51%)	No (58%)	NS	NS	NS	NS
Ketotifen	1A2	No (90%)	Yes (91%)	139.473	9.355	3.488	C22 e N19
	2A6	ND	Yes (ND)	ND	ND	ND	C22 e C12
	2B6	ND	No (ND)	NS	NS	NS	NS
	2C8	ND	No (77%)	NS	NS	NS	NS
	2C9	No (ND)	Yes (ND)	21.787	0.107	0.359	C22
	2C19	Yes (ND)	Yes (ND)	50.763	5.845	1.612	C22
	2D6	Yes (55%)	Yes (ND)	21.857	27.720	10.146	C22, C12, C7, C3 e N19
	2E1	ND	No (76%)	NS	NS	NS	NS
	3A4	No (90%)	Yes (92%)	53.902	4.131	8.507	C22, N19 e C12

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Methylprednisolone	1A2	No (97%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98)	NS	NS	NS	NS
	2B6	ND	Yes (47%)	ND	ND	ND	C25
	2C8	ND	No (66%)	NS	NS	NS	NS
	2C9	No (99%)	No (98%)	NS	NS	NS	NS
	2C19	No (95%)	No (99%)	NS	NS	NS	NS
	2D6	No (95%)	No (95%)	NS	NS	NS	NS
	2E1	ND	No (78%)	NS	NS	NS	NS
	3A4	Yes (46%)	Yes (98%)	92.176	37.806	45.527	C7, C25 e C13
Prednisolone	1A2	No (97%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	Yes (46%)	ND	ND	ND	C24 e C12
	2C8	ND	No (77%)	NS	NS	NS	NS
	2C9	No (99%)	No (98%)	NS	NS	NS	NS
	2C19	No (97%)	No (95%)	NS	NS	NS	NS
	2D6	No (95%)	No (95%)	NS	NS	NS	NS
	2E1	ND	No (80%)	NS	NS	NS	NS
	3A4	Yes (38%)	Yes (92%)	83.372	24.021	31.981	C7, C24 e C12
Prednisone	1A2	No (97%)	No (90%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	Yes (49%)	ND	ND	ND	C23
	2C8	ND	No (80%)	NS	NS	NS	NS
	2C9	No (99%)	No (98%)	NS	NS	NS	NS
	2C19	No (98%)	No (95%)	NS	NS	NS	NS
	2D6	No (95%)	No (95%)	NS	NS	NS	NS
	2E1	ND	No (84%)	NS	NS	NS	NS
	3A4	Yes (46%)	Yes (98%)	74.801	11.076	16.436	C7, C23

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Propanolol	1A2	No (61%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (98%)	NS	NS	NS	NS
	2B6	ND	No (73%)	NS	NS	NS	NS
	2C8	ND	No (92%)	NS	NS	NS	NS
	2C9	No (92%)	No (98%)	NS	NS	NS	NS
	2C19	No (65%)	No (99%)	NS	NS	NS	NS
	2D6	No (55%)	Yes (87%)	3.901	1.458	2.990	C17
	2E1	ND	No (98%)	NS	NS	NS	NS
	3A4	No (90%)	No (58%)	NS	NS	NS	NS
Rosiglitazone	1A2	No (90%)	No (91%)	NS	NS	NS	NS
	2A6	ND	No (63%)	NS	NS	NS	NS
	2B6	ND	No (78%)	NS	NS	NS	NS
	2C8	ND	Yes (83%)	ND	ND	ND	C23 e C4
	2C9	Yes (63%)	Yes (66%)	117.900	8.272	5.122	S19, C23, C4 e C5
	2C19	Yes (23%)	Yes (82%)	15.478	0.964	0.872	C23, S19 e C9
	2D6	No (95%)	Yes (56%)	9.274	11.191	9.654	S19, C4 e C23
	2E1	ND	Yes (70%)	ND	ND	ND	S19, C4 e C23
	3A4	No (67%)	Yes (82%)	35.382	4.836	15.171	C23 e S19
Salmeterol	1A2	Yes (72%)	No (97%)	NS	NS	NS	NS
	2A6	ND	No (94%)	NS	NS	NS	NS
	2B6	ND	No (83%)	NS	NS	NS	NS
	2C8	ND	No (99%)	NS	NS	NS	NS
	2C9	No (90%)	No (90%)	NS	NS	NS	NS
	2C19	No (91%)	No (72%)	NS	NS	NS	NS
	2D6	Yes (70%)	Yes (72%)	3.054	37.871	99.216	C7, C20, C11, C23 e C27
	2E1	ND	No (82%)	NS	NS	NS	NS
	3A4	Yes (51%)	No (51%)	NS	NS	NS	NS

Drug	CYP Enzyme	Inhibitor	Substrate	Km	V_{max}	CL	Sites of metabolism
Theophylline	1A2	No (86%)	Yes (91%)	173.268	1.901	0.570	C12 e C7
	2A6	ND	Yes (82%)	ND	ND	ND	C12
	2B6	ND	No (75%)	NS	NS	NS	NS
	2C8	ND	No (99%)	NS	NS	NS	NS
	2C9	No (99%)	No (72%)	NS	NS	NS	NS
	2C19	No (98%)	No (95%)	NS	NS	NS	NS
	2D6	No (95%)	No (95%)	3.054	37.871	99.216	C7, C20, C11, C23 e C27
	2E1	ND	Yes (49%)	ND	ND	ND	C7, C10 e C12
	3A4	No (81%)	No (38%)	NS	NS	NS	NS

ND, not defined; NS, no substrate

Appendix B: Chapter II

Table B1. PBPK models and respective individuals' characteristics.

