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Population pharmacokinetics of fluticasone propionate/salmeterol using two different dry powder inhalers

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ABSTRACT

The combination of fluticasone propionate (FLP) and salmeterol (SAL) is often used in clinical practice for the treatment of pulmonary disorders. The purpose of this study was to explore the pharmacokinetics (PK) of inhaled FLP and SAL, after concomitant administration, in healthy male and female subjects using two dry powder inhalers. Plasma concentration (C)–time (t) data were obtained from a single dose, two-sequence, two-period, crossover (2 × 2) bioequivalence (BE) study. Activated charcoal was co-administered in order to prohibit absorption from the gastrointestinal tract. A number of 60 subjects were recruited, while 57 of them completed the study and were included in the PK analysis. Initially, PK parameters of FLP and SAL were estimated using the classic non-compartmental methods. Subsequently, BE assessment was applied to the estimated PK parameters of the two dry powder inhalers. Special focus was placed on the population PK analysis of the C–t data, which were pooled together. 'Treatment' (i.e., test or reference) and 'period' of the BE study were considered as covariates. A variety of structural and residual error models were tested to find the one which best described the plasma C–t data of FLP and SAL. Demographic data were also evaluated for their impact on the PK parameters. Several goodness-of-fit criteria were utilized. The non-compartmental PK estimates of this study were in agreement with previously reported values. The population PK analysis showed that FLP data were described by a two-compartment model with first-order absorption and elimination kinetics. Body weight was found to affect significantly absorption rate constant, inter-compartmental clearance, and volume of distribution of the peripheral compartment. As body weight increases, the values of these PK parameters also rise. For SAL, the best results were obtained when a two-compartment disposition model was used assuming very rapid absorption kinetics (like intravenous bolus) and first-order elimination kinetics. Gender was found to be a significant covariate on clearance, with men exhibiting higher clearance than women.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a debilitating chronic lung disorder with a wide prevalence and considerable morbidity worldwide. COPD is characterized by a progressive airflow limitation, which in some cases is not fully reversible (Csikesz and Gartman, 2014; Goldcopd, 2014; Hassett et al., 2014). The management of COPD focuses primarily on reducing exposure to risk factors, alleviating respiratory symptoms, preventing exacerbations, and treating COPD-related co-morbidities. First-line pharmacological treatment of COPD mainly consists of bronchodilators, such as β_2-adrenergic receptor agonists, corticosteroids and long acting anticholinergics, administered systemically or via inhalation (Calverley et al., 2007; Falk et al., 2008; Hassett et al., 2014).

In this context, oral powder inhalers are considered very attractive drug delivery systems for the treatment of COPD, since they provide three major clinical benefits: a) minimization of systemic adverse effects, due to the reduced systemic exposure, b) rapid onset of action, and c) reduction of the administered dose (Frijlink and De Boer, 2004;

Abbreviations: 90% CI, 90% confidence interval; ANOVA, analysis of variance; AUCinf, area under the C–t curve from time zero extrapolated to infinity (pg/mL/h); AUCt, area under the concentration–time curve from time zero to the last sampling point or the last measurable concentration, whichever occurs earlier (pg/mL/h); BE, bioequivalence; BMI, body mass index (kg/m^2); BSV%, between subject variability (in %); BW, body weight (kg); C, concentration (pg/mL); CL, clearance of the drug (L/h); Clast, last quantifiable concentration (pg/mL); Cmax, the first recorded maximum plasma concentration value (pg/mL); COPD, chronic obstructive pulmonary disease; CV%, percent coefficient of variation; C–t, concentration–time; EM, expectation-maximization algorithm; F, fraction of bioavailable dose; FLP, fluticasone propionate; GMR, geometric mean ratio; IPRED, individual predicted concentrations; IWRES, individual weighted residuals; Ka, first order absorption rate constant (h^{-1}); λz, apparent terminal rate constant (h^{-1}); min, minutes; PK, pharmacokinetic; Q, intercompartmental clearance (L/h); R, reference product (Diskus® 500/50 μg/inhalation, GSK); RSE%, relative standard error (in %); SAEM, stochastic approximation expectation maximization; SAL, salmeterol; SD, standard deviation; t, time (min or h); T, test product (Elpenhaler® 500/50 μg/inhalation, ELPEN); Tmax, the time (h) at which Cmax occurs; VPC, visual predictive checks; V_1/F, apparent volume of drug distribution of the central compartment (L); V_2/F, apparent volume of drug distribution of the peripheral compartment (L).

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Olsson et al., 2011). The co-administration of inhaled corticosteroids with long-acting β_2-agonists is usually preferred in the treatment of patients with severe COPD, since they combine both anti-inflammatory and bronchodilator activity (Chrystyn, 2007; Kirby et al., 2001; Labiris and Dolovich, 2003). Besides, any adverse events that may occur are usually pharmacologically predictable and dose-dependent. One such combination is the fluticasone propionate (FLP)/salmeterol (SAL) dry powder inhaler (Fenton and Keating, 2004; Jenkins et al., 2009; Shapiro et al., 2000). FLP is a potent inhaled corticosteroid with an important anti-inflammatory activity (Crim et al., 2001), while SAL is an effective long-acting bronchodilator that acts locally in the lung (Fenton and Keating, 2004; Verberne and Fuller, 1998). It has been shown that for an optimal pharmacodynamic interaction, these two drugs should reach the target cells together and in adequate concentrations (Nelson et al., 2003).

Due to the combined action, knowledge of the pharmacokinetic (PK) properties following co-administration of FLP and SAL is highly important. It should be noted that the pharmacokinetic behavior of inhaled drugs is much more complicated compared to more conventional ways of administration (Cazzola et al., 2002; Crim et al., 2001; Möllmann et al., 1998; Reynolds et al., 2005; Singh et al., 2003; Weber and Hochhaus, 2013). In addition, the very low systemic drug levels reached following inhalation of therapeutic doses require analytical methods with high sensitivity and specificity (Callejas et al., 1998; Krishnaswami et al., 2000). Previous studies have shown that after inhalation the absolute bioavailability of FLP is 10–30%, while oral bioavailability of inhaled FLP is negligible (less than 1%) due to both low absorption from the gastrointestinal tract and high hepatic first-pass metabolism (Möllmann et al., 1998; Reynolds et al., 2005; Singh et al., 2003). Similarly, following administration of SAL via inhalation, plasma concentrations of the drug are very low or even undetectable (Cazzola et al., 2002; Nelson et al., 2003). The pharmacokinetics of SAL and FLP, when administered concomitantly through the same inhaler, are very similar to those of the two agents when administered separately and no pharmacokinetic interaction between the two agents occurs (Kirby et al., 2001; Reynolds et al., 2005).

The purpose of this study was to explore the pharmacokinetics of inhaled FLP and SAL after concomitant administration in healthy male and female subjects. The concentration–time (C–t) data were obtained from a bioequivalence (BE) study on two dry powder inhalers. Initially, a conventional non-compartmental methodology was applied. Furthermore, population PK analysis was applied to FLP and SAL. In this context, several structural and residual error models were tested to find the one that described best the plasma C–t data of FLP and SAL. Also, the population PK analysis examined various subject demographic characteristics to elucidate the variability of the PK parameters.

