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Pharmacokinetic analysis of inhaled salmeterol in asthma patients: Evidence from two dry powder inhalers

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Abstract

Salmeterol (SAL) is a long-acting β2-adrenergic agonist, which is widely used in the therapy of asthma. The aim of this study was to investigate the pharmacokinetics (PK) of inhaled salmeterol in asthma patients using two different dry powder inhalers. This analysis was based on data from 45 subjects who participated in a two-sequence, four-period crossover bioequivalence (BE) study after single administration of the test (T) and reference (R) products. In order to mimic more closely the real treatment conditions, activated charcoal was not co-administered. Plasma concentration–time (C–t) data were initially analysed using classic non-compartmental PK approaches, while the main objective of the study was to apply population PK modeling. The relative fraction of the dose absorbed via the lungs (R_L) was set as a parameter in the structural model. The plasma C–t profiles of salmeterol showed a biphasic time course indicating a parallel pulmonary and gastrointestinal (GI) absorption. A two-compartment disposition model with first order absorption from the GI and very rapid absorption from lungs (like an i.v. bolus) was found to describe successfully the C–t profiles of salmeterol. The estimated R_L value was 13% suggesting a high gut deposition of inhaled salmeterol. Women were found to exert less capability to eliminate salmeterol than men, while body weight (in allometric form) was found to be an important covariate on the peripheral volume of distribution.

KEYWORDS

dry powder inhalers, parallel absorption, population pharmacokinetics, salmeterol xinafoate

1 | INTRODUCTION

Asthma is a highly prevalent, chronic inflammatory disease of the airways. It is characterized by airway inflammation, hyper-responsiveness and progressive airflow obstruction, which result in wheezing, coughing and shortness of breath (Gerritsen, Koeter, Postma, Schouten, & Knol, 1989; Murdoch & Lloyd, 2010). Inhaled combination therapy with anti-inflammatory and bronchodilating agents has been shown to improve lung function in patients with varying degrees of asthma severity (Calzetta, Rinaldi, Cazzola, & Matera, 2016; Nelson, Chapman, Pyke, Johnson, & Pritchard, 2003; Shrewsbury, Pyke, & Britton, 2000). Treatment with inhalation devices allows the administration of relatively low doses of drugs, since these are delivered directly to the site of inflammation, achieving high local pulmonary concentrations. This in turn leads to a high therapeutic ratio and minimization of the systemic adverse effects (Lipworth, 1996).

Salmeterol (SAL) xinafoate is a β_2-adrenergic agonist which acts locally in the lung by providing a long-lasting (ca. 12 h) bronchodilation (Johnson et al., 1993). Salmeterol offers effective protection against histamine-induced bronchoconstriction, by decreasing airway resistance and improving the ventilation of patients (Mahler et al., 1999; Ricciardolo, Blasi, Centanni, & Rogliani, 2015). However, similarly to other β_2-adrenoceptor agonists, the use of inhaled salmeterol has been associated with dose-related cardiovascular and systemic effects such as increased heart rate, palpitations, tremor and changes in plasma glucose and potassium levels (Guhan et al., 2000). Since the main adverse effects of inhaled salmeterol relate to its systemic activity, knowledge of its pharmacokinetics is important for optimizing the use in everyday clinical practice. At the same time, investigation of the pharmacokinetics of inhaled salmeterol may serve as a valuable tool for determining its lung deposition and bioavailability, thus providing useful information for optimizing drug delivery.

Population pharmacokinetic (PK) analyses describing the full pharmacokinetic profiles of other β_2-adrenergic agonists following inhaled administration can be found in the literature (Ambery, Wielders, Ludwig-Sengpiel, Chan, & Riley, 2015; Borghardt et al.,

2016; Derks, van den Berg, van der Zee, Braat, & van Boxtel, 1997; Diderichsen, Cox, Martin, Cleton, & Ribbing, 2013; Goyal et al., 2014; Maier, Rubino, Hsu, Grasela, & Baumgartner, 2007). However, only limited data regarding salmeterol pharmacokinetics have been published (Advair Diskus®, 2004; Cazzola, Testi, & Matera, 2002; Kempsford, Handel, Mehta, De Silva, & Daley-Yates, 2005). This lack of information is most likely attributed to the very low systemic concentrations obtained following drug inhalation and the technical difficulties in developing sensitive enough bioanalytical methods that can assay these concentrations (Cazzola et al., 2002). In the case of salmeterol, the results of a population pharmacokinetic analysis were recently published using data obtained from a crossover bioequivalence (BE) study in healthy volunteers (Soulele, Macheras, Silvestro, Rizea Savu, & Karalis, 2015). In that previous study, the subjects received a single dose of the fluticasone propionate (FLP)/salmeterol (500/50 μg/inhalation) combination via two different inhalation devices, while activated charcoal was co-administered in order to prevent any gastrointestinal absorption of salmeterol.

In the present study, the PK analysis of salmeterol is extended to an asthma patient group, since the investigation of the influence of the airway disease state on the pharmacokinetics of inhaled salmeterol is very important. The data used for this analysis come from a two-sequence, four-period, crossover bioequivalence study in asthma patients receiving the FLP/SAL combination via two different inhalation devices. Activated charcoal was not co-administered in the bioequivalence study in order to mimic more closely the real treatment conditions and to examine the total systemic exposure. Besides, fluticasone propionate levels were not measured in the bioequivalence study and instead of them, the systemic exposure of cortisol was determined as a safety measure related to fluticasone propionate treatment. The classic non-compartmental methodology and a population PK analysis were applied to the salmeterol concentration–time (C–t) data. The non-compartmental analysis was further extended to a classic bioequivalence assessment of the estimated PK parameters. The novelty of this work relies on the following issues: (a) to reveal the pharmacokinetics of inhaled salmeterol in asthma patients, for example, to determine the fraction of inhaled salmeterol that is deposited at the gastrointestinal tract, (b) to apply population pharmacokinetic analysis as a surrogate for bioequivalence investigation of two medicinal products, (c) to investigate and identify demographic characteristics (e.g. gender, body weight) and study related factors (e.g. period, treatment) as potential covariates influencing the salmeterol pharmacokinetics.

2 | MATERIALS AND METHODS

2.1 | Study design

Salmeterol plasma C–t data were obtained from a single dose, two-sequence, four-period, crossover (2 × 4) bioequivalence study using two dry powder inhalers of the FLP/SAL xinafoate combination: the multi-dose Seretide® Diskus™ (250/50 μg/inhalation, GlaxoSmithKline (GSK), Brentford, UK) and a single-dose device Rolenium® Elpenhaler® (250/50 μg/inhalation, ELPEN Pharmaceuticals, Attica, Greece) under fasting conditions. In the bioequivalence study, instead of fluticasone propionate levels, cortisol plasma levels were determined as a measure of safety related to the fluticasone treatment. A washout period of 5 days was set between each treatment to allow for the complete removal of salmeterol from the body and to prevent carry-over effects. The study was performed in accordance with ICH E6 Good Clinical Practice Consolidated Guidance and the Declaration of Helsinki, by 3S–Pharmacological Consultation and Research (Harpstedt, Germany).

Forty-eight controlled or partly controlled (according to the GINA 2009 classification of Level Asthma Control) asthma male and female patients, with varying degrees of symptoms severity, were enrolled in the study. All patients were informed about the purpose, protocol and potential risks of the study and each participant signed a written consent form before entering the study. The subjects were aged between 18 and 65 years and met the inclusion criteria specified in the study protocol, such as body mass index (BMI) within 18.5–30 kg/m^2, controlled or partly controlled asthma with mild to moderate exacerbations, regular asthma therapy with inhaled glucocorticosteroids (except fluticasone) alone or in combination with long-acting β_2-agonist bronchodilators, (except salmeterol), absence of other than respiratory diseases, non-smokers, non-pregnant and non-lactating women. The main exclusion criteria referred to intolerance or hypersensitivity to the study drugs or lactose, poor clinical asthma control, hospitalization for any other reason or donation of ≥450 ml of blood within 2 months prior to study initiation, any recent history of drug or alcohol abuse, upper respiratory tract infection within 6 weeks prior to the study, ECG changes or any clinical significant abnormalities, positive AIDS or hepatitis B/C tests results. Vital signs, measured before and after the study drugs administration in each study period, were analysed and all reported adverse effects were recorded. Three subjects were considered as drop-outs due to positive pregnancy test results, concomitant medication or personal reasons. Therefore, 45 subjects completed the study and their salmeterol C–t data were analysed and included in our study.

On the treatment days, after at least 8 h of fasting, each patient received either one dose of Rolenium® Elpenhaler® 250/50 μg/inhalation (test product, T) or one dose of Seretide® Diskus™ 250/50 μg/inhalation (reference product, R), according to the randomization scheme. Activated charcoal, for gastrointestinal absorption blockade, was not co-administered in order to compare the total systemic exposure of salmeterol and to investigate the safety of the drug in the real treatment conditions. Blood samples (6 ml each) were collected before drug administration (i.e. time 0) and at 2, 4, 7, 10, 15, 30 and 45 min, as well as at 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 9, 12, 16, 24, 36, 48 and 72 h post-dose. The elimination half-life of salmeterol is 5.5 h (Serevent Diskus, 2014), which implies that most of the drug (ca. 90%) will be removed from the body within 27.5 h after its administration. However, blood samples were collected at 36, 48 and 72 h post-dose and analysed to confirm the complete elimination of the drug after 24 h. After 5 days of the washout period, patients received the alternate product and blood samples were again drawn and analysed using the same procedures. The entire procedure was repeated for each subject since the study had a fully replicate design.

2.2 | Assay methodology

The identification and quantification of salmeterol in plasma were performed by a validated liquid chromatography/mass spectrometry (LC–MS/MS) method, showing adequate sensitivity, precision, accuracy, specificity and linearity. Separations were performed on a reversed phase column Ascentis Phenyl, 10 cm × 2.1 mm, 5 μm (Merck), in isocratic conditions using a mobile phase composition of 90% acetonitrile and 10% ammonium acetate 15 mM in water, with a flow rate of 1 ml/min. The utilized technique was a re-validated version of the previously published method (Silvestro, Savu, Savu, Tudoroniu, & Tarcomnicu, 2012). The lower limit of quantification for salmeterol was 1.00 pg/ml.

2.3 | Pharmacokinetic analysis

2.3.1 | Non-compartmental pharmacokinetic analysis

For the purposes of the study, the salmeterol C–t data were initially analysed using non-compartmental pharmacokinetic approaches. The PK parameters of salmeterol were calculated and the bioequivalence between the two inhalation devices was further assessed using WinNonlin® (v.5.0.1, Pharsight Corp., Menlo Park, CA). The estimated PK parameters referred to the area under the concentration–time curve from time zero to the last quantifiable sample (AUC_t), the area under the C–t curve from time zero extrapolated to infinity (AUC_{inf}), the first recorded maximum plasma concentration value (C_{max}), and the time (T_{max}) at which C_{max} occurs. The AUC_t was calculated using the linear trapezoidal rule, whereas AUC_{inf} was calculated as $AUC_t + C_{last}/\lambda_z$, where C_{last} is the last quantifiable concentration and λ_z refers to the apparent terminal elimination rate constant. The constant λ_z was determined by linear regression analysis applied to the terminal log-linear phase of the C–t curve. Two of these parameters were further used in the bioequivalence assessment of the two inhalers. In this respect, the AUC_t and C_{max} estimates were assessed according to the current European Medicines Agency (EMA) guideline on the investigation of bioequivalence by applying a general linear model (analysis of variance) (EMA CHMP, 2010). Data derived from the 45 subjects, who completed all four periods of the study, were included in the statistical analysis. Ninety percent confidence intervals (CI) around the geometric mean ratio (GMR) of T over R product (T/R) were constructed using the residual error from ANOVA. The two inhalers were considered bioequivalent if the 90% CIs of both AUC_t and C_{max} were within the predetermined equivalence range of 80–125% (EMA CHMP, 2010).

2.4 | Population pharmacokinetic analysis

The population PK analysis was applied to the entire set of data of the 45 subjects who completed all four periods of the bioequivalence study, providing a final dataset of 180 C–t profiles for the analysis. Since the data were derived from a 2 × 4 crossover bioequivalence study, plasma C–t data from both T and R products were incorporated in the analysis by setting the 'treatment' and 'period' effects as potential covariates. Similar methodologies in population PK analyses using datasets from bioequivalence studies have been proposed in the literature (Fradette, Lavigne, Waters, & Ducharme, 2005; Karlsson & Sheiner, 1993; Panhard & Mentre, 2005; Soulele et al., 2015). The entire population PK analysis was implemented in Monolix® v.2016R1 (Lixoft, Orsay, France).

The first stage of the analysis included the determination of the structural model. In this context, one- and two-compartment models were evaluated. Due to the fact that activated charcoal was not co-administered, apart from the pulmonary absorption of salmeterol, systemic absorption from the gastrointestinal (GI) tract could also be present. Therefore, the kinetics of salmeterol absorption was modeled in a way capable of describing the parallel lung and GI absorption. An MIxtran code was created incorporating the two parallel absorption processes of salmeterol, namely, the very rapid (like i.v. bolus) kinetics of the pulmonary absorption and the assumed first-order kinetics for the GI absorption. In addition, the relative fraction of the administered dose absorbed via the lungs (R_L) was included as a term into the model. Estimation of R_L allowed also the knowledge of the remaining amount of salmeterol which is swallowed/deposited in the GI (R_{GI}) and is capable of being absorbed, since their sum equals to unity. Thus, in the case of the one-compartment model, the structural PK model was parameterized in terms of the apparent first-order absorption rate constant (K_a), R_L (or equivalently R_{GI}), the apparent volume of distribution (V_{ap}/F), and the apparent systemic drug clearance (CL/F), where the term F refers to the bioavailable fraction of dose. For the two-compartment model, the PK parameters incorporated in the model were K_a, R_L (or R_{GI}), CL/F, the apparent volume of drug distribution of the central (V_c/F) and the peripheral (V_p/F) compartments, as well as the apparent inter-compartmental clearance (Q/F). The individual pharmacokinetic parameters were assumed to follow log-normal distribution. In all cases, elimination was considered to take place in the central compartment following first-order kinetics.

The between-subject variability (BSV) in the pharmacokinetic parameters was also assumed to follow log-normal distribution. Several residual error models (constant, proportional, exponential and combined) were tested in order to describe the unexplained variability of the structural model. The variability in the pharmacokinetic parameters between occasions (Inter-Occasion Variability, IOV) was also evaluated. Finally, the possibility of covariance between the PK parameters was assessed and the effect of the administered product (test or reference) on PK parameters was evaluated through the inclusion of the 'treatment' as an additional component in the model.

The stochastic approximation expectation maximization (SAEM) algorithm was used for the estimation of the population parameters. The SAEM algorithm is a powerful stochastic algorithm which allows the estimation of the maximum likelihood estimators of the population PK parameters (Lavielle & Mentre, 2007; Maltezou et al., 2012; Savic & Lavielle, 2009). In the present analysis, the following settings were applied in the SAEM algorithm: (i) the maximum numbers of SAEM iterations K1 and K2 did not exceed 500 and 200, respectively, (ii) the number of Markov chains was set equal to two, (iii) the simulated annealing version of SAEM was used (i.e. the variances were constrained to decrease or increase slowly during the first iterations of SAEM), and finally (iv) the Monte-Carlo sizes for the prediction distribution graphic, visual predictive check (VPC) plots, and the log-likelihood estimation were set to 100, 500 and 20000, respectively.

During the model building procedure, different initial values were tested for the PK parameters. Random or values derived from previously published data were tested and the estimated model PK parameters were evaluated and compared (Soulele et al., 2015). The 'fixed' option, in the initialization frame of Monolix, was also used during the initial model optimization process for some of the PK parameters (i.e. V_c/F, V_p/F) where the level of PK estimates was not known.

Missing concentration data, which were below the lower limit of quantitation (BLQ), were treated as censored data using the appropriate setting of the software and were replaced by the lower limit of quantitation value of the bioanalytical method (1 pg/ml). However, not all missing data up to 72 h of sampling could be treated as censored, for computational reasons. The latter arose from the fact that almost all drug was eliminated from the body at 24 h (the elimination half-life is 5.5 h) and after this time point the vast majority of concentration data were missing (Serevent Diskus, 2014). Therefore, truncation at 36 h was applied, while any missing observations up to 36 h were treated as censored, whereas C–t data after 36 h were omitted.

For the evaluation of the best final PK model, numerical and graphical selection criteria, as well as the physiological soundness of the PK estimates were taken into consideration. All models were evaluated in terms of the values of the −2LL (log-likelihood) function, the Akaike and Bayesian information criteria (AIC and BIC), the model predicted estimates and the percent relative standard error (RSE%) of these estimates, as well as the BSV and residual error values. The graphical criteria included the adequacy of fitting to the actual C–t data through the visual inspection of the individual fits, and goodness of fit plots such as the predicted–observed plots, the individual weighted residuals (IWRES) and the normalized prediction distribution error (NPDE) vs the individual predicted (IPRED) concentrations values, and finally the visual predictive check (VPC) plots (Gabrielsson & Weiner, 2007; Post, Freijer, Ploeger, & Danhof, 2008).

After determining the best structural model, patient covariates were tested for their contribution into the model. The investigated covariates included patient demographic characteristics such as gender, age, body weight (BW), height and BMI. In all cases, the continuous covariates were examined either untransformed or centered around their 'mean' value. Allometric scaling with body weight as the size descriptor and fixed exponents (1 for the central and peripheral volume of distribution and 0.75 for clearance) was also evaluated. A combination of forward addition and backward elimination methods was implemented for investigation of all potential covariates. Initially, the backward elimination method was applied. A stepwise procedure was followed that involved the initial inclusion of all covariates in the model and the progressive deletion of non-significant covariates using the pre-defined model selection criteria. The removal of each covariate should improve the model (e.g. a decrease in the −2LL by at least 3.84), and the process was repeated until no further improvement of the model was possible. After completing this procedure, a forward addition method was utilized by adding some covariates that might have a significant impact on the PK variability. The covariates examined at this stage referred to those quoted in the literature for similar studies or that had been identified as significant during some steps of the backward elimination process (but not in the final model).

The selection of the best final model was based on the selection criteria described above and the significance and physiological soundness of each covariate. Finally, the most important intermediate models were further tested with regard to the initial settings, including different initial values for the fixed effects and settings of the algorithm.

3 | RESULTS

The salmeterol C–t data of the 45 male and female patients, who completed the four periods of the study, were included in the PK and statistical analysis. Since the fluticasone propionate levels were not measured in the bioequivalence study, our PK modeling analysis was solely based on the salmeterol data. The mean age of the study population was 45 years (age range 23–64 years), mean body weight 75.1 kg (range 52–100 kg), mean height 168 cm (range 150–187 cm) and mean BMI was 26.5 kg/m^2 (range 18.9–29.9 kg/m^2). The tolerability of both products was acceptable, since a total of 13 adverse events were recorded in 10 patients; namely, 7 (R: 3, T: 4) were of mild intensity and 6 (R: 4, T: 2) characterized of moderate intensity. No serious adverse event occurred during treatment with either the T or R products. Also, no statistically significant difference in the incidence of adverse events between the two treatments was observed. The subjects that encountered these adverse events completely recovered before the end of the study. No clinically significant abnormalities on physical examination, vital sign measurements or electrocardiographic recordings were reported.

3.1 | Non-compartmental pharmacokinetic analysis

The mean salmeterol C–t profiles following a single administration of the T and R products to the 45 patient volunteers are presented in Figure 1. Despite the wide time-range of the entire sampling period, the plasma C–t profiles show a biphasic time course. These double-peak C–t profiles were obtained for both products and indicate a parallel pulmonary and GI absorption for salmeterol due to the absence of oral activated charcoal.

Table 1 summarizes the estimates of the PK parameters (AUC_t, AUC_{inf}, C_{max}, T_{max} and λ_z) of salmeterol accompanied by their descriptive statistics, namely, mean, standard deviation (SD), percent coefficient of variation (CV%), median, minimum and maximum. The mean C_{max}, AUC_t, AUC_{inf} and λ_z values of the T product appear quite close to those of the R device. The T_{max} of the R product is 1.7 times slower than that of T. The derived CV% values ranged from 49.9% to 71.9% for all PK parameters.

Subsequently, a bioequivalence assessment was performed for the AUC_t and C_{max} estimates, quoted in Table 1, following the EMA methodology (EMA CHMP, 2010). These results are listed in Table 2. For both PK parameters, the 90% CIs were within the acceptance range of 80–125%, indicating that the two products are bioequivalent. The point estimates of the GMR(%) of AUC_t was 97.96%, while the 90% CI ranged from 92.88% to 103.32%. With

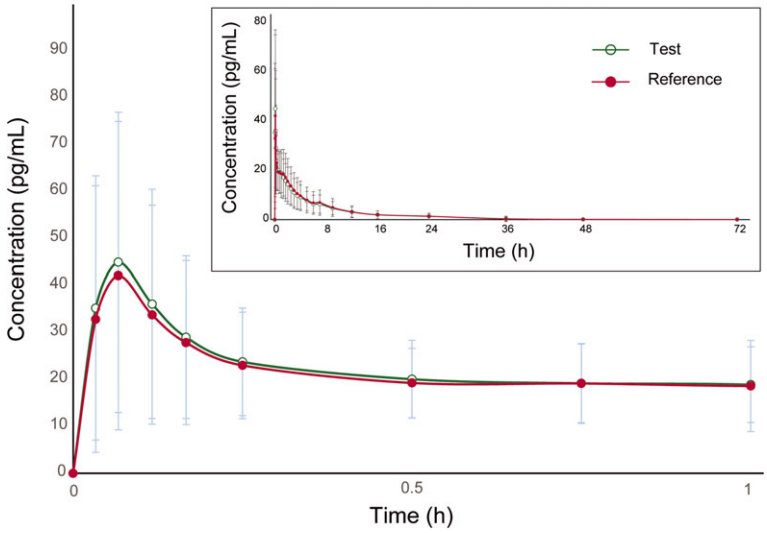

FIGURE 1 Mean plasma concentration–time profiles of salmeterol for the test and reference dry powder inhalers up to 1 h after inhalation. The first peak referring to lung absorption is obvious. The error bars refer to the standard deviation of the concentration values at each time-point. The inset shows the entire time profile up to 72 h

TABLE 1 Pharmacokinetic (PK) parameters and statistical descriptive criteria for the plasma concentration–time data of inhaled salmeterol (test and reference products)

PK parameter[a]	Mean	SD[b]	CV%[c]	Median	Min	Max
Test						
AUC_t (pg/ml/h)	136.333	84.509	62.0	115.029	28.530	622.572
C_{max} (pg/ml)	47.897	30.090	62.8	40.473	10.959	135.164
AUC_{inf} (pg/ml/h)	156.041	90.414	57.9	133.733	43.608	655.111
T_{max} (h)	0.240	-	-	0.067	0.033	1.667
λ_z (h^{-1})	0.096	0.048	50.3	0.092	0.032	0.294
Reference						
AUC_t (pg/ml)	140.502	100.959	71.9	119.725	38.923	883.011
C_{max} (pg/ml)	46.543	30.935	66.5	37.639	9.976	131.266
AUC_{inf} (pg/ml/h)	160.726	107.254	66.7	137.039	46.096	919.744
T_{max} (h)	0.411	-	-	0.067	0.033	2.000
λ_z (h^{-1})	0.102	0.051	49.9	0.099	0.008	0.288

[a]AUC_t, area under the concentration–time curve from time zero to the last quantifiable sample; C_{max}, the first recorded maximum plasma concentration value; AUC_{inf}, area under the concentration–time curve from time zero extrapolated to infinity; T_{max}, the time at which C_{max} occurs; λ_z, apparent terminal elimination rate constant.

[b]Standard deviation.

[c]Percent coefficient of variation.

TABLE 2 Bioequivalence results for salmeterol administered via two different dry powder inhalers

Pharmacokinetic parameter	GMR(%)[a]	Lower 90% CI[b]	Upper 90% CI[b]	Statistical power (%)[c]
AUC_t (pg/ml/h)	97.96	92.88	103.32	100.00
C_{max} (pg/ml)	106.71	95.97	118.66	94.08

[a]GMR refers to the geometric mean ratio of the test over reference pharmacokinetic metric.

[b]The 90% confidence interval (CI) around the GMR.

[c]Statistical power of the study computed using: the estimated GMR, the residual error of the study, level of significance 5%, number of subjects 45 and a 2 × 4 clinical design.

regard to C_{max}, the GMR(%) value was 106.71% with a 90% CI ranging from 95.97% to 118.66%. In addition, no significant sequence, treatment or period effects were observed for any PK parameter of salmeterol (data not shown).

3.2 | Population pharmacokinetic analysis

A total of 180 (= 4 periods × 45 subjects) C–t profiles of SAL were included in the dataset. As reported above, careful examination of the individual plasma C–t profiles of salmeterol reveals the form of a parallel absorption, which in our case can be attributed to the pulmonary and GI absorption of the drug. For this reason, the appropriate MIxtran code was developed describing the parallel pulmonary and GI absorption. Many scenarios were examined in order to determine the final model that describes best the pharmacokinetics of salmeterol. All the possible combinations of conditions tested during the population PK analysis were evaluated according to the selection criteria described in the 'Materials and Methods' section. In order to guide the reader on some important intermediate steps of the analysis, a relevant table with some selected representative model program executions accompanied by the corresponding −2LL, AIC and BIC estimates is presented (Table 3). These numerical criteria along with

TABLE 3 Information criteria and some basic steps of the analysis in order to find the final best pharmacokinetic (PK) model of salmeterol

Model ID	Model short description	Statistic criterion[a]			Comments
		-2LL	AIC	BIC	
1	1-compartment Covariates: none	20958.60	21016.60	21109.19	The basic one-compartment model from which the analysis was initiated Information criteria and goodness-of-fit plots were not satisfactory
2	2-compartment Covariates: none	18799.49	18885.49	19022.79	The two-compartment model was tested Numerical criteria and goodness-of-fit plots were better than model '1'
3	2-compartment Covariates: none Other initial PK values	17688.07	17726.07	17786.74	Several different values were tested for the initial PK estimates. The estimates that led to the best information criteria and goodness-of-fit plots were selected
4[b]	2-compartment Covariates: all available on all parameters	17586.21	17720.21	17934.14	The effect of all covariates was investigated on the PK parameters and a combination of backward elimination and forward addition methodologies was applied in order to elucidate the role of each covariate in salmeterol kinetics
5[c]	2-compartment Covariates: body weight on V_c/F (using an allometric exponent of 1)	18687.56	18725.56	18786.23	The performance of this model is worse than the simple model (i.e. '3'). Thus, the allometric body weight on V_c/F should be omitted
6[d]	2-compartment Covariates: body weight on V_p/F (using an allometric exponent of 1)	17647.37	17685.37	17746.04	The performance of this model is better than the simple model (i.e. '3'). Thus, the allometric body weight on V_p/F is important
7[c]	2-compartment Covariates: body weight on CL/F using an allometric exponent of 0.75	18675.10	18713.10	18773.77	The performance of this model is worse than the simple model (i.e. '3'). Thus, the allometric body weight on CL/F should be omitted
8[e]	2-compartment Covariates: treatment and occasion on all parameters	17624.70	17710.70	17848.00	The 'treatment' effect (i.e. T and R products) and study 'Period' were tested as potential covariates on all PK parameters. Subsequently, combinations of backward elimination and forward addition methodologies were applied
9[f]	2-compartment Covariates: body weight on V_p/F (using an allometric exponent of 1), treatment on V_c/F	17621.37	17661.37	17725.23	One of the final models elucidating the role of 'body weight' and 'treatment' on the PK parameters
10[g]	2-compartment Covariates: body weight on V_p/F (using an allometric exponent of 1), treatment on V_c/F, Gender on CL/F	17541.93	17583.93	17650.98	The final PK model of salmeterol In this model, the further inclusion of 'gender' effect on CL/F in the previous model (no. '9') was found to improve the information criteria and goodness-of-fit plots

[a]The terms −2LL, AIC and BIC refer to −2 log-likelihood function, Akaike information criterion and Bayesian information criterion, respectively.

[b]Gender was found to be a significant covariate on CL/F, while body weight was found to be a significant covariate on V_p/F.

[c]The inclusion of body weight was found to increase −2LL and was omitted as a covariate.

[d]The use of body weight was significant and decreased −2LL and for this reason was included as a covariate on V_p/F.

[e]The only significant covariate was 'Treatment' on V_c/F.

[f]Both body weight on V_p/F and 'Treatment' on V_c/F were found to be a significant.

[g]The final model with the three significant covariates.

K_a, first order absorption rate constant (h^{-1}); F, fraction of bioavailable dose; V_c/F, apparent volume of drug distribution (l) of the central compartment; CL/F, apparent clearance (l/h), PK, pharmacokinetic.

additional goodness-of-fit criteria and diagnostic plots led to the determination of the final PK model of salmeterol.

The salmeterol plasma concentrations were best described by a two-compartment disposition model combining two parallel absorption processes, a first order absorption from the GI and a very rapid absorption (like i.v. bolus) from the lungs. Elimination was considered to take place in the central compartment following first order kinetics. Figure 2 depicts the structural model of the pharmacokinetics of salmeterol following inhaled administration. The model was parameterized in terms of the GI absorption rate constant (K_a), apparent volume of distribution in the central (V_c/F) and the peripheral (V_p/F) compartment, as well as apparent clearance (CL/F) and inter-compartmental clearance (Q/F). The relative fraction of dose swallowed and deposited at the GI (R_{GI}) was also set as a parameter estimated by the optimization process. The residual error model that led to the optimum performance was a proportional error model:

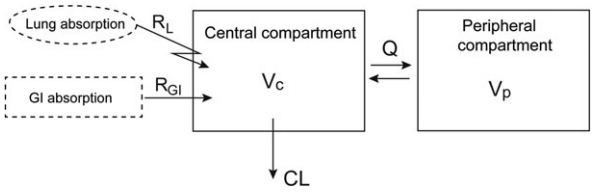

FIGURE 2 Structural representation of the two-compartment model used to describe the pharmacokinetics of salmeterol after inhalation when no activated charcoal is administered. Two input sources are shown: lung and gastrointestinal (GI) absorption. A relative part of the drug (R_L) is absorbed via the lungs, while the remaining part (R_{GI}) is finally swallowed and deposited in the GI. V_c, volume of drug distribution (l) of the central compartment; V_p, volume of drug distribution (l) of the peripheral compartment; Q, inter-compartmental clearance of the drug (l/h); CL, clearance from the central compartment (l/h)

$$C_{ij} = f_{ij} + b \cdot f_{ij} \cdot \varepsilon_{ij} \quad (1)$$

where C_{ij} is the jth observed concentration of salmeterol for the ith individual, f_{ij} is the jth model predicted value for ith subject, b is the parameter of the residual error model, and ε_{ij} is the random error which is assumed to be normally distributed with mean 0 and variance 1. Also, any combination of covariance terms between the PK parameters did not lead to better fittings or significant correlations between the PK parameters.

Table 4 lists the estimates of the population parameters of salmeterol, their inter-individual variability (BSV%) and the RSE% estimates for each parameter. The estimated first order GI absorption rate constant was 0.33 h^{-1}, the relative fraction of dose absorbed from the GI was 0.87, the apparent clearance was equal to 392 l/h and the mean apparent inter-compartmental clearance was 340 l/h. The population values of the apparent volume of distribution of the central compartment and peripheral compartment were equal to 177 and 3160 litres, respectively. Relatively high BSV% values were observed for almost all estimated PK parameters, which ranged from 9% to 73%.

Covariates that may be important determinants of the PK model were also identified. Gender was found to be a significant covariate on *CL/F* (p = 0.016), with male patients exhibiting about 27% higher clearance values compared with females. Body weight was also found to be a significant covariate on V_p/F. The latter was implemented by using an allometric relationship between V_p/F and body weight (normalized by a fixed value of 70) and setting fixed the allometric exponent at unity. Finally, a 'treatment' effect (T or R product) was observed on V_c/F. Therefore, the model functions for the covariates are:

$$CL/F = \theta_1 \cdot \exp(-0.31) \quad (2)$$

$$V_c/F = \theta_2 \cdot \exp(-0.17) \quad (3)$$

where θ_1 refers to the typical apparent drug clearance estimate for the male subjects and θ_2 refers to the typical apparent volume of distribution of the central compartment for the reference product. The 'period' effect was not found to be a significant (p > 0.05) covariate on any PK parameter.

TABLE 4 Population pharmacokinetic parameters for the best population PK model applied to the salmeterol data

Parameter	Mean (RSE%)	BSV% (RSE%)
K_a (h^{-1})	0.33 (6)	43.69 (11)
R_{GI}	0.87 (1)	9.32 (11)
CL/F (l/h)	392 (10)	43.69 (12)
V_c/F (l)	177 (11)	73.19 (12)
V_p/F (l)	3160 (9)	60.69 (12)
Q/F (l/h)	340 (9)	63.88 (12)
Covariate effects		
Gender on CL/F[a]	−0.31 (41) (p = 0.016)	-
Treatment on V_c/F[b]	−0.17 (40) (p = 0.013)	-
Allometric exponent on V_p/F[c]	1	-
Residual error model		
b	0.16 (1)	-

[a]Male was considered as the 'control' group.

[b]The reference product was considered as the 'control' group.

[c]Allometric scaling exponent fixed at value '1'.

K_a, first order absorption rate constant from the gastrointestinal tract (h^{-1}); R_{GI}, relative fraction of dose undergoing swallowing and being deposited in the gastrointestinal tract; F, fraction of bioavailable dose; V_c/F, apparent volume of drug distribution (l) of the central compartment; V_p/F, apparent volume of drug distribution (l) of the peripheral compartment; Q/F, apparent inter-compartmental clearance of the drug (l/h); CL/F, apparent clearance from the central compartment (l/h); b, residual error parameter for the proportional error model (Equation 1); RSE%, relative standard error of the calculation of the population pharmacokinetic estimate; BSV%, between subject variability.

Goodness-of-fit plots for the final model are depicted in Figures 3–5. Figure 3 illustrates the individual predicted SAL concentrations vs their observed concentration values for the final population PK model. As shown in Figure 3, there is a reasonable agreement between the predicted and observed concentrations. The overall distribution of points around the line of unity looks random and roughly symmetric. This is also supported by the balanced distribution around the zero line in the individual weighted residuals (IWRES) and normalized prediction distribution error (NPDE) vs predicted concentration plots in Figure 4. Finally, the VPC plot is presented in Figure 5. The observed concentrations seem to be reproduced adequately by the model, indicating that the utilized structural/error models are appropriate for describing the plasma C–t profile of salmeterol.

4 | DISCUSSION

It can generally be considered that the airway disease state of asthma patients may alter the pulmonary disposition and absorption of salmeterol, similarly to that observed with other β_2-agonists (Lipworth & Clark, 1997; Vaisman et al., 1987). However, relatively limited data have been published on the PK behavior of salmeterol. Thus, the aim of this study was to investigate the salmeterol pharmacokinetics in patients with controlled or partly controlled asthma after inhalation by two different dry powder devices.

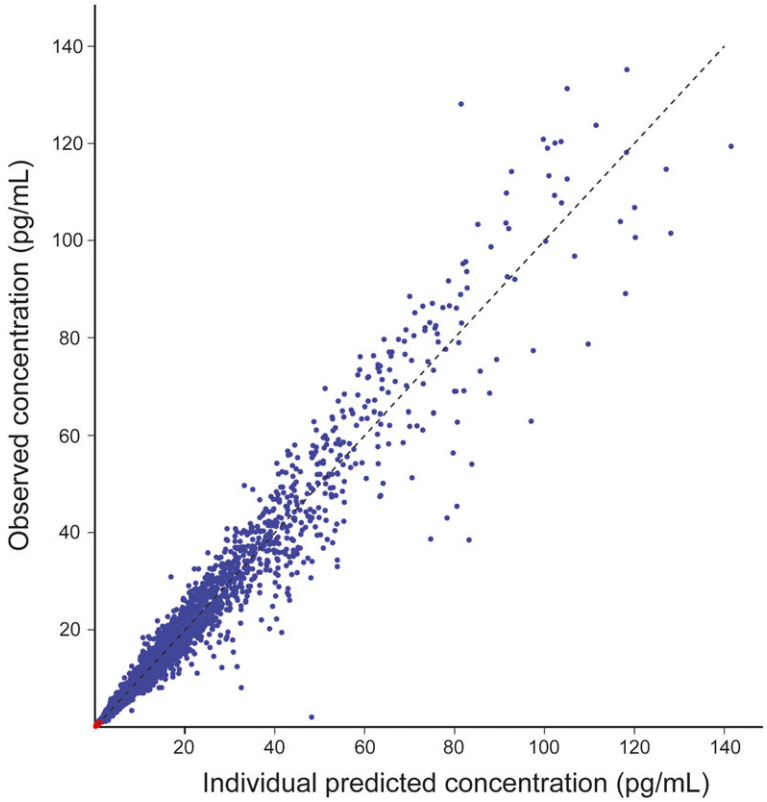

FIGURE 3 Individual predicted salmeterol concentrations from the population pharmacokinetic model vs the observed salmeterol plasma concentration values. The diagonal dashed line represents the line of unity, namely, the ideal situation. Points in red color refer to the missing data due to censoring

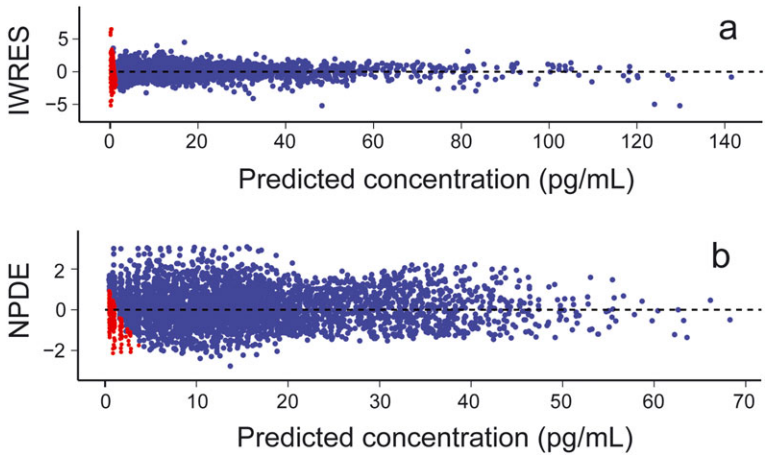

FIGURE 4 Graphical representation of: (a) the individual weighted residuals (IWRES) vs. the individual predicted concentrations (IPRED) and (b) the normalized prediction distribution error (NPDE) vs. the individual predicted concentrations (IPRED) for the final model of salmeterol. Points in red color refer to the missing data due to censoring

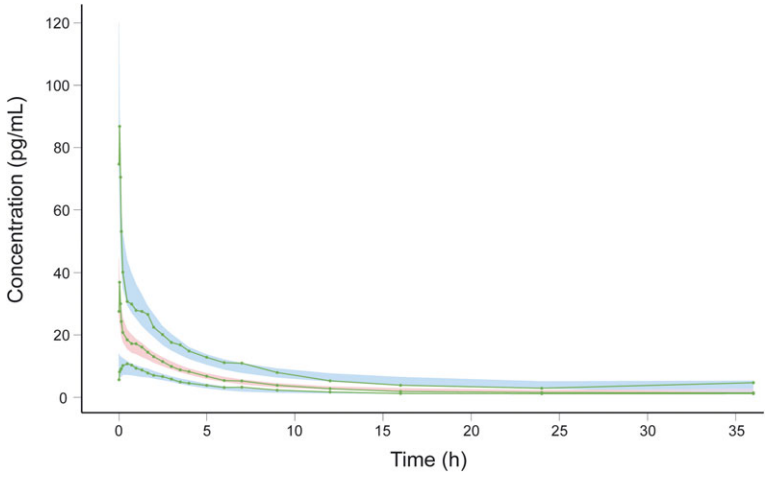

FIGURE 5 Visual predictive check of the final model for salmeterol. Solid lines refer to the 10th, 50th, and 90th percentiles of the empirical data; shaded areas refer to the 95% prediction intervals around each theoretical percentile

4.1 | Non-compartmental pharmacokinetic analysis

In the first part of our study, the C–t data obtained from the 45 patients, completing all four periods of the bioequivalence study, were analysed using the classic non-compartmental PK methodology. The blood sampling scheme was considered appropriate to adequately characterize salmeterol pharmacokinetics after a single inhaled administration. Another important point is that highly variable C–t profiles were observed among the 45 subjects (Figure 1). Likely contributors to this variation are the inclusion of asthma patients with varying degrees of symptom severity, the high variability associated with patients' inhalation and the inadequate understanding of device-administration interactions (Smaldone, 2005). In addition, other factors that contribute to this high variability may refer to subjects' pharmacokinetics, namely differences in absorption, distribution and elimination processes. Possible differences in demographic characteristics, such as gender, age and body weight among the treated population may also be considered as additional sources of variation. Despite this high variability, similar PK parameters (C_{max}, AUC_t, AUC_{inf}, T_{max}, λ_z) were obtained for the two products under evaluation (Table 1). The estimated PK parameters were generally in agreement with previously reported values (Cazzola et al., 2002; Kempsford et al., 2005), as well as with the results from our previous PK study in healthy volunteers (Soulele et al., 2015).

The results of the bioequivalence assessment for the two inhalers (T, R) are listed in Table 2. Based on these results, it can be concluded that the two products are bioequivalent in terms of the extent and rate of absorption; this finding permits one to conclude that the two dry powder inhalers lead to comparable total systemic drug exposure. For both PK parameters (AUC_t and C_{max}), the 90% CIs lie within the acceptance interval of 80–125% (EMA CHMP, 2010). To this point, it should be mentioned that no universal guidance exists for the establishment of bioequivalence of locally acting inhaled drugs (Apiou-Sbirlea et al., 2013). The EMA currently suggests a stepwise evaluation of *in vitro* and *in vivo* pharmacokinetic and pharmacodynamics studies, while the US-FDA endorses an 'aggregate weight of evidence' approach for establishing the bioequivalence of inhalation drugs (Apiou-Sbirlea et al., 2013; Lu et al., 2015). Even though the performance of a bioequivalence study is not always considered sufficient to establish therapeutic equivalence between two locally acting orally inhaled drugs (Lu et al., 2015), and certain critical issues when conducting such studies exist (Thakkar, Mhatre, Jadhav, Goswami, & Shah, 2015), PK studies are still considered the most sensitive methodology in detecting differences between two inhalation drug products (Hochhaus, Horhota, Hendeles, Suarez, & Rebello, 2015).

4.2 | Population pharmacokinetic analysis

In the second part of the study, the same C–t data were analysed in terms of population PK methodology. Our aim was not limited to providing a description of the subjects' C–t profiles, but also to provide an in-depth analysis of salmeterol kinetics. In this vein, the argument made in this work aims at: (a) discussing the structural model, (b) unveiling the complex mechanisms of lung absorption, as well as the parallel absorption from the GI, (c) highlighting the relative fraction of dose absorbed either through the lungs or the GI tract, (d) commenting on the large salmeterol volume of distribution, (e) justifying the existence of possible covariates, and finally (f) commenting on the evaluation of the intermediate and final models.

4.2.1 | On the structural model

Data from the 45 subjects and the four periods of the study for both products (T and R) were pooled together, producing a dataset of 180 C–t profiles for the analysis. Several combinations were examined, including a variety of structural and error models, different absorption kinetics and initial estimates. Some indicative models are listed in Table 3. Finally, a two-compartment disposition model with first order absorption from the GI and very rapid absorption (like an i.v. bolus) from the lungs was found to describe successfully the plasma salmeterol C–t data in asthma patients, with elimination from the central compartment following first order kinetics (Figure 2). The estimates of the population PK parameters, their BSV% values, along with their RSE% estimates are listed in Table 4. It should be mentioned that a two-compartment disposition model was also developed for salmeterol in our previous PK study, where healthy subjects received a single dose of inhaled salmeterol in the presence of activated charcoal (Soulele et al., 2015). Besides, similar two-compartment PK models have been also described for the disposition kinetics of other β_2-agonists, including formoterol (Derks et al., 1997), albuterol (Anderson et al., 1998; Maier et al., 2007), batefenterol (Ambery et al., 2015) and vilanterol (Goyal et al., 2014).

4.2.2 | Parallel absorption – Complex mechanisms

In our study, following a single inhaled administration of salmeterol, the plasma C–t profiles showed a two-peak pattern with a very short-lived, high peak concentration within the first 10 min after inhalation and a lower second peak concentration, occurring at 30–90 min. It is generally considered that the fraction of dose reaching the airways is absorbed systemically in the same way as an intravenous dose, while the swallowed fraction of an inhaled SAL xinafoate dose is absorbed similarly to an oral formulation (Cazzola et al., 2002; Harrison, Novak, Needham, & Ratner, 2011). The very rapid absorption from the respiratory system is in accordance with previous findings where absorption can only occur through the lungs (Anderson et al., 1998; Grekas, Athanassiou, Papataxiarchou, Savu, & Silvestro, 2014; Soulele et al., 2015). The second concentration peak observed in the later phase indicates a slower absorption process, which can be mainly attributed to the swallowed portion of drug finally absorbed from the GI. The assumption that the second concentration peak primarily characterizes gastrointestinal absorption of SAL xinafoate is further supported by findings in PK studies of other inhaled β_2-agonists (Derks et al., 1997; Dhand et al., 1999).

Besides, it has been suggested that for lipophilic substances, such as salmeterol, delayed pulmonary dissolution may lead to a prolonged pulmonary absorption and also contribute to the later absorption phase observed for salmeterol (Horhota et al., 2015; Weber & Hochhaus, 2015). Likewise, the presence of flip-flop kinetics in the

later absorption phase and a possible mismatching of the estimated oral absorption rate constant with the disposition parameters cannot be excluded as well. The so-called, in this study, oral absorption rate constant, is actually a hybrid parameter expressing both slow dissolution of the remaining drug in the lungs and oral absorption from the GI. In our analysis, the pulmonary dissolution and GI absorption were described by a single PK parameter (Krishnaswami, Hochhaus, Möllmann, Barth, & Derendorf, 2005; Liang & Derendorf, 1998). However, the delayed drug absorption from the lungs is not believed to contribute to a great extent to the second absorption phase of salmeterol. This is further supported by the absence of a second peak concentration in the C–t profile of salmeterol in the presence of activated charcoal in a previous study (Soulele et al., 2015).

Complex absorption kinetics for inhaled drugs, like multiple parallel pulmonary absorption processes, has been described in the literature (Bartels, Looby, Sechaud, & Kalser, 2013; Weber & Hochhaus, 2013, 2015). However, the development of these models requires the use of data derived from both intravenous and inhaled administrations, or the collection of plasma and urine samples (Bartels et al., 2013; Borghardt et al., 2016; Mobley & Hochhaus, 2001). But, in our case, based on a stand alone study with only plasma samples derived from salmeterol inhalation and no i.v. administration, it was not possible to differentiate explicitly between the portion of drug absorbed through the lungs or the GI tract. It is, therefore, acknowledged that the estimated absorption PK parameters (e.g. K_a and R_{GI}) approximate the underlying more complex drug absorption kinetics. In fact, the actual phenomenon is rather complicated since for example: (a) not all the amount of salmeterol present in the GI enters the systemic circulation due to first-pass effect and (b) a part of the inhaled drug is eliminated by the lungs by mucociliary clearance and eventually is swallowed and deposited again in the GI tract. Irrespective of the complexity of the absorption process, the structural model should be able to describe the two parallel pulmonary and gastrointestinal absorption processes.

4.2.3 | Relative absorption from lungs and gastrointestinal system

It is evident that the systemic drug levels of salmeterol result from the absorption from both the respiratory epithelium and the GI tract, with a variable contribution of each absorption site among the patients. The estimated R_{GI} value was equal to 87%, which implies that the remaining fraction of the administered dose absorbed via the lungs was around 13%. This low R_L value implies that most of the inhaled drug does not undergo pulmonary absorption, but is deposited (either after swallowing or from the mucociliary clearance) in the GI tract and can enter the general circulation. This finding is in line with other literature studies which showed that, even with an optimal inhalation condition, most of the drug (60–90%) is impacting the oropharynx and the upper airways and is subsequently swallowed, with a much smaller fraction (10–20%) reaching the lungs (Cazzola et al., 2002; Lipworth, 1996). For example, following inhalation of salbutamol, another β_2-adrenergic agonist, most of the dose (60–80%) was found to be delivered to the oropharynx and hence to the gut after swallowing, whereas not more than 20% of the dose was deposited in the lungs (Newnham, McDevitt, & Lipworth, 1993). It has been also reported that the mean total lung deposition of radiolabelled terbutaline in ten asthmatics, using a dry powder inhaler, was in the range 9.1%–16.8% of the inhaled dose (Newman, Moren, Trofast, Talaee, & Clarke, 1991), while similar results have been reported for salbutamol, using another dry powder inhaler device, with a range of 11.7%–16.1% of the inhaled dose deposited in the lungs (Pitcairn, Lunghetti, Ventura, & Newman, 1994). An important determinant for the relative absorption from the lungs or the GI tract following inhaled administration is the drug particle size, with the reduction of particle size within an optimal size range (0.5–5 μm) leading to increased pulmonary deposition (Labiris & Dolovich, 2003; Mobley & Hochhaus, 2001; Tena & Clarà, 2012). The lung bioavailability of an inhaled drug is also dependent on asthma severity and the associated airway calibre of asthma patients (Lipworth & Clark, 1997). The mouth and throat deposition is highly individual in such cases and in some subpopulations of asthma patients can be extremely high (Svartengren et al., 1994). In line with the above reports, the estimated R_{GI} value of 87% in our study suggests a high gut deposition of inhaled salmeterol xinafoate in asthma patients.

4.2.4 | Volume of distribution

The apparent volume of distribution of salmeterol was large for both the central (117 litres) and the peripheral (3160 litres) compartment (Table 4). After absorption from the lungs, it is likely that salmeterol rapidly distributes into tissues and membranes due to its high lipophilicity (Kirby et al., 2001). In a disposition study in laboratory animals, radioactive salmeterol was widely distributed throughout the body tissues in rats and dogs following intravenous and oral administration (Manchee et al., 1993). In that study, the salmeterol volume of distribution was significantly greater than the total body water in both species and indicated high tissue uptake of the drug. Furthermore, even though a direct comparison of the PK behavior of salmeterol between asthma patients and healthy volunteers cannot easily be performed, this study confirms the extensive distribution of salmeterol into tissues observed in healthy volunteers (Soulele et al., 2015). Finally, CL/F and Q/F were also high for salmeterol. Similar values for both parameters have also been reported for another inhaled β_2-agonist, (R)-albuterol (Maier et al., 2007).

4.2.5 | The role of covariates

In order to explain the observed inter-individual variability, covariate effects such as subject demographics, the administered medicinal product ('treatment') and the occasion ('period') effects were tested for their impact on the model parameters. A gender effect was found on CL/F (Table 4). Males were found to exhibit about 27% higher clearance values compared with female subjects. This difference is also depicted when comparing the three main PK parameters (C_{max}, AUC_t, AUC_{inf}) between male and female subjects (Table A1 in the Appendix). Comparison of these parameters shows that male subjects have about a 30–35% lower C_{max}, AUC_t and AUC_{inf} values compared with the females. The gender effect on clearance might be attributed to the higher enzymatic capacity of men to metabolize salmeterol, as well as possible differences in lung deposition between males and females

(Cazzola et al., 2002). A similar gender effect on salmeterol clearance has been also observed in healthy volunteers, where women exerted a lower capability to eliminate salmeterol than men by 21% (Soulele et al., 2015). Body weight, in terms of an allometric relationship, was also found to significantly influence the volume of distribution of the peripheral compartment (Table 4). It appears that salmeterol, due to its high lipophilicity, distributes to the extravascular space of body, and this distribution can be further facilitated with an increased body weight. Significant effects of gender and body weight on disposition parameters, CL/F and apparent volumes of distribution (of the central and two peripheral compartments), have been also reported for a new long acting β_2-agonist, PF-00610355 (Diderichsen et al., 2013).

It should be mentioned that in the final model, the 'period' effect was not found to be a significant ($p > 0.05$) covariate on any PK parameter. The 'treatment' effect (i.e. T or R) was found to be significant only in the case of V_c/F; however, no physiological meaning can be ascribed to this finding, since drug formulation cannot be related to the volume of distribution. This absence of correlation between the administered products and the PK parameters is in accordance with the obtained bioequivalence results, which suggest that administration of the two inhaled products will result in similar PK behavior for salmeterol.

To this point, it should be stated that, since the original purpose of the clinical study was the investigation of the bioequivalence between two medicinal products, a relatively homogenous sample of subjects, in terms of demographic characteristics, was used in the study and were further included in our population PK analysis. In any case, the present analysis was still capable of identifying the role of body weight and gender on salmeterol pharmacokinetics. An increased sample size and a more heterogeneous group of subjects could provide better information for the potential covariate effects. In addition, other physiological or physical factors, such as lung function parameters, drug particle size distribution and patient status, could potentially explain part of the remaining variability in the estimated PK parameters, however, no such kind of information were available in the current analysis.

4.2.6 | Model evaluation

The evaluation of the intermediate and final population PK models was made using several principles such as goodness-of-fit criteria, visual inspections of plots and the physiological soundness of the PK values. Some representative goodness-of-fit plots of the final PK model are shown in Figures 3–5. In Figure 3, the individual predicted salmeterol concentration values were compared directly with the observed data. The distribution of points around the line of identity appears to be random and roughly symmetric, which implies a good agreement between the observed and the model predicted drug plasma concentrations. The two diagnostic graphs presented in Figure 4 further support this finding: the individual weighted residuals (IWRES) vs the individual predicted (IPRED) concentrations (Figure 4a) and the NPDE vs the IPRED concentrations (Figure 4b). In both cases, no actual trend was observed and the data (either IWRES or NPDE) were almost symmetrically distributed around zero. Finally, the goodness-of-fit of the final model was evaluated by a visual predictive check (VPC) plot (Figure 5). The VPC was performed on the basis of 500 model-based simulations in order to evaluate the model's performance. Visual inspection of the VPC plot reveals that most of the time the three empirical percentiles (median, 10% and 90%) were within the relevant confidence intervals of the simulated percentiles, despite the large variability of the data (Tables 1 and 4). We cannot also disregard the fact that a number of 180 observations correspond to each time-point, while the C–t data used in the analysis derived from a patient population with varying degrees of asthma severity. Overall, the obtained plots indicate that the developed model allows for an adequate description of the pharmacokinetics of salmeterol following administration via inhalation.

5 | CONCLUSION

The aim of this study was to investigate the pharmacokinetics of inhaled salmeterol in asthma patients using two different dry powder inhalers. A classic non-compartmental and a population PK modeling approach were applied to a set of C–t data of 45 patients participating in a 2 × 4 bioequivalence study. The population PK analysis led to the same finding with regard to equivalence of the PK parameters of the two inhalation devices. The plasma C–t profiles generally showed a two-peak pattern with a very early C_{max}, which is followed by a lower second peak. For this reason, the salmeterol C–t data were modeled assuming parallel lung absorption (very rapid like an i.v. bolus) and a slower first order absorption. The population PK analysis showed that a two-compartment PK model, with parallel (GI and lung) absorption describes successfully the C–t profile of salmeterol in asthma patients. Elimination was considered to take place in the central compartment following first order kinetics. The relative amount of dose absorbed via the lungs was around 13%, which indicates that most of the drug is swallowed and deposited in the GI. Women were found to exert less capability to eliminate salmeterol than men, while body weight was found to be an important covariate for the volume of distribution of the peripheral compartment. A proportional residual error model led to the optimum performance.

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CONFLICT OF INTEREST

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. K. Soulele, P. Macheras and V. Karalis declare that they have no conflict of interest.

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APPENDIX

TABLE A1 Mean values of the main pharmacokinetic (PK) parameters for the plasma concentration–time data of inhaled salmeterol between male and female subjects

Pharmacokinetic parameter[a]	Male subjects (n = 20) [b]	Female subjects (n = 28)	% difference [c]
C_{max} (pg/ml)	38.69	54.04	33.11
AUC_t (pg/ml/h)	111.35	160.07	35.90
AUC_{inf} (pg/ml/h)	131.77	179.67	30.76
		Overall	33.26

[a]AUC_t, area under the concentration–time curve from time zero to the last quantifiable sample; C_{max}, the first recorded maximum plasma concentration value; AUC_{inf}, area under the concentration–time curve from time zero extrapolated to infinity; mean values of the pharmacokinetic parameters are used.

[b]The term 'n' refers to the number of subjects (either male or female).

[c]Difference values for each parameter derive from the equation: % Difference = [(Females – Males)/(Females + Males)/2] × 100.