PBPK Model	Population	Gender	Age (years)	Health Status	Height (cm)	Weight (kg)	BMI (kg/m^2)	Body Fat (%)
1	American	Male	30	Healthy	176.43	75.0	24.0944	23.41
2	American	Female	30	Healthy	162.20	65.0	24.7065	33.18
3	American	Female	5	Healthy	111.18	20.42	16.5197	21.17
4	American	Female	10	Healthy	142.08	39.43	19.5326	26.17
5	American	Female	20	Healthy	162.71	60.0	22.6633	31.74
6	American	Female	65	Healthy	156.24	60.0	24.5791	33.20
7	American	Male	5	Healthy	111.65	20.06	16.0921	16.17
8	American	Male	10	Healthy	145.23	38.9	18.4432	17.18
9	American	Male	20	Healthy	176.10	75.0	24.1848	23.58
10	American	Male	65	Healthy	173.76	75.0	24.8406	24.08
11	Japanese	Male	30	Healthy	167.16	62.57	22.3924	22.03
12	Japanese	Female	30	Healthy	155.78	50.59	20.8469	26.01
13	Chinese	Female	30	Healthy	159.26	56.03	22.0906	28.07
14	Chinese	Male	30	Healthy	171.00	68.37	23.3816	23.52
7	American	Male	30	Healthy (Overweight)	176.43	85.00	27.3070	26.21
8	American	Male	30	Healthy (Obese)	176.43	105.0	33.7322	31.80

Table B2. Basic population pharmacokinetic models of salbutamol inhaler.

Project Name	Model Description	Error model	BIC
PK_I_01	Two compartments, no delay, first order, linear elimination	Combined 1	–6671.31
PK_I_02	Two compartments, lag time, first order, linear elimination	Combined 1	–6614.15
PK_I_03	Two compartments, transit compartment, first order, linear elimination	Combined 1	–6178.33
PK_I_04	Two compartments, no delay, first order, Michelis-Menten elimination	Combined 1	–6407.67
PK_I_05	Two compartments, no delay, zero order, linear elimination	Combined 1	–6671.32
PK_I_06	Three compartments, no delay, zero order, linear elimination	Combined 1	–6648.67
PK_I_07	Three compartments, no delay, first order, linear elimination	Combined 1	–7147.89
PK_I_08	Three compartments, lag time, first order, linear elimination	Combined 1	–6659.8

BIC: Bayesian information criteria; ΔOFV: Based on the BIC values, PK_I_07 (yellow) was the selected model for the study, due to their best description of the population pharmacokinetic profile.

Table B3. Basic population pharmacokinetic models of salbutamol DPI.

Project Name	Model Description	Error model	BIC
PK_DPI_01	Three compartments, no delay, first order, linear elimination	Combined 1	–9367.13
PK_DPI_02	Two compartments, no delay, first order, linear elimination	Combined 1	–9516.26
PK_DPI_03	Two compartments, no delay, first order, linear elimination	Proportional	–9710.73

ΔOFV: Based on the BIC values, PK_DPI_03 (yellow) was the selected model for the study, due to their best description of the population pharmacokinetic profile.

Table B4. Basic population pharmacokinetic models of salbutamol nebulizer.

Project Name	Model Description	Error model	BIC
PK_NEB_01	Two compartments, no delay, first order, linear elimination	Combined 1	–9559.95
PK_NEB_02	Three compartments, no delay, first order, linear elimination	Combined 1	–10854.47
PK_NEB_03	Two compartments, lag time, first order, linear elimination	Proportional	–9619.75
PK_NEB_04	Three compartments, no delay, first order, linear elimination	Combined 1	–9520.73

ΔOFV: Based on the BIC values, PK_DPI_03 (yellow) was the selected model for the study, due to their best description of the population pharmacokinetic profile.

Table B5. Basic population pharmacokinetic models of salbutamol oral syrup.

Project Name	Model Description	Error model	BIC
PK_OS_01	One compartment, no delay, first order, linear elimination	Combined 1	–21880
PK_OS_02	Two compartments, no delay, first order, linear elimination	Combined 1	–24056.66
PK_OS_03	Two compartments, lag time, first order, linear elimination	Combined 1	–23084.58

ΔOFV: Based on the BIC values, PK_OS_03 (yellow) was the selected model for the study, due to their best description of the population pharmacokinetic profile. This model has already incorporated covariates.

Table B6. Basic population pharmacokinetic models of salbutamol oral tablet.

Project Name	Model Description	Error model	BIC
PK_OT_01	One compartment, no delay, first order, linear elimination	Combined 1	–18838.91
PK_OT_02	One compartment, lag time, first order, linear elimination	Combined 1	–18981.49
PK_OT_03	One compartment, lag time, first order, linear elimination	Combined 2	–19229.76

ΔOFV: Based on the BIC values, PK_OS_03 (yellow) was the selected model for the study, due to their best description of the population pharmacokinetic profile.

Table B7. Basic population pharmacokinetic models of salbutamol IV.

Project Name	Model Description	Error model	BIC
PK_IV_01	One compartment, no delay, first order, linear elimination	Combined 1	–80713.37
PK_IV_02	One compartment, lag time, first order, linear elimination	Combined 1	–35471.54
PK_IV_03	Three compartments, no delay, first order, linear elimination	Combined 1	–104108.17
PK_IV_04	Three compartments, no delay, first order, linear elimination	Proportional	–113447.24

ΔOFV: Based on the BIC values, PK_OS_03 (yellow) was the selected model for the study, due to their best description of the population pharmacokinetic profile.