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Drivers of absolute systemic bioavailability after oral pulmonary inhalation in humans

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Abstract

There are few studies in humans dealing with the relationship between physico-chemical properties of drugs and their systemic bioavailability after administration via oral inhalation route (Fpulm). Getting further insight in the determinants of Fpulm after oral pulmonary inhalation could be of value for drugs considered for a systemic delivery as a result of poor oral bioavailability, as well as for drugs considered for a local delivery to anticipate their undesirable systemic effects. To better delineate the parameters influencing the systemic delivery after oral pulmonary inhalation in humans, we studied the influence of physico-chemical and permeability properties obtained in silico on the rate and extent of Fpulm in a series of 77 compounds with or without marketing approval for pulmonary delivery, and intended either for local or for systemic delivery. Principal component analysis (PCA) showed mainly that Fpulm was positively correlated with Papp and negatively correlated with %TPSA, without a significant influence of solubility and ionization fraction, and no apparent link with lipophilicity and drug size parameters. As a result of the small sample set, the performance of the different models as predictive of Fpulm were quite average with random forest algorithm displaying the best performance. As a whole, the different models captured between 50 and 60% of the variability with a prediction error of less than 20%. Tmax data suggested a significant positive influence of lipophilicity on absorption rate while charge apparently had no influence. A significant linear relationship between Cmax and dose ($R^2 = 0.79$) highlighted that Cmax was primarily dependent on dose and absorption rate and could be used to estimate Cmax in humans for new inhaled drugs.

Keywords: Systemic bioavailability; Oral pulmonary inhalation; Physico-chemical properties; Permeability; Bioavailability prediction; Humans

1 Introduction

Pulmonary administration is underused because this route of administration is quite challenging, whether it is used for a local or for a systemic delivery of drugs. The main hurdles rely on the formulation of the drug (i.e., excipients and particle size) and on the delivery device that should allow a low retention in the device as well as a regular and highly efficient drug deposition in the lungs. Advances in inhaled technologies are developed to optimize the lung deposited dose (LDD) which is a fraction of the metered dose (MD) and of the delivered dose (DD) [1].

When used for a local delivery, depending on the target diseases, different levels of delivery of drugs within the broncho-alveolar tree are required (i.e., upper levels for asthma and COPD, and lower levels for pulmonary infections). Moreover, other features are needed such as a high potency allowing extremely low dose to administer and a prolonged lung residence time. The prolonged residence time of pharmacologically active free concentrations has been obtained by increase in basicity and polarity (e.g., for anticholinergics and β_2 -agonists) and/or decrease in aqueous solubility (e.g., corticosteroids). A low oral bioavailability, a rapid plasma clearance and a high plasma protein binding are also desirable to reduce systemic side effects [2].

When used for a systemic delivery, a lower level delivery to the alveolar area is required because of its high surface area ($>100 \text{ m}^2$), significant blood perfusion by the entire cardiac output, small aqueous volume at the absorptive surface ($0.07 \mu\text{m}$ fluid) and relative thin membrane ($0.1\text{--}0.2 \mu\text{m}$) so that the lung has the shortest diffusion distance of all organs [3].

Pulmonary administration for a systemic delivery is interesting for drugs requiring a rapid onset of action (within seconds or minutes) and for those with poor oral bioavailability as a result of unfavorable oral absorption properties (solubility and/or permeability) and/or significant intestinal and hepatic first-pass effect. Even though in much lower concentrations in the lungs, drug-metabolizing enzymes and transporters are also to be considered at the pulmonary level [4,5]. As physiological variables operational at the epithelial-luminal interface, transporters may influence the local residence time of drugs and their access to intracellular targets and potentially influence the systemic drug absorption profiles [6]. Among the nineteen transporters identified in human lung tissue OCTN1 was the most abundantly expressed protein. High expression of MRP1, BCRP and PEPT2 protein have also been reported [4].

To our knowledge there are few studies dealing with the relationship between physico-chemical properties and systemic bioavailability of drugs administered via oral inhalation route in humans. Very relevant studies using a panel of 10 molecules of different physico-chemical properties have been carried out in rats in isolated perfused model [7] and in vivo [8]. The absorption rate was found to correlate with molecular polar surface area, hydrogen bonding potential and apparent permeability (Papp) in Caco-2 cell monolayers. However, the influence of physico-chemical properties on the extent of absorption could not be explored since all compounds in the dataset has high systemic bioavailability. More recently, a QSAR model based on the isolated perfused respiring rat lung model was developed with a data set of 98 compounds [9]. The model built on the percentage of solubilized dose in perfusate, and not on the total dose in perfusate, allowed the prediction of the ability of a drug to cross the lung. Given the parameter on which the model was built, the prediction was more an estimation of the absorption rate than that of the extent of absorption. This model considered solubility as a driver of pulmonary absorption, and found that the percentage of solubilized dose was positively associated with permeability and hydrophobicity and that descriptors associate with charge, ionization and size were negatively correlated. These studies bring some convergent informations on the drivers of pulmonary absorption rate in animals, without significant informations on the drivers of extent of absorption.