2. Materials and Methods

2.1. Study Design and Volunteers

Plasma C–t data were obtained from a single dose, two-sequence, two-period, crossover 2 × 2 BE study using two dry powder inhalers: the traditional multi-dose (fluticasone/salmeterol via Diskus® 500/50 μg/inhalation, GSK) and a novel single-dose device (fluticasone/salmeterol via Elpenhaler® 500/50 μg/inhalation, ELPEN) under fasting conditions. Both devices are currently commercially available in various European countries. A wash-out period of 8 days was set between each treatment to allow for the complete removal of the drug from the body.

The study was performed in compliance with ICH E6 Good Clinical Practice Consolidated Guidance, by 3S-Pharmacological Consultation & Res. Srl in Romania. Sixty healthy male and female subjects were enrolled in the study. All subjects were informed about the purpose, protocol, and risks of the study and a written consent form was provided by each participant before entering the study. The subjects aged between 18 and 45 years and met the inclusion criteria, such as body mass index (BMI) within 19–29 kg/m^2, good general health, no clinically significant or relevant abnormalities of medical history, normal physical examination or laboratory values, non-smokers and non-lactating women. The main exclusion criteria included intolerance or hypersensitivity to the study drugs, hospitalization or donation of ≥450 mL of blood within two months prior to study initiation, intake of any medication two weeks prior to dosing, history of bronchial asthma or other bronchospastic conditions, positive AIDS or hepatitis B/C tests results, etc. Vital signs, measured before and after the study drugs administration in each study period, were analyzed and all reported adverse effects were recorded. Finally, 57 subjects completed the study and further analyzed. The three subjects who were considered as drop-outs referred to either positive pregnancy or alcohol test results.

On the treatment days, after at least 10 h of fasting, each subject received either one dose of Elpenhaler® 500/50 μg/inhalation (test formulation, T) or one dose of Diskus® 500/50 μg/inhalation (reference formulation, R), according to the randomization plan.

Activated charcoal was co-administered at specified time points in order to prevent any absorption from the gastrointestinal tract. More specifically, it was administered 2 min pre-dosing and at 2, 60, 120 and 180 min post-dose. Blood samples (of 5 mL each) were collected before drug administration (time 0) and at 10, 20, 30, 45 min and 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, 48, and 72 h post-dose. After the eight days of the washout period, the subjects received the alternate formulation, and blood samples were again drawn and analyzed using the same procedures.

2.2. Assay Methodology

The identification and quantification of FLP and SAL in plasma were performed by validated Liquid Chromatography/Mass Spectrometry (LC–MS/MS) methods, showing adequate sensitivity, precision, accuracy, specificity, and linearity. The lower limits of quantification were 1.500 pg/mL and 2.500 pg/mL for SAL and FLP, respectively (Silvestro et al., 2012).

2.3. Pharmacokinetic Analysis

2.3.1. Non-compartmental Pharmacokinetic Analysis

Initially, the PK parameters of FLP and SAL were evaluated using non-compartmental methods (WinNonlin® v.5.0.1/Pharsight Corp., Menlo Park, CA). These parameters referred to the area under the concentration–time curve from time zero to the last quantifiable sample (AUCt), the area under the C–t curve from time zero extrapolated to infinity (AUCinf), the first recorded maximum plasma concentration value (Cmax), and the time at which Cmax occurs (Tmax). AUCt was calculated using the linear trapezoidal rule. Values for AUCinf, were calculated as AUCt + Clast / λz, where Clast is the last quantifiable concentration and λz refers to the apparent terminal elimination rate constant. The latter was determined by a least squares regression analysis applied to the terminal log-linear phase of the C–t curve. Descriptive statistics (arithmetic mean, standard deviation, coefficient of variation, median and range) were also calculated for these PK parameters.

The conventional non-compartmental analysis was extended by performing a BE assessment of the estimated PK parameters. For this reason, the AUCt and Cmax estimates were analyzed according to the methodology proposed by the European Medicines Agency (EMA, 2010). Data from the 57 subjects, who completed all periods of the study, were included in the statistical analysis. Bioequivalence assessment was made using the typical general linear model (analysis of variance, ANOVA). Ninety percent confidence intervals (90% CI) around the geometric mean ratio (GMR) of T over R formulation (T/R) were constructed using the residual error from ANOVA. The two inhalers were considered bioequivalent if the 90% CIs of both AUCt and Cmax were within the predetermined equivalence range of 80.00–125.00% (EMA,

2010). The entire computing work was implemented in WinNonlin® v.5.0.1 (Pharsight Corp., Menlo Park, CA).

2.3.2. Population Pharmacokinetic Analysis

The population PK analysis was performed using a non-linear mixed effects modeling approach (Sheiner and Beal, 1983; Mandema et al., 1992; Ette and Williams, 2004; Bonate, 2005). The entire computational work was implemented in Monolix® v.4.2 (Lixoft, Orsay, France).

Due to the fact that the data refer to a 2 × 2 crossover design, the C–t data of the T and R products were pooled together and the 'treatment' was set as a covariate. This procedure was followed separately for FLP and SAL. The population PK analysis was in line with other published works (Combrink et al., 1997; Dubois et al., 2012; Fradette et al., 2005; Karlsson and Sheiner, 1993; Panhard and Mentré, 2005).

Several structural models were evaluated which included one-, two-, and three-compartment models. Absorption kinetics was assumed to be either first-order or bolus since these types of kinetics had also been reported in the literature for FLP and SAL. The choice of first-order absorption kinetics was selected based on previous findings from other published studies (Rohatagi et al., 1996; Simon et al., 1998; Wu et al., 2008; Xu et al., 2010). Oral absorption rate constant was also excluded from both models due to the co-administration of activated charcoal which prohibited any systemic absorption from the gastrointestinal tract.

In all cases, elimination was considered to take place in the central compartment and follow first-order kinetics. For example, in case of the two-compartment models, the structural PK models were parameterized in terms of the absorption rate constant (Ka), apparent clearance (CL/F), apparent intercompartmental clearance (Q/F), apparent volume of drug distribution of the central (V_1/F) and the peripheral (V_2/F) compartment. In all cases, the term F refers to the bioavailable fraction of dose. The between-subject variability (BSV) in the pharmacokinetic parameters was assumed to follow log-normal distribution. The possibility of covariance between the PK parameters was also assessed and the effect of each product (test and reference) on PK parameters was evaluated through the inclusion of the 'treatment' as a covariate. Finally, several residual error models (constant, proportional, exponential, and combined) were examined to describe the unexplained variability of the structural model.

The stochastic approximation expectation maximization (SAEM) algorithm was used, which allowed the estimation of the maximum likelihood estimators of the population PK parameters (Comets et al., 2007; Delyon et al., 1999; Maltezou et al., 2012; Lavielle and Mentré, 2007; Samson et al., 2007; Savic and Lavielle, 2009; Savic et al., 2011). The stochastic approximation method of the standard expectation-maximization (EM) process relies on the fact that the usual E-step of EM is replaced by a stochastic procedure. For the purposes of the current analysis, the maximum numbers of SAEM iterations K1 and K2 did not exceed 500 and 200, respectively, whereas a simulated annealing version of SAEM was used to estimate the population parameters (i.e., the variances were constrained to decrease or increase slowly during the first iterations of SAEM). No burning iterations were tested and the number of Markov chains was set equal to unity. Finally, 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 20,000, respectively.

A step-by-step procedure was applied to find the models that best describe the available FLP and SAL plasma C–t data. All models were tested in terms of the values of the −2LL (Log-Likelihood) function, the physiological soundness of the PK estimates, the adequacy of fitting of the model predicted estimates to the actual C–t data, the BSV and residual error values, the percent relative standard error (RSE%) values of the estimates, the Akaike and Bayesian information criteria, as well as, the goodness of fit plots such as predicted–observed plots, the individual weighted residuals (IWRES) versus the individual predicted (IPRED) concentrations values, and the VPC plots (Bonate, 2005; Gabrielsson and Weiner, 2007; Post et al., 2008). In some cases, the visual comparison of the results did not make possible to identify which model leads to a better fitting to the actual C–t data. In these cases, our selection criteria were the values of the −2LL, as well as the AIC and BIC estimates. In order to guide the reader to some important results of the analysis, a relevant table with the −2LL, AIC, and BIC estimates is included.

After the appropriate structural model for each drug was identified, several covariates were tested. The covariates examined in this study referred to subject specific characteristics and in particular: gender, age, body weight (BW), height, and BMI. Each covariate was assessed either alone or in combination with other covariates. Initially, the effect of each covariate on reducing the BSV of a PK parameter was tested separately and solely for each PK parameter. This step allowed the identification of covariates which might have significant impact on the variability. After this step, a backward elimination method of the previously 'important' covariates was applied. Finally, the selection of the best model was based on the model selection criteria described above and on the significance and physiological soundness of each covariate. In all cases, the continuous covariates were examined either untransformed or centered around the 'mean' covariate value. Also, the program executions of the most important models were carried out by defining many different initial settings such as initial estimates of the PK parameters, and number of iterations.

All population analyses were applied to the entire set of data of the 57 subjects who completed the study. Missing concentration data, which were below the lower limit of quantitation, were modeled as censored data using the appropriate setting in Monolix®. In case of FLP all censored observations (i.e., up to the last sampling point 72 h) were replaced with the lower limit of quantitation value (2.5 pg/mL). For SAL, not all missing data could be treated as censored, due to computational reasons. Thus, any missing observations up to 16 h were modeled as censored and were replaced by the lower limit of quantitation value (1.5 pg/mL). It should be mentioned that other missing concentration data (i.e., not lower than the limit of quantitation) were treated with the typical methodology, namely, using the 'missing' option of the software.

3. Results

A total of 57 male and female subjects finally completed the study and were included in the PK analysis. The mean age of subjects was 30 years (18–44 years), mean height was 171 cm (154–190 cm), mean BW was 71.6 kg (50–98 kg), and mean BMI was 24.3 kg/m^2 (19.2–29 kg/m^2). Seven non-serious adverse events were recorded in the study; three of moderate and four of mild intensity. According to the clinical study report, there were no statistically significant differences in the incidence of adverse events between the T and R treatments.

3.1. Non-compartmental Pharmacokinetic Analysis

The mean plasma C–t curves of FLP and SAL after a single inhaled dose of the T and R formulations are shown in Fig. 1A and B, respectively. The mean PK parameters (i.e., AUCt, AUCinf, Cmax, Tmax, and λz) accompanied by their statistical descriptive criteria (mean, standard deviation (SD), percent coefficient of variation (CV%), median, minimum, and maximum) are summarized in Table 1 for FLP and Table 2 for SAL. For FLP, the peak concentration was 82.62 pg/mL for the T and 88.36 pg/mL for the R product. In the case of SAL, the Cmax values were 50.38 pg/mL and 47.17 pg/mL, for the T and R inhalers, respectively. Also, comparable values between the two tested formulations were obtained for AUCt for both FLP (T: 801.29 pg/mL/h vs. R: 785.10 pg/mL/h) and SAL (T: 79.36 pg/mL/h vs. R: 78.37 pg/mL/h). Besides, the derived CV% values were between 40 and 60% for almost all PK parameters for the two dry powder inhalers and active substances. In addition, both products exhibited similar mean terminal

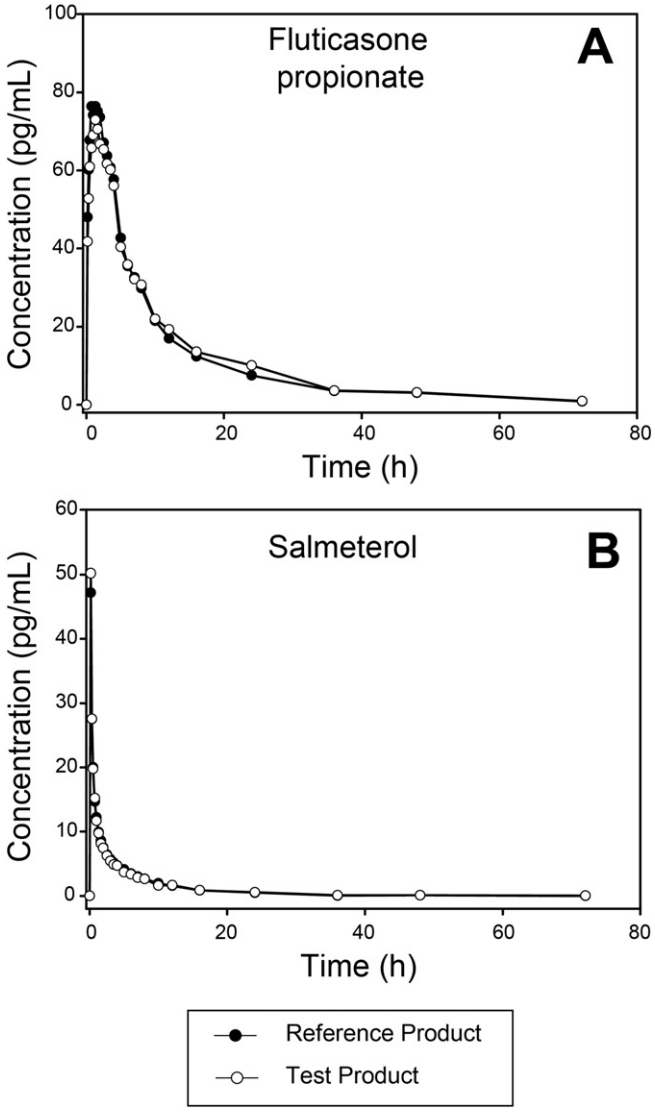

Fig. 1. Mean plasma concentration–time profiles of fluticasone propionate (A) and salmeterol (B) for the test and reference dry powder inhalers.

slope values for FLP (T: $\lambda z = 0.064\ h^{-1}$ vs. R: $\lambda z = 0.075\ h^{-1}$) and SAL (T: $\lambda z = 0.122\ h^{-1}$ vs. R: $\lambda z = 0.121\ h^{-1}$) (Tables 1 and 2).

The PK parameters for FLP and SAL were further analyzed following the BE assessment procedure of the EMA (EMA, 2010). The results are listed in Table 3. In case of FLP, the percent GMR of AUCt was 107.3%, while the 90% CI ranged from 96.22% to 119.66%. For SAL, the relevant estimates were 100.9% and 88.43–115.14%. The estimated statistical power of the study was found to be 95.73% and 87.37%, for FLP and SAL, respectively. The coefficient of variation of the within-subject variability was 36% for FLP and 44% for SAL.

Table 3 also quotes the BE results for Cmax. For FLP, the GMR value was 92.84% (90% CI: 85.13–101.25%), whereas for SAL the GMR was found equal to 110.51% (90% CI: 99.74–122.44%). The statistical power values were 99.45% for FLP and 97.3% in case of SAL. Finally, the coefficients of variation values of the within-subject variability were 28% for FLP and 34% for SAL.

3.2. Population Pharmacokinetic Analysis

A plethora of program executions took place in order to examine as many as possible combinations of conditions at each step of analysis. Many different models and scenarios were tested even starting from poor initial PK parameter estimates. Apart from the visual inspection of the individual C–t plots for FLP and SAL, the selection of the final model was based on the criteria described in the 'Materials and methods' section (Bonate, 2005; Gabrielsson and Weiner, 2007). Obviously, not all results can be presented, and for this reason some representative model program executions along with the corresponding estimates of −2LL, AIC, and BIC are listed in Table 4. Using all goodness-of-fit information and a step-by-step methodology, we finally ended up with the models which best described the available C–t data of FLP and SAL.

In case of FLP, the structural model that was best fitted to the C–t data was a two-compartment model with first order absorption and elimination kinetics (model number '6' in Table 4). The residual error model that led to the optimum performance was a combined (i.e., additive & proportional) model:

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

where C_{ij} is the jth observed concentration (of either FLP or SAL) for the ith individual, a and b are the parameters of the residual error model,

Table 1
Pharmacokinetic (PK) parameters and statistical descriptive criteria for the plasma concentration–time data of inhaled fluticasone propionate (test and reference products).

PK parameter[a]	Mean	SD[b]	CV%[c]	Median	Min	Max
Test						
AUCt (pg/mL/h)	801.293	391.837	48.901	712.057	319.698	2215.581
Cmax (pg/mL)	82.616	32.611	39.473	77.112	28.901	162.751
AUCinf (pg/mL/h)	917.493	426.157	46.448	779.859	332.714	2287.245
Tmax (h)	1.417	–	–	1.333	0.500	4.000
λz (h^{-1})	0.064	0.037	57.860	0.057	0.014	0.249
Reference						
AUCt (pg/mL)	785.100	522.574	66.561	681.769	99.371	3596.052
Cmax (pg/mL)	88.361	35.211	39.849	83.395	35.740	226.911
AUCinf (pg/mL/h)	863.396	552.464	63.987	782.419	125.134	3879.131
Tmax (h)	1.197	–	–	1.000	0.167	3.500
λz (h^{-1})	0.075	0.044	58.024	0.061	0.028	0.260

[a] AUCt: area under the concentration–time curve from time zero to the last quantifiable sample; Cmax: the first recorded maximum plasma concentration value; AUCinf: area under the concentration–time curve from time zero extrapolated to infinity; Tmax: the time at which Cmax occurs; λz: apparent terminal elimination rate constant.
[b] Standard deviation.
[c] Coefficient of variation in %.

Table 2
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						
AUCt (pg/mL/h)	79.358	40.066	50.488	75.773	7.803	226.869
Cmax (pg/mL)	50.377	20.491	40.676	47.258	16.862	104.599
AUCinf (pg/mL/h)	105.431	59.184	56.136	94.116	9.725	356.014
Tmax (h)	0.177	–	–	0.167	0.167	0.750
λz (h^{-1})	0.122	0.124	100.996	0.101	0.015	0.923
Reference						
AUCt (pg/mL)	78.368	37.512	47.867	66.782	9.213	172.378
Cmax (pg/mL)	47.171	24.061	51.009	40.616	9.299	147.611
AUCinf (pg/mL/h)	102.130	51.088	50.023	83.048	12.527	236.574
Tmax (h)	0.167	–	–	0.167	0.167	0.167
λz (h^{-1})	0.121	0.092	76.250	0.110	0.021	0.590

[a] AUCt: area under the concentration–time curve from time zero to the last quantifiable sample; Cmax: the first recorded maximum plasma concentration value; AUCinf: area under the concentration–time curve from time zero extrapolated to infinity; Tmax: the time at which Cmax occurs; λz: apparent terminal elimination rate constant.
[b] Standard deviation.
[c] Coefficient of variation in %.

Table 3
Bioequivalence results for the fluticasone propionate and salmeterol study.

Pharmacokinetic parameters	GMR (%)[a]	Lower 90% CI[b]	Upper 90% CI[b]	Statistical power (%)[c]	Residual CV%[d]
Fluticasone propionate					
AUCt (pg/mL/h)	107.3	96.22	119.66	95.73	36%
Cmax (pg/mL)	92.84	85.13	101.25	99.45	28%
Salmeterol					
AUCt (pg/mL/h)	100.9	88.43	115.14	87.37	44%
Cmax (pg/mL)	110.51	99.74	122.44	97.3	34%

[a] GMR refers to the geometric mean ratio of the test over reference pharmacokinetic metric.
[b] The 90% confidence interval (90% 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%, a number of 57 subjects, and a 2 × 2 clinical design.
[d] The percent values of the coefficient of variation (CV%) of the residual error.

f_{ij} is the jth model predicted value for ith subject, 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.

The estimates of the population parameters of FLP, their BSV% values, along with their RSE% estimates for each parameter are listed in Table 5. The estimated mean first order absorption rate constant for the study population was 3.87 h^{-1}, the mean apparent clearance was equal to 659 L/h and the mean apparent intercompartmental clearance equal to 259 L/h. The apparent volume of distribution of the central compartment V_1 was 5690 L and that of the peripheral compartment equal to 5550 L. The 'treatment' effect was not found to be a significant ($p > 0.05$) covariate on any PK parameter, while body weight (i.e., mass) was significant on Ka ($p = 1.4 \cdot 10^{-7}$), Q/F ($p = 0.00086$) and V_2/F ($p = 0.0048$). The model functions for the covariates are:

$$Q/F = \theta_1 \cdot \exp(0.0207 \cdot (BW - Mean\ BW)) \quad (2)$$

$$Ka = \theta_2 \cdot \exp(0.0215 \cdot (BW - Mean\ BW)) \quad (3)$$

$$V_2/F = \theta_3 \cdot \exp(0.0315 \cdot (BW - Mean\ BW)) \quad (4)$$

where the term θ_1 refers to the typical apparent intercompartmental clearance estimate for a subject with the 'mean' body weight, θ_2 reflects the typical first order absorption rate constant and θ_3 the typical apparent volume of distribution of the peripheral compartment. Eqs. (2)–(4) reveal that Ka, Q/F and V_2/F rise with the increase of BW.

The residual error parameters for the combined error model (Eq. (1)) were: $a = 1.91$ and $b = 0.117$. Finally, the BSV% estimates were found to exhibit moderate to relatively high values which ranged approximately from 21% to 46% (Table 5).

For SAL, the final best model (number '12' in Table 4) was obtained when a two-compartment disposition model was used, assuming very rapid absorption kinetics (like intravenous bolus) and first-order elimination kinetics. Similar to FLP, a combined (additive & proportional) error model was found to describe best the residual variability.

The estimated population parameters and the BSV% values along with their RSE% are quoted in Table 6. The mean apparent clearance was found to be 678 L/h, the apparent volumes of distribution were 891 L and 2570 L for the central and the peripheral compartment, respectively. The estimated apparent intercompartmental clearance was found equal to 1270 L/h. As in the case of FLP, the 'treatment' effect was not found to be significant ($p > 0.05$) for any parameter. However,

Table 4
Information criteria for the selection of the final best model for fluticasone propionate and salmeterol. In all cases, 'treatment' and 'period' were used as covariates.
Key: PK = pharmacokinetic; IV = intravenous; Ka = first order absorption rate constant (h^{-1}); F = fraction of bioavailable dose; Q/F = intercompartmental clearance of the drug (L/h); CL/F = drug clearance (L/h), V_1 = volume of drug distribution of the central compartment; V_2 = volume of drug distribution of the peripheral compartment.

Model number	Model short description	Statistic criterion[a]		
		−2LL	AIC	BIC
Fluticasone propionate				
1	1-Compartment *Covariates*: none	17,612.23	17,646.23	17,692.74
2	1-Compartment *Covariates*: on all parameters	17,553.87	17,617.87	17,705.43
3	2-Compartment *Covariates*: none	17,236.49	17,290.49	17,364.36
4[b]	2-Compartment *Covariates*: on all parameters	17,149.78	17,253.78	17,396.07
5[c]	2-Compartment *Covariates*: body weight as covariate on Ka, Q/F, CL/F, and V_2/F	17,192.94	17,254.94	17,339.76
6	2-Compartment *Covariates*: body weight as covariate on Ka, Q/F, and V_2/F	17,195.45	17,255.45	17,337.54
Salmeterol				
7	1-Compartment *Covariates*: none	11,477.23	11,501.23	11,534.07
8	1-Compartment *Covariates*: on all parameters	11,444.77	11,492.77	11,558.44
9	2-Compartment *Covariates*: none	7333.53	7377.53	7437.73
10[d]	2-Compartment *Covariates*: on all parameters	7302.92	7386.92	7501.84
11[e]	2-Compartment *Covariates*: gender on CL/F, body weight on Q/F	7327.63	7375.63	7441.30
12	2-Compartment *Covariates*: gender on CL/F	7326.46	7372.46	7435.39

[a] The terms −2LL, AIC, and BIC refer to −2 Log-Likelihood function, Akaike information criterion, and Bayesian information criterion, respectively.
[b] Only *body weight* was found to be a significant covariate for Ka, Q/F, CL/F, and V_2/F.
[c] *Body weight* was not significant for CL/F.
[d] Significant covariates were *gender* on CL/F and *body weight* on Q/F.
[e] *Body weight* was not found to be significant.

Table 5
Fluticasone propionate population pharmacokinetic parameters for the final best model. Key: Ka = first order absorption rate constant (h^{-1}); F = fraction of bioavailable dose; V_1/F = apparent volume of drug distribution (L) of the central compartment; V_2/F = apparent volume of drug distribution (L) of the peripheral compartment; Q/F = intercompartmental clearance of the drug (L/h); CL/F = drug clearance (L/h); *a* and *b* = residual error parameters for the combined error model (Eq. (1)); RSE% = relative standard error of the calculation of the population pharmacokinetic estimate; BSV% = between subject variability.

Parameter	Mean (RSE%)	BSV% (RSE%)
Ka (h^{-1})	3.87 (8)	21.23 (33)
CL/F (L/h)	659 (8)	39.19 (16)
V_1/F (L)	5690 (7)	30.37 (15)
V_2/F (L)	5550 (23)	45.64 (50)
Q/F (L/h)	259 (12)	31.87 (31)
Covariates effects[a]		
Body weight on Ka[b]	0.0215 (19) ($p = 1.4 \cdot 10^{-7}$)	–
Body weight on Q/F[b]	0.0207 (30) ($p = 0.00086$)	–
Body weight on V_2/F[b]	0.0315 (35) ($p = 0.0048$)	–
Residual error model		
a	1.91 (5)	–
b	0.117 (3)	–

[a] The p-values for the 'treatment' effect were 0.074, 0.16, 0.11, 0.36, and 0.66 for Ka, CL/F, V_1/F, Q/F, and V_2/F, respectively.
[b] The covariate of 'weight' was centered around the mean weight.

for the C–t data of this study, gender was found to exert a significant effect on CL/F (Eq. (5)):

$$CL/F = \theta_4 \cdot \exp(-0.235) \quad (5)$$

where θ_4 refers to the typical population PK parameter estimate for the male subjects. In other words, male subjects exhibit higher clearance values than females.

The residual error parameters for the combined error model (Eq. (1)) were $a = 0.2$ and $b = 0.125$ (Table 6). In the same context, the BSV% estimates were found to exhibit moderate to relatively high values ranging approximately from 26% to 37%.

Fig. 2 illustrates the predicted vs. observed concentration values for the final population PK models, namely models numbered as '6' and '12' in Table 4 in case of FLP and SAL, respectively. For both FLP and SAL, an adequate degree of linearity can be observed in their predicted–observed plots. Another goodness-of-fit criterion utilized in this study was the graphical representation of IWRES versus IPRED (Fig. 3). The latter showed a satisfactory distribution of the residuals around zero. Finally, the VPCs of the final models of FLP and SAL are depicted in Fig. 4 and these findings are in line with the above mentioned results.

4. Discussion

Dry powder inhalation devices are convenient and efficient drug delivery systems, because they are capable of delivering large doses of the drug(s) into the lung with limited systemic exposure. Fluticasone propionate and salmeterol are two very valuable compounds for the treatment of COPD. Therefore, knowledge of the PKs of these two drugs, as well as the factors which might affect them is of special importance. The objective of this study was to explore the PKs of the combination FLP/SAL, when co-administered via inhalation, using data from two dry powder inhalers.

The individual C–t data analyzed in this work were obtained from a 2 × 2 crossover BE study. Plasma drug levels were low for both agents, with Cmax values up to around 80 pg/mL for FLP and 50 pg/mL for SAL (Fig. 1). The limited systemic absorption is consistent with previously published data, where especially in the case of SAL very low plasma levels were reached following inhalation of therapeutic doses (Advair, 2004; Cazzola et al., 2002; Möllmann et al., 2001). This low systemic absorption is however desirable, since dry powder inhalation devices focus mainly on the topical treatment of lung inflammations, and the potential of any systemic adverse events (mainly cardiovascular and non-pulmonary effects) should be minimized.

Visual inspection of the plasma C–t profiles of FLP and SAL (Fig. 1) reveals a similar general profile. It should not be disregarded that these plasma C–t data can only be ascribed to the systemic absorption of the two drugs through the lungs, since gastrointestinal absorption cannot occur due to the co-administration of activated charcoal.

Initially, a non-compartmental PK analysis was applied to the FLP and SAL C–t data in order to estimate the basic PK parameter estimates of the studied sample of volunteers (Tables 1 and 2). Data from periods I and II of the BE study were combined into one group for each drug; thus, a dataset of 57 individuals was available for FLP and SAL. This manipulation was feasible since both treatments (at periods I and II) were held under exactly the same conditions. Tables 1 and 2 reveal that similar PK estimates (e.g., Cmax, AUCt, AUCinf) were obtained for the T and R products. The PK parameters were generally in agreement with previously reported values (Advair, 2004; Cazzola et al., 2002; Möllmann et al., 2001). Peak plasma concentrations of FLP were achieved between 1 and 2 h following inhalation, while the absorption of SAL was much faster with maximum drug concentrations observed within 10 min after inhalation.

Our analysis continued with the PK comparison of the two dry powder inhalers in terms of BE assessment. The regulatory frame regarding the comparisons between inhaled medicinal products is a field of ongoing evolution. In fact it is suggested that the conduct of a BE study may not always be sufficient to establish therapeutic equivalence of locally acting oral inhaled drugs (Lu et al., 2015). In this vein, not all regulatory authorities share the same thinking on the approaches used to demonstrate equivalence. Even though, the EMA suggests a stepwise approach, other agencies like the US FDA and Health Canada recommend an aggregated weight of evidence method (Hendeles et al., 2015; Lu et al., 2015). In a recent article discussing the reports form the 'Orlando inhalation conference', it was noted that pharmacokinetic studies may serve as the most appropriate methodology for assessing BE (Hochhaus et al., 2015). In addition, the issue, whether PK studies represent the most sensitive marker of BE, is currently under extensive discussion (Hendeles et al., 2015). Thus, some critical points have been identified regarding the proof of BE in case of orally inhaled drug products (Thakkar et al., 2015).

The BE results for the FLP and SAL data utilized in this analysis are listed in Table 3. These results indicate that the two dry powder inhalers

Table 6
Salmeterol population pharmacokinetic parameters for the final best model. Key: F = fraction of bioavailable dose; V_1/F = apparent volume of drug distribution (L) of the central compartment; V_2/F = apparent volume of drug distribution (L) of the peripheral compartment; Q/F = intercompartmental clearance of the drug (L/h); CL/F = drug clearance (L/h); *a* and *b* = residual error parameters for the combined error model (Eq. (1)); RSE% = relative standard error of the calculation of the population pharmacokinetic estimate; BSV% = between subject variability.

Parameter	Mean (RSE%)	BSV% (RSE%)
CL/F (L/h)	678 (7)	26.34 (31)
V_1/F (L)	891 (9)	36.76 (14)
V_2/F (L)	2570 (7)	27.29 (12)
Q/F (L/h)	1270 (8)	35.88 (12)
Covariates effects[a]		
Gender on CL/F[b]	−0.235 (33) ($p = 0.0024$)	–
Residual error model		
a	0.2 (4)	–
b	0.125 (3)	–

[a] The p-values for the 'treatment' effect were 0.94, 0.058, 0.69, and 0.47 for CL/F, V_1/F, Q/F, and V_2/F, respectively.
[b] Male was considered as the 'control' group.

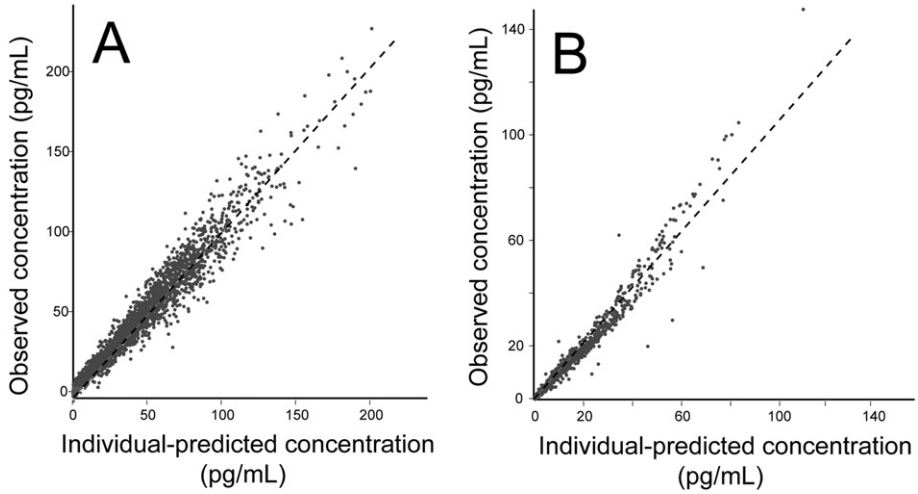

Fig. 2. Individual predicted–observed plasma concentration values in case of the population pharmacokinetic studies applied to the C–t data of: A) fluticasone propionate and B) salmeterol. The diagonal dashed line represents the line of unity, namely, of the ideal situation.

are bioequivalent, since in all cases the 90% CI for AUCt and Cmax lie within the acceptance interval of 80–125% (EMA, 2010). It is worth mentioning that BE is proved despite the high within-subject variability (ranges from 28% to 44%) of the drug. Also, the derived statistical power for each PK parameter is well above the limiting value of 80% (Table 3).

Apart from typical non-compartmental analysis, a population PK analysis was also applied to the C–t data of FLP and SAL. Data for the T and R products were combined, while period (i.e., occasion) and treatment (i.e., T or R) effect were considered as covariates in the population models. Thus, a dataset of 114 individuals was available for analysis in the case of both FLP and SAL. Many runs using several scenarios such as a variety of structural and error models, initial estimates, and combination of covariates, were examined. The evaluation of the results obtained was made using the goodness-of-fit criteria (visual inspections of several types of plots and statistical criteria) presented in the 'Materials and methods' section.

In case of FLP, the C–t data of the current BE study were best described by a two-compartment model with first-order absorption and elimination kinetics (model '6' in Table 4). A similar model for FLP has also been suggested in the literature (Krishnaswami et al., 2005; Wu et al., 2008). A one-compartment disposition model has also been described in the literature for fluticasone propionate (Rohatagi et al., 1996; Simon et al., 1998; Xu et al., 2010). Perhaps, this divergence might be attributed to the different sample size of the trials and the health status (healthy or asthmatic patients) of the study participants. The data, we analyzed in this study, came from a BE study where 57 healthy volunteers are analyzed. In contrast, the studies of Rohatagi et al. (1996), Simon et al. (1998) and Xu et al. (2010) included asthmatic patients where the FLP kinetics can be different. It should be mentioned that in the recent 'Orlando inhalation conference', it was suggested that BE studies should be preferably be conducted in healthy adult volunteers, since variability is reduced and the deposition of drug to tissues is not hampered (Hochhaus et al., 2015).

The estimates of the population PK parameters, their BSV% values, along with their RSE% estimates are quoted in Table 5. It should be noted here that previous studies have shown that for lipophilic

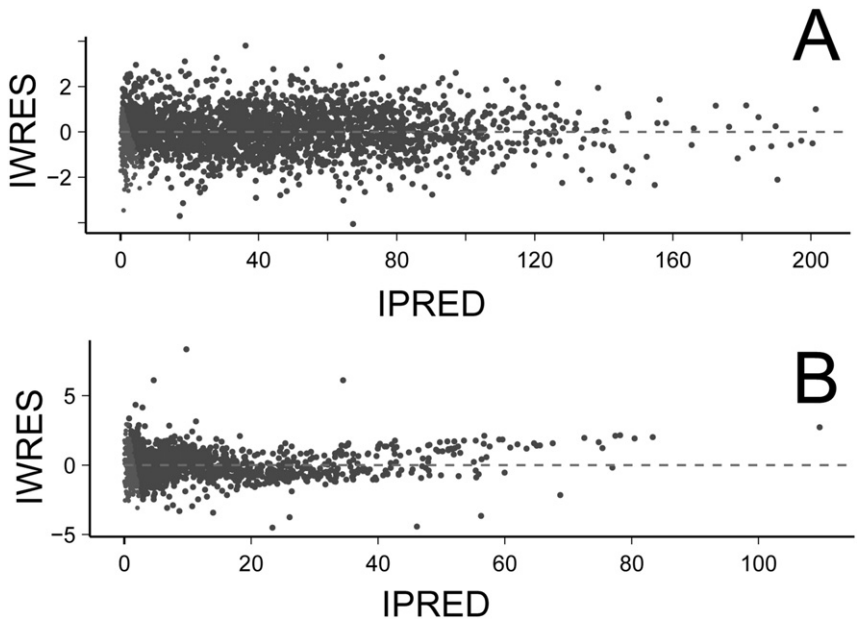

Fig. 3. Graphical representation of the individual weighted residuals (IWRES) versus the individual predicted concentrations (IPRED) for the two final best models in case of: A) fluticasone propionate and B) salmeterol.

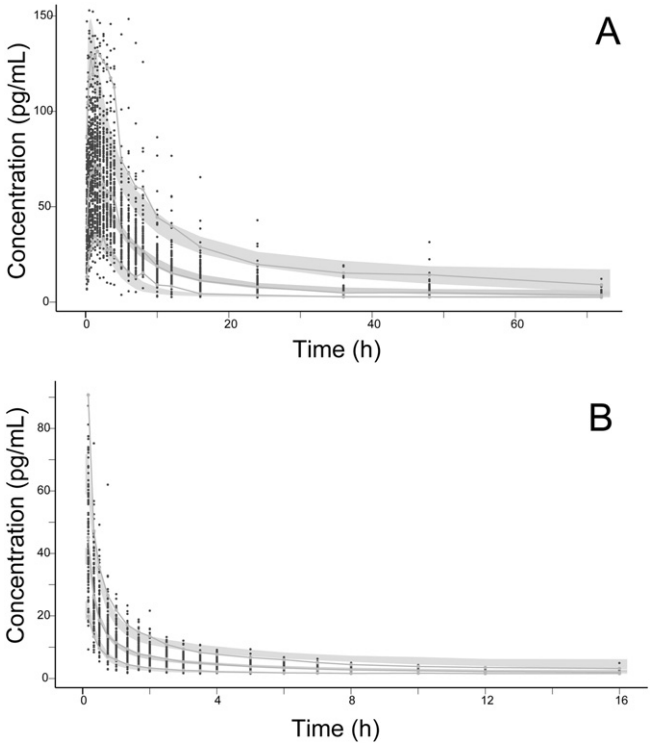

Fig. 4. Visual predictive check of the final models for FLP (A) and SAL (B). Key: closed circles are the observed individual concentration–time data; solid lines refer to the 5th, 50th, and 95th percentiles of the empirical data; shaded areas refer to the 95% prediction intervals around each theoretical percentile.

substances, as in case of FLP, pulmonary dissolution acts in essence as the rate limiting step in the entire process of pulmonary absorption (Hochhaus et al., 1997). Thus, the so-called in this study as absorption rate constant (i.e., Ka), is actually a hybrid parameter expressing both slow dissolution of the lipophilic FLP in the lungs and its passing through the alveolar–capillary interface. In the current analysis, the pulmonary dissolution and absorption were considered as a single process that was described by a single PK estimate, i.e., the absorption rate constant. The latter is in accordance with other published studies (Rohatagi et al., 1996; Simon et al., 1998; Wu et al., 2008; Xu et al., 2010).

To this point, a clarification should be made towards the use of simple first-order kinetics to describe the FLP absorption. More complex absorption kinetics for inhaled drugs, like multiple parallel pulmonary absorption processes, has also been suggested in the literature started twenty years ago and updated quite recently (Bartels et al., 2013; Falcoz et al., 1995; Weber and Hochhaus, 2013; Weber and Hochhaus, 2015). In these articles a discrimination between a central and a peripheral lung region presenting different absorption rates was discussed. However, the development of more complex models and the incorporation of multiple absorption processes through the lung require the use of rich data from both intravenous and inhaled administrations (Bartels et al., 2013; Krishnaswami et al., 2005; Mobley and Hochhaus, 2001). The characterization of the PK models used in this analysis was based solely on data after inhalation which restricted any differentiation between parallel absorption processes. It is worth mentioning that until now, the PK models appeared in the literature have treated fluticasone propionate and salmeterol, assuming simple first order absorption kinetics. It is therefore acknowledged that the current models are, like any model, only a simplification of the true drug kinetics in the lung. These models could of course be extended with the incorporation of intravenous data and the acquisition of more evidence regarding the pulmonary physiology and the complex underlying absorption processes.

Another point that requires special attention is the fact that due to slow dissolution of FLP in the lungs and the non-existence of intravenous data, there is a difficulty to distinguish whether this situation is flip-flop or not. This issue of potential mis-assignment of the inhaled fluticasone propionate PK parameters was highlighted in the study of Krishnaswami and colleagues who investigated the pharmacokinetics of FLP after single- and multiple-dose administration in 14 healthy volunteers (Krishnaswami et al., 2005). Based on these findings, there can be a mismatching of the absorption with the disposition parameters. In our study, the estimate of Ka (3.87 h^{-1}), was considered to reflect truly the absorption rate constant, since it is quite close to the reported Ka value (4.07 h^{-1}) in the more recent study of Xu et al. which utilizes a simpler PK model applied to 32 asthmatic patients (Xu et al., 2010). Besides, our estimated Ka value is also close to the Ka estimate (2.79 h^{-1}) reported in the study of Wu et al. where a two-compartment model fitted to the C–t data of 14 healthy subjects (Wu et al., 2008).

The derived FLP volume of distribution for the central and the peripheral compartments were found to be equal to 5690 L and 5550 L for V_1 and V_2, respectively (Table 5). It should be stated that in the study of Xu et al. (in 32 asthmatic patients), the apparent volume of distribution was found to be even larger, namely, 9800 L (Xu et al., 2010). Even though a direct comparison of the pharmacokinetic behavior of FLP between asthmatic patients and healthy volunteers cannot be easily performed, this study confirms the extensive distribution of FLP into tissues which appears to be consistent with the high lipid solubility and tissue binding of the drug (Harrison and Tattersfield, 2003; Thorsson et al., 1997). The extensive distribution of FLP may be the reason for its delayed elimination from the body. The latter is reflected on the fact that FLP plasma concentrations can be detected for more than 24 h after inhalation (Fig. 1A).

This population analysis also examined the significance of several covariates on the PK parameters. Initially, it should be stated that 'treatment' and 'period' effect were not found to exert a significant impact on any PK parameter at the 5% significance level. This finding, that 'treatment' effect was not found to be significant, is in line with the results derived from the BE study which suggests that administration of the two inhaled formulations will result in similar pharmacokinetic profiles for FLP. For the remaining tested covariates, only body weight (centered around mean) was found to significantly influence Ka ($p = 1.4 \cdot 10^{-7}$), intercompartmental clearance ($p = 0.00086$) and peripheral volume of distribution ($p = 0.0048$) (Table 5). These findings suggest that as body weight increases, absorption rate, drug intercompartmental clearance and peripheral volume of distribution also rise. Fluticasone propionate appears to be restricted to the extracellular space and the extravascular distribution of the drug could be facilitated by the increased fluid associated with an increased body weight. The latter may explain the high volume of distribution estimates found in this study. Also, a literature search revealed that a gender effect on volume of distribution and clearance has been reported, but these data come from a study which did not include healthy subjects, but asthmatic patients (Simon et al., 1998). Again in asthmatic patients, two other studies did not identify any differences between male and female subjects (Advair, 2004; Xu et al., 2010).

The increase in FLP absorption rate with higher body weight might be attributed to a larger lung size, which offers a wider absorption surface. Besides, the estimated increase in Q/F as body weight rises seems reasonable due to the physicochemical properties of FLP. A similar effect of body weight on Q/F has been reported for propofol (which is also a lipophilic drug) using allometric scaling (Knibbe et al., 2005). Nevertheless, it should be reminded that the current population PK analysis was applied to a relatively homogenous sample of subjects, since it comes from a BE study. An increased sample size and a more heterogeneous pool of subjects would carry more information regarding the effect of covariates.

In the case of SAL, visual inspection of Fig. 1B reveals that its peak plasma levels are reached almost instantaneously. In particular, Tmax estimates are observed almost 10 min (Table 2) after inhalation. The

best fitting results (model number '12' in Table 4) were obtained when a two-compartment disposition model was used assuming very rapid absorption kinetics (like intravenous bolus) and first-order elimination kinetics from the central compartment. In order to verify our findings, population PK analysis was also applied assuming first-order input. The latter led to very high Ka estimates equal to $2.22 \cdot 10^5\ h^{-1}$ (data not shown). For this reason, it was decided to consider an instantaneous absorption in order to be able to estimate more accurately the remaining parameters. Besides, the rapid absorption of SAL is in agreement with literature reports (Cazzola et al., 2002). The choice between one- and two-compartment models was based on the C–t fittings and goodness-of-fit criteria.

The apparent volume of distribution of SAL for the central compartment was large (891 L) and it was found even higher for the peripheral compartment (2570 L), Table 6. These findings indicate an extensive distribution of SAL within the body, which can be ascribed to its high lipophilicity.

A gender effect was found on CL/F (Table 6). Males were found to have higher parameter values for these PK parameters compared to female subjects. The gender effect on clearance might be attributed to the higher enzymatic capacity of men to metabolize SAL and a difference in lung deposition between males and females (Cazzola et al., 2002). Again, in accordance with the results from the BE study, the 'treatment' effect was not found to exert a significant impact on any PK parameter of SAL at the 5% significance level. This finding implies that administration of either T or the R product would lead to similar PK profiles of SAL.

Some indicative goodness-of-fit plots of the final models for FLP and SAL are shown in Fig. 2A and B. The plots of individual predicted–observed concentration values, shown in Fig. 2, reveal that the data are mostly randomly distributed around the line of identity. This finding implies a good agreement between the observed and the model predicted drug plasma concentrations. Furthermore, no trend was observed in the diagnostic plot of IWRES versus the IPRED concentrations for both FLP and SAL (Fig. 3). The individual weighted residuals were almost symmetrically distributed around zero.

Finally, the goodness-of-fit of the final models was evaluated by visual predictive checks (Fig. 4A and B). Even though, some observations lie outside the 5th or 95th, the majority of them is between. The large concentration values of FLP and SAL are in line with the descriptive statistical criteria quoted in Tables 1 and 2. For example, the Cmax (pg/mL) estimates of FLP range from 28.9 to 162.7 for the test product and from 35.7 to 226.9 in case of the reference dry powder inhaler. We should also bear in mind that a number of 114 observations correspond to each time-point.

5. Conclusions

The purpose of this study was to explore the pharmacokinetics in healthy male and female subjects of FLP and SAL after concomitant administration using data from two dry powder inhalers. Classic non-compartmental approaches, as well as, population pharmacokinetic analyses were applied separately to FLP and SAL. The classic PK analysis allowed the estimation of the individual Cmax, AUCt, AUCinf, Tmax, and λz values as well as their descriptive statistics. In a second step, a BE assessment was applied to the estimated PK parameters of the two dry powder inhalers, which showed their bioequivalence. According to the population pharmacokinetic analysis, a two-compartment model was found to best describe the C–t data of FLP assuming first-order absorption and elimination kinetics from the central compartment. In case of SAL, the best results were found when a two-compartment disposition model was used assuming very rapid absorption kinetics (like intravenous bolus) and first-order elimination kinetics from the central compartment. For both FLP and SAL situations, a combined residual error model led to the optimum performance.

Conflict of Interest

The authors did not receive any funding for writing and/or preparing this manuscript.

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