The translatability of findings in preclinical species to humans can be questioned. Several models are available to study the pulmonary disposition of drugs [10], and in vivo models are preferred for studying drug disposition [11,12]. Extrapolation of in vivo data from pre-clinical species to humans should be made with caution given the huge differences in lung anatomy, physiology and biochemical features that may impact drug disposition [13]. Due to the high complex pulmonary deposition, dissolution, and absorption processes, sophisticated mathematical approaches are needed [14], and PB-PK modeling might be an option.

Even though pharmacokinetic modeling has been used successfully to predict pulmonary pharmacokinetics from in vitro properties [15], several obstacles to in vitro-in vivo correlations in pulmonary drug delivery have been identified [10,16,17]. Pharmacokinetic modeling from microfluidics technologies (e.g. human-on-a-chip) and 3D bioprinting could be tools of interest for assessing specific pharmacokinetic parameters of drugs [18]. Modeling in combination with isolated perfused rat lung (IPL) has shown better performance compared to those based on in vitro data [19]. Furthermore, prediction of human dose for oral inhalation from preclinical in vivo data have been successfully obtained [20,21], as well as prediction of drugs concentrations in human lungs [22].

If much attention has been paid to local drug disposition of pulmonary drugs, the drivers of systemic drug disposition after oral inhalation are less clearly delineated and deserve further understanding. This is of paramount importance both for drugs aimed to act locally to minimize their undesirable systemic effects, as well as for drugs aimed to act systematically to maximize and hence decrease the variability of their systemic delivery after oral inhalation. Moreover, oral inhalation is currently an option for new chemical entities (NCE) as well as for repositioned drugs [23].

To get further insight in the drivers of systemic delivery after oral pulmonary inhalation in humans, we study the influence of physico-chemical and membrane permeability properties on the systemic bioavailability (i.e., rate and extent) of a series of compounds administered via the oral pulmonary route. Absolute bioavailability after oral pulmonary administration has been obtained from a literature survey leading to a data set of 77 compounds with or without marketing authorization for pulmonary delivery, and intended either for local or for systemic delivery.

2 Materials and methods

2.1 Literature search for pulmonary absolute bioavailability

The determination of the absolute systemic bioavailability after pulmonary administration (Fpulm) is not frequently the aim of studies, and in some studies the absolute bioavailability was not calculated even though data were available to do so. Hence to be sure to capture the largest panel of compounds with information on Fpulm we performed a search using “pulmonary pharmacokinetics” or “inhalation pharmacokinetics”. The search was limited to articles published in English language from up to Mai 2020. Titles, abstracts, and full-text articles were screened for systemic exposure data after inhalation and IV administration.

An additional search was performed on clinical trial database (<https://clinicaltrials.gov/ct2/home>) for investigational drugs for which data on pulmonary bioavailability were not published in peer review journals but were available in this database.

A panel of 77 compounds (essentially marketed or investigational drugs) with human pulmonary bioavailability data were retrieved (Table 1). When several studies displayed different values of Fpulm for a specific compound, a weighted arithmetic mean was computed taking into account the number of subjects involved in the individual clinical studies.

Table 1

List of compounds used in the study with the intended site of action (local: L or systemic: S), the regulatory status (M: marketed, Inv: investigational drugs, Misc: miscellaneous), and the drug class and main indication.

Drugs	Action (local or systemic, L/S)	Marketed (M), Investigational drugs (Inv), Miscellaneous (Misc)	Drug Class	Main Indication
Abediterol	L	M	β_2 -adrenoreceptor agonist	Asthma and COPD
Aclidinium bromide	L	M	Anticholinergic drug	Asthma and COPD
Adroglide Hydrochloride	S	Inv	D1 - dopamine agonist	Parkinson

Amikacin	L	M	Antibiotic	Pulmonary infection
Amiloride hydrochloride	L	Misc	Sodium channel blocker	Cystic Fibrosis
Amphotericin B liposomal	L	Misc	Antifungal	Pulmonary infection
Atropine	S	Misc	Anticholinergic drug	Organo-phosphate antidote
Aztreonam	L	M	Antibiotic	Pulmonary infection
Batefeneterol succinate	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Beclomethasone dipropionate	L	M	Glucocorticoid	Asthma and COPD
Beclomethasone 17-MP	L	Misc	Glucocorticoid	Asthma and COPD
Bimosiamose	L	Misc	Pan-selectin antagonist	Antiinflammatory
Budesonide	L	M	Glucocorticoid	Asthma and COPD
Caffeine	S	Misc	Stimulant	Energy drink
Cannabidiol	S	Misc	Psychoactive	Recreational drug
Ceftazidime	L	Misc	Antibiotic	Pulmonary infection
Ciclesonide	L	M	Glucocorticoid	Asthma and COPD
Ciclosporin	L	Misc	Immunosuppressant	Lung transplant
Ciprofloxacin	L	Misc	Antibiotic	Pulmonary infection
Cocaine	S	Misc	Psychoactive	Recreational drug
Colistin MS	L	Misc	Antibiotic	Pulmonary infection
Cromoglycate sodium	L	M	Anti-inflammatory, nonsteroidal	Allergic Asthma
Denufosal	L	Misc	P2Y2 receptor agonists	Cystic Fibrosis
DTPA99m Technecium	L	Misc	gamma ray emitting radionuclide	Radiopharmaceutical agent
Dihydroergotamine	S	M	serotonin (5-HT) receptor agonist	Migraine
Duramycin	L	Misc	Antibiotic	Pulmonary infection
Epinephrine	S	Misc	Adrenergic alpha- and beta-Agonist	imminent anaphylactic reactions
Ergotamine tartrate	S	M	serotonin (5-HT) receptor agonist	Migraine
Esketamine	S	M	N-methyl-D-aspartate receptor antagonist	Pain
Fenoterol	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Fentanyl	S	M	Narcotic	Pain
Flumazenil	S	Misc	Benzodiazepine Antagonist	Benzodiazepine overdose
Flunisolide	L	M	Glucocorticoid	Asthma and COPD
Fluticasone furoate	L	M	Glucocorticoid	Asthma and COPD
Fluticasone propionate	L	M	Glucocorticoid	Asthma and COPD
Fumaryl diketopiperazine	L	Misc	Excipient	-
Gentamicin	L	Misc	Antibiotic	Pulmonary infection
Glycopyrronium bromide	L	M	Anticholinergic drug	Asthma and COPD
Heroin	S	Misc	Recreational drug	Recreational drug
Iloprost	L	Misc	Prostacyclin analogues	Pulmonary arterial hypertension
Indacaterol acetate	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Ipratropium bromide	L	M	Anticholinergic drug	Asthma and COPD
Itraconazole	L	Inv	Anti-fungal agent	Pulmonary infection
Levofloxacin	L	M	Antibiotic	Pulmonary infection
Lidocaine	L	Misc	Local anesthetic	Intubation
Mannitol	L	M	Mucus clearance modifier	Cystic Fibrosis
Metamphetamine	S	Misc	Psychoactive	Recreational drug
Mometasone furoate	L	M	Anticholinergic drug	Asthma and COPD
Morphine M6G	S	Misc	Narcotic	Pain
Morphine sulfate	S	Misc	Narcotic	Pain
Nedocromil sodium	L	M	Anti-inflammatory agent	Asthma
Nemoralisib hemisuccinate	L	M	Anti-inflammatory agent	COPD
Nicotine	S	Misc	nicotinic cholinergic receptor agonist	Smoke addiction
Nitroglycerin - glyceryl trinitrate	S	Misc	Vasodilator	Angina pectoris
Olodaterol	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Oxytocin	S	Misc	Vasoconstrictor	Prevention of postpartum hemorrhage
Oxytropium bromide	L	M	Anticholinergic drug	Asthma and COPD
Pentamidine	L	Misc	Anti-infective agents	Pneumocystis carinii infection prevention
Prochlorperazine	S	M	D2 receptor-antagonist	Treatment of acute migraine attacks
Revefenacin	L	M	Anticholinergic drug	COPD
Salbutamol sulfate	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Salmeterol xinofoate	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Terbutaline sulfate	L	M	β2-adrenoreceptor agonist	Asthma and COPD
Tetrahydrocannabinol	S	Misc	Psychoactive	Recreational drug
Tiotropium bromide	L	M	Anticholinergic drug	Asthma and COPD
Tobramycin	L	Misc	Antibiotic	Pulmonary infection
Treprostinil sodium	S	M	Prostacyclin	Pulmonary arterial hypertension
Triamcinolone acetonide	L	M	Glucocorticoid	Asthma and COPD
Umeclidinium bromide	L	M	Anticholinergic drug	Asthma and COPD
Vilanterol trifenate	L	M	β2-adrenoreceptor agonist	Asthma
Zaleplon	S	M	Allosteric benzodiazepine receptor activator	Sleep induction
Zanamivir	S	M	Antiviral	influenza A and B virus neuraminidase inhibitor
AZD5423	L	Inv	Non-steroidal glucocorticoid receptor modulator	Asthma
AZD7594	L	Inv	Non-steroidal glucocorticoid receptor modulator	Asthma

GLP-1 MKC253	S	Inv	Glucagon-like peptide 1	Diabetes
HMR1031	L	Inv	Integrin VLA-4 ($\alpha 4\beta 1$) binding antagonist	Asthma
IVL745	L	Inv	VLA-4 antagonist	Asthma

2.2 Calculation of pulmonary absolute bioavailability

The systemic bioavailability after pulmonary administration/oral inhalation ($F_{systemic}$) is the sum of the pulmonary bioavailability (F_{pulm}) and of the oral bioavailability (F_{oral}) secondary to drug swallowing.

The absolute systemic bioavailability after pulmonary administration (F_{pulm}) (i.e., fraction of the dose deposited in and absorbed from the lung) was calculated from the systemic availability ($F_{systemic}$) and oral availability (F_{oral}) using the following equation (24):

$$F_{pulm} = (F_{systemic} - F_{oral}(1 - f_{ret}))/1 - F_{oral}$$

where f_{ret} is the fraction of the nominal dose retained in the device. These calculations require the assumption that no metabolism occurred locally in the lungs.

In each report, attention was paid to the exact calculation of F_{lung} , especially to be sure that the amount actually deposited to the lung was estimated (i.e., 2D or 3D-gamma scintigraphy, exhalation into a supplied exhalation filter to collect any exhaled aerosol, measure of the remaining drug in the device used for administration, administration via endotracheal intubation...). Given a potential systemic participation of the oral absorption, a correction was performed with the oral bioavailability excepted when a charcoal-blocked oral absorption was carried out.

2.3 Permeability descriptor

In vitro lung permeability data in humans, in animal or in pulmonary immortalized cell-lines are available only for few of the compounds studied. Furthermore, as shown by Larregieu and Benet [25], there is a huge interlaboratory variability in in vitro Caco-2 permeability values with large variations for hydrophilic drugs (27-fold for terbutaline) compared to hydrophobic drugs (2.7-fold for propranolol).

For these reasons, we choose to use an in silico parameter for passive permeability as intrinsic Caco-2 permeability measurements have been shown to give good predictions of the passive transcellular permeability of lung epithelium to drugs [26].

Hence, a calculated apparent permeability (P_{app} , cm/sec) has been obtained from ADMETlab software (web interface at <http://admet.scbdd.com>, CBDD Group, Xiangya School of Pharmaceutical Sciences & Central South University). The ADMETlab web platform is designed based on the Django framework in Python [27]. The dataset of Caco-2 cell permeability used in the model has been collected from a QSAR study from 1,182 compounds [28]. For ionized compounds, the P_{app} was computed for the ionized form of the active compound and not for the salt.

2.4 Physico-chemical descriptors

2-D and 3-D physico-chemical descriptors were computed using ACD/Labs Percepta software (Advanced Chemistry Development Inc., Version 2018.2.1, Toronto, ON, Canada). The physico-chemical descriptors used were molar volume (cm^3), LogD (pH 7.4), LogP, topological polar surface area (TPSA in %), polarizability ($10^{-24} cm^3$), Log S (mol/L), solubility at pH 7.4 (mg/ml), and hydrogen bonding (HB, HBD and HBA). The ionized fraction at pH 7.4 (%) was not calculated directly but was estimated from the graph showing the net charge as a function of pH. The percentage of polar surface area was calculated from TPSA and from surface area (PSA in \AA^2) given by from Dragon 6 software (Talete, Milano, Italy) [29].

The charge at pH 7.4 was estimated from the graph showing the protonation status as a function of pH.

2.5 Principal component analysis

A principal component analysis (PCA) including physico-chemical and permeability descriptors was performed to define the most important determinants of the pulmonary bioavailability (F_{pulm}). PCA is a multivariate analysis method allowing its principal components (PC's) to contain most of the variance from a data set albeit in a much lower dimensional space. PC1 is defined along the direction of maximum variance of the whole data set (i.e., maximal elongation direction), PC2 to the direction orthogonal to PC1 explaining the maximal variance, and PC3 to the maximal variance orthogonal to both PC1 and PC2. After choosing the two PC's accounting for the main part of the information present in the original data set, a plane projection of the points lead to an exhaustive summary of the original 3-dimensional information. PCA was performed by using the XTSTAT statistical software (Addinsoft®, Paris, France, 2019).

2.6 Model construction for F_{pulm} prediction

2.6.1 Predictive variables

Quite frequently, several variables can be highly correlated (as evidenced on ACP plots) so that multicollinearity may occur during modelisation steps. To avoid this problem, ACP axes will be used as predictive variables instead of the initial variables since by definition ACP axes are uncorrelated. ACP axes that will be considered are those that capture 95% of the information contained in the initial variables. The main drawback of this procedure is that resulting models will be harder to interpret.

2.6.2 Algorithms used

The following algorithms were used to establish predictive models:

- Multiple linear regression (MLR),
- Beta regression: Beta regression is useful for situations where the variable of interest is continuous and restricted to the interval (0, 1) and is related to other variables through a regression structure. The underlying assumption is that the response variable follows the beta law. Its mean is related to unknown parameters and covariates through a link function [30].
- Random forest algorithm: Random Forest algorithm (RF) is a supervised classification algorithm used for both classification and regression problems. RF combines a forest of uncorrelated trees created with the CART (Classification And Regression Trees) procedure. Each tree is constructed by a randomly selected subset of training data. The remaining training data are used to estimate prediction error and variable importance. Bootstrap sampling of samples and random selection of input features are used in the algorithm to make sure that the classification trees grown in the forest are dissimilar and uncorrelated from each other.
- Gradient boosting trees: Gradient boosted regression and classification is an additive training tree classification method where trees are built in series (iteratively) and compared to each other based on a mathematically derived score of splits.
- Support vector machine (SVM): SVM regression is considered as a nonparametric technique because it relies on kernel functions. The kernel-based approach to regression is to transform the variable to some vector space, then perform a linear regression in that vector space that is somewhat different than ordinary least squares.

Both these models were used to predict F_{pulm} either as percentage or as its logit (excepted for beta regression that can only be used for percentages) since distribution of the pulmonary bioavailability may not be symmetric. For models trained with logit values of F_{pulm} ($\text{logit } F_{pulm} = \log(F_{pulm} / (1 - F_{pulm}))$), predictions are transformed by logit inverse to return back to F_{pulm} as percentage.

2.6.3 Performance evaluation

In order to develop statistically significant and robust models, the whole dataset is usually divided into a training set, a validation set and a test set; the latter being used to objectively evaluate the predictability of a model. In the current study, as a result of a rather limited size of the data-set, we have used a 10-fold cross validation procedure. Briefly, in this iterative procedure, at each step 9 blocks (90% of the observations) are used to construct the model (as well as pre-treatment such as ACP) and the 10th bloc being used to validate the model. This procedure is used 10-times iteratively so that the 10 blocks are successively used as test data-sets. This cross-validation procedure is repeated 5-times to avoid that the results are linked to the block division.

The performance indicators used were: PCC (Pearson's correlation coefficient) to account for the association between observed and predicted values, R2 (coefficient of determination), RMSE (root mean square error) and MAE (mean absolute error).

2.7 Statistical analysis

The performance indicators of the predictive models were: PCC (Pearson's correlation coefficient) to account for the association between observed and predicted values, R2 (coefficient of determination), RMSE (root mean square error) and MAE (mean absolute error). T_{max} were compared by using Kruskal-Wallis rank test. A p-value lower than 0.05 was considered as significant.

3 Results

3.1 Profile of sample set compounds

Our search allowed the identification of 77 compounds for which data on F_{pulm} were available (24 compounds for a systemic delivery and 53 for a local delivery, Table 1). Marketed or investigational drugs (n = 45–58%), were mainly intended for a local delivery (n = 37–82%). Within miscellaneous compounds (n = 30–39%), the pulmonary route was mainly used for systemic delivery (n = 14 – 47%).

Most of the compounds were intended for treatment of asthma and/or COPD (n = 31–42%) and belonged mainly (77%) to the 3 main therapeutic classes (anticholinergic drugs n = 8, glucocorticoids n = 7 and $\beta 2$ -adrenoreceptor agonists n = 9).

The biopharmaceutical and physico-chemical properties of the compounds are given in Table 2. The compounds of interest have a rather small molecular weight (average MW: 529 g/mol), and a large majority were in ionized form (75%) at pH 7.4. About 72% of the molecules have a polar surface area lower than 140\AA^2 , considered as the upper threshold to have a good bioavailability by oral route [31]. Most of the compounds (60%) can be considered as soluble ($\geq 1 \text{ mg/ml}$).

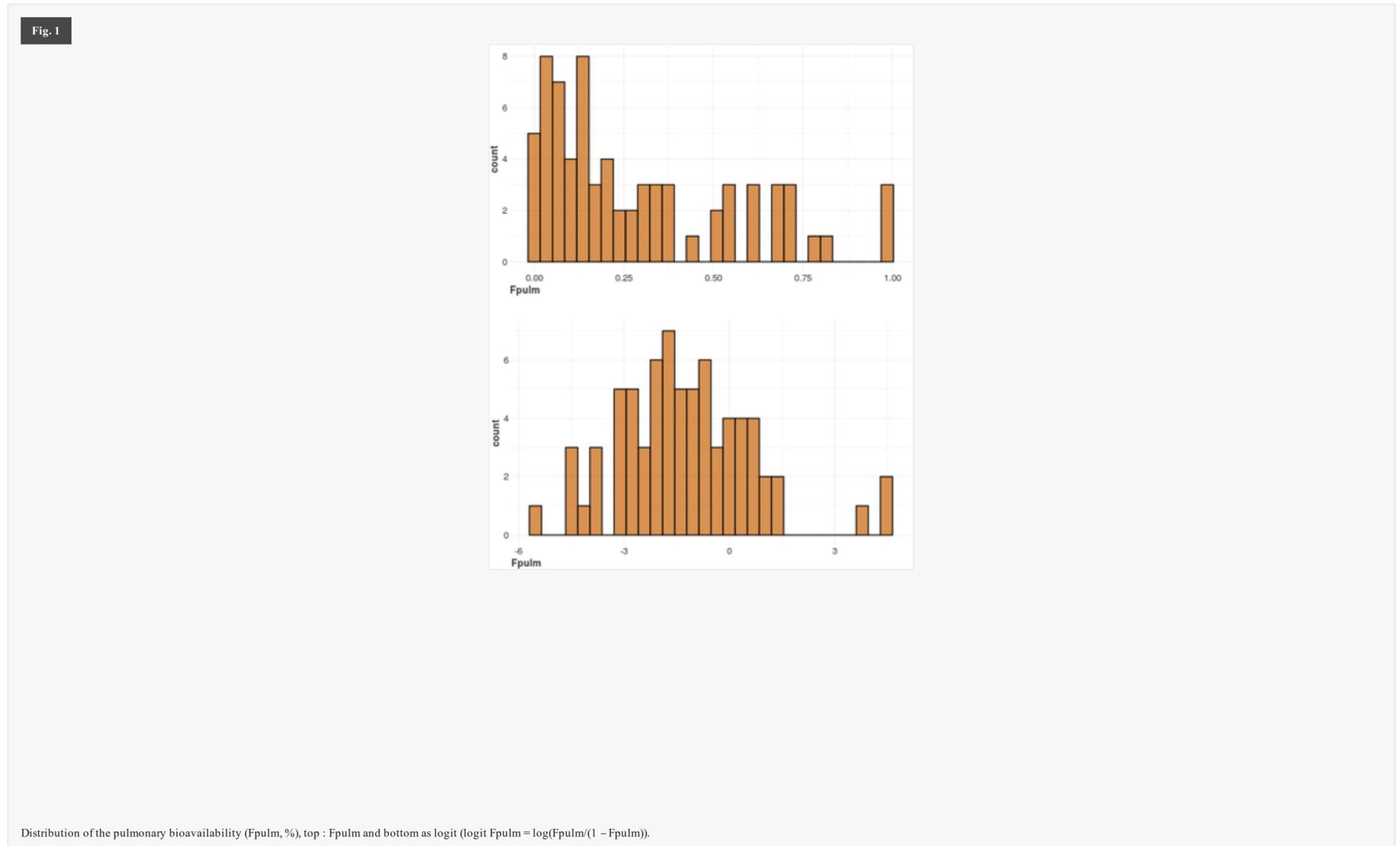
Table 2

Compound	Fp _{ml} (%)	T _{max} (min)	Papp of the active moiety (10 ⁻⁶ cm/sec)	MW (g/mol)	MV (cm ³)	Log D (pH 7.4)	Log P	TPSA (Å ²)	% TPSA	Polariz-ability (10 ⁻²⁴ cm ³)	HBD	HBA	HB	Log S (mol/L)	Ionised Fraction (pH 7.4, %)	Cationic/Anionic/Neutral/Zwitterion	Ref Fp _{ml} and T _{max}
Abediterol	4	30	2.6	461	371	1.9	3.8	91	15.2	48	4	6	10	-2.7	99	Cationic	[32,33]
Aclidinium bromide	4.4	5	6.1	564	432	0.5	0.5	112	16.2	51	1	5	6	-4.0	100	Cationic	[34,35]
Adrogolide Hydrochloride	19.8	5	25.7	436	328	2.9	3.8	93	18.3	43	1	5	6	-3.4	96	Cationic	[36]
Amikacin	6.92	130	0.1	586	364	-9.8	-4.5	332	37.7	54	17	18	35	0.2	50	Cationic	[37][38]
Amiloride hydrochloride	14	30	1.6	266	109	-1.1	-0.9	159	49.8	20	8	8	16	-2.8	20	Cationic	[39]
Amphotericin B liposomal	0	ND	0.3	924	689	-2.2	0.3	320	21.3	95	13	18	31	-3.0	5	Anionic	[40][41]
Atropine	71.9	15	7.1	289	242	-0.5	1.9	50	11.9	32	1	4	5	0.3	99	Cationic	[42,43]
Aztreonam	16	60	0.3	435	237	-6.0	-1.2	238	42.2	38	5	13	18	0.4	100	Anionic	[44]
Batefenterol succinate	1.6	50	0.4	858	524	3.9	4.7	161	21.2	80	6	12	18	-5.9	65	Cationic	[45]
Beclomethasone dipropionate	2	12	8.9	521	410	4.2	4.2	107	14.3	53	1	7	8	-4.9	0	Neutral	[46,47]
Beclomethasone 17-MP	36	12	10.1	465	357	3.5	3.5	101	15.0	47	2	6	8	-4.7	0	Neutral	[46,47]
Bimosiamose disodium	5	30	0.4	907	609	-2.0	2.8	273	22.0	88	10	16	26	-1.9	100	Cationic	[48]
Budesonide	38	20	10.4	431	336	3.0	3.0	93	15.1	45	2	6	8	-4.0	0	Neutral	[24,49]
Caffeine	60	10	43.7	194	133	0.3	0.3	58	25.7	20	0	6	6	-0.8	0	Neutral	[50]
Cannabidiol	31	30	11.9	314	307	6.4	6.4	40	7.9	38	2	2	4	-5.2	1	Anionic	[51]
Ceftazidime	50	240	1.3	548	440	-6.9	-4.2	242	36.8	50	5	13	18	-3.1	100	Anionic	[52]
Ciclesonide	18	30	10.0	541	437	5.5	5.5	99	12.9	57	1	7	8	-4.6	0	Neutral	[53,54]
Ciclosporin	8.2	40	3.5	1203	1184	1.8	1.8	279	14.2	130	5	23	28	-5.0	0	Neutral	[55,56]
Ciprofloxacin	54	80	6.8	331	227	-2.5	-0.3	73	19.2	33	2	6	8	-3.2	90	Zwitterion	[57]
Cocaine	62.4	2.4	31.8	303	248	1.5	2.8	56	13.5	32	0	5	5	-1.1	93	Cationic	[58,59]
Colistin MS	6.7	300	0.8	1634	1109	-16.0	-13.5	734	27.7	152	23	44	67	-0.2	96	Anionic	[60]
Cromoglycate sodium	15	5	8.8	512	289	-3.0	1.8	166	31.0	43	3	11	14	0.3	100	Anionic	[61,62]
Denufosal tetrasodium	2.6	15	0.2	861	324	-15.1	-8.6	422	50.2	56	10	26	36	0.1	99	Anionic	[63]
Dihydroergotamine	77	10	2.3	584	401	2.6	2.8	118	16.4	63	3	10	13	-1.9	16	Cationic	[64,65]
DTPA99m Technecium	71	90	22.4	393	255	-6.5	-0.5	196	30.8	34	5	13	18	0.4	100	Anionic	[66]
Duramycin	0	ND	0.3	2015	1385	-12.8	-10.3	850	30.9	203	30	48	78	-7.0	8	Cationic	[67]
Epinephrine	6.6	20	1.2	183	143	-2.6	-0.7	73	24.7	20	4	4	8	0.7	95	Cationic	[68,69]
Ergotamine tartrate	43.3	3	2.3	732	393	2.4	2.6	118	24.5	63	3	10	13	-1.6	16	Cationic	[70]
Esketamine	70	20	61.9	238	203	2.1	2.1	29	8.9	26	1	2	3	-2.3	35	Cationic	[71,72]
Fenoterol	11	120	4.0	303	235	-0.5	1.2	93	20.1	34	5	5	10	0.5	93	Cationic	[73]
Fentanyl	98	0.3	23.8	336	309	3.0	4.1	24	4.8	41	0	3	3	-2.7	83	Cationic	[74,75]
Flumazenil	72	1	36.6	303	217	1.0	1.0	64	18.1	31	0	6	6	-2.6	0	Neutral	[76]
Flunisolide	34	10	8.1	435	326	2.5	2.5	93	15.5	43	2	6	8	-3.8	0	Neutral	[77]
Fluticasone furoate	13.4	10	11.6	539	386	3.9	3.9	119	17.7	51	1	6	7	-5.2	0	Neutral	[78,79]
Fluticasone propionate	14.5	90	9.9	501	377	3.7	3.7	106	16.0	48	1	5	6	-5.3	0	Neutral	[80,24,81]
Fumaryl diketopiperazine	23.1	7.5	0.5	452	353	-7.5	-2.8	191	29.6	44	6	12	18	0.3	100	Anionic	[82]
Gentamicin	15.5	180	0.8	478	367	-7.9	-2.2	200	27.3	49	11	12	23	0.3	51	Cationic	[83]
Glycopyrronium bromide	52.6	5	14.9	398	315	-0.8	-0.8	47	8.5	36	1	4	5	-1.9	100	Cationic	[84,85]
Heroin	53	2	32.6	369	274	1.3	1.9	65	14.2	39	0	6	6	-2.1	81	Cationic	[86,87,88]
Iloprost	80	5	8.5	360	298	1.0	3.6	78	13.0	42	3	4	7	-1.0	100	Anionic	[89]
Indacaterol acetate	34	15	2.9	453	307	2.1	3.6	82	18.7	45	4	5	9	-1.9	95	Cationic	[90]
Ipratropium bromide	6.9	10	14.8	412	332	-1.6	-1.6	47	8.1	38	1	4	5	-2.4	100	Cationic	[91]
Itraconazole	1.5	240	9.4	706	502	5.1	5.2	101	12.4	75	0	12	12	-6.6	99	Cationic	[92]
Levofloxacin	60	15	20.0	361	244	-2.1	0.2	73	17.3	36	1	7	8	-2.8	11	Anionic	[93,94,95]
Lidocaine	68.9	7	40.8	234	228	1.8	2.3	32	8.6	29	1	3	4	-1.3	76	Cationic	[96]
Mannitol	59	80	1.5	182	114	-3.3	-3.3	121	34.7	15	6	6	12	0.7	0	Neutral	[97]
Metamphetamine	81.0	150	48.6	149	164	-0.6	2.2	12	4.8	19	1	1	2	0.8	100	Cationic	[98,99]
Mometasone furoate	2.6	120	11.5	521	379	4.0	4.0	94	13.7	52	1	6	7	-5.1	0	Neutral	[100,101]
Morphine M6G	6	70	6.1	461	280	-3.8	-1.2	149	24.9	44	5	10	15	0.0	16	Anionic	[102]
Morphine sulfate	69.3	7	6.1	285	198	-0.1	0.7	53	27.9	31	2	4	6	-1.5	85	Cationic	[103,104,105]
Nedocromil sodium	7.8	20	23.4	371	252	-2.6	2.2	121	26.1	36	2	8	10	0.4	100	Anionic	[61,106]
Nemiralisib hemisuccinate	32.4	5	7.0	441	344	2.8	3.4	77	14.5	52	2	7	9	-2.9	80	Cationic	[107,108]
Nicotine	56	30	34.6	162	157	-0.4	0.8	16	6.7	20	0	2	2	0.8	89	Cationic	[109]
Nitroglycerin	0.45	7.8	23.1	227	136	2.4	2.4	165	51.7	16	0	12	12	-2.4	0	Neutral	[110]
Olodaterol	30	15	2.7	386	309	0.1	1.8	100	18.3	42	4	7	11	-0.8	93	Cationic	[111,112]
Oxytocin	4.6	20	0.5	1007	793	-4.4	-4.4	450	32.5	101	16	24	40	-2.7	11	Cationic	[113]
Oxytropium bromide	12.4	40	10.2	412	314	-2.1	-2.1	59	11.2	35	1	5	6	-1.4	100	Cationic	[91]
Pentamidine	5	55	1.0	340	281	-1.1	1.9	118	24.8	38	6	6	12	-2.8	100	Cationic	[114]
Prochlorperazine	100	0.3	19.0	374	307	4.2	4.8	35	8.5	43	0	3	3	-4.0	15.1	Cationic	[115]
Revefenacin	3	20	7.0	598	474	2.8	3.6	108	13.4	68	3	9	12	-5.2	99	Cationic	[116]
Salbutamol sulfate	19.1	15	3.4	239	208	-1.5	0.6	73	29.3	27	4	4	8	0.6	3	Cationic	[117,118]
Salmeterol xinofoate	13	10	3.4	604	373	1.9	3.9	82	15.3	48	4	5	9	-1.3	95	Cationic	[119]

Terbutaline sulfate	9.1	17	3.1	225	192	-1.6	0.5	73	30.8	25	4	4	8	0.7	5.3	Cationic	[120]
Tetrahydrocannabinol	21	5	17.9	314	310	7.2	7.2	29	6.0	38	1	2	3	-5.6	0	Neutral	[121,122]
Tiotropium bromide	19.5	5	9.0	472	333	-1.1	-1.1	116	21.3	40	1	5	6	-2.1	100	Cationic	[123,124]
Tobramycin	13.3	100	0.2	468	306	-9.4	-4.1	268	38.9	44	15	14	29	0.3	76	Cationic	[125,126,127]
Treprostinil sodium	68	10	8.2	391	337	1.3	4.9	87	13.8	43	3	5	8	-1.7	100	Anionic	[128]
Triamcinolone acetonide	10.4	40	8.5	435	325	2.6	2.6	93	15.5	43	2	6	8	-4.0	0	Neutral	[129]
Umeclidinium bromide	12.8	5	7.2	508	423	0.9	0.9	29	4.3	51	1	3	4	-5.0	100	Cationic	[130]
Vilanterol trifenate	27.3	7	3.0	486	387	2.0	4.0	91	13.1	51	4	6	10	-2.0	95	Cationic	[131]
Zaleplon	100	2	36.4	305	244	1.5	1.5	74	19.0	36	0	6	6	-3.8	0	Neutral	[132]
Zanamivir	11.4	100	0.3	332	190	-5.8	-3.3	201	40.7	28	9	11	20	-0.5	0	Neutral	[133]
AZD5423	38.3	10	8.8	487	366	5.2	5.2	65	11.8	48	1	6	7	-6.4	0	Neutral	[134,135]
AZD7594	29.6	15	4.7	607	430	3.0	4.6	113	15.8	61	2	10	12	-4.4	1	Anionic	[136]
GLP-1 MKC253	1	5	0.5	3355	2267	-24.3	-9.4	1420	29.7	331	56	87	143	-3.5	37	Cationic	[137]
HMR1031	24.6	45	3.9	628	491	1.1	4.3	148	17.0	69	4	11	15	-1.6	100	Anionic	[138]
IVL745	26.3	30	1.0	593	456	-0.5	2.7	156	19.4	64	4	12	16	-1.3	100	Anionic	[139]
Mean	30.5	ND	11.0	529	386	-0.8	1.0	154	21	53	4.8	9.9	15	-2.3			
SD	28.0	ND	13.0	441	305	5.3	3.8	201	11	43	7.9	11.9	20	2.1			
Median	19.5	15.0	7.0	436	325	0.5	1.9	99	18	43	2	6	8	-2.1	80		
Min	0.0	0.3	0.1	149	109	-24.3	-13.5	12	4	15	0	1	2	-7.0	0		
Max	100	300	61.9	3355	2267	7.2	7.21	1420	52	331	56	87	143	0.83	100		

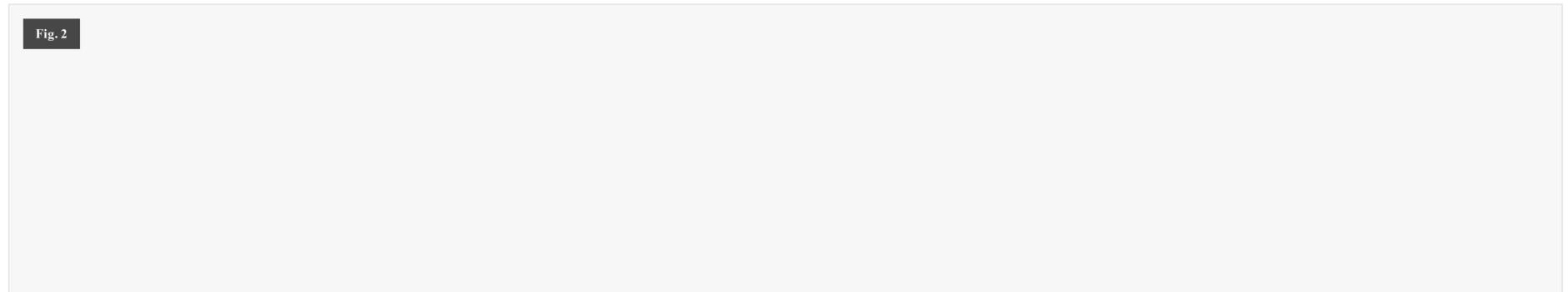
The mean in silico permeability was $11.0 \pm 13.0 \cdot 10^{-6}$ cm/sec. Our set of compounds contained 3 of the model drugs proposed by the FDA for permeability classification, namely caffeine in the high permeability group, and amiloride and terbutaline in the moderate permeability group [140]. The in silico permeability of caffeine ($43.7 \cdot 10^{-6}$ cm/sec) was much higher than that of amiloride ($1.6 \cdot 10^{-6}$ cm/sec) and terbutaline ($3.1 \cdot 10^{-6}$ cm/sec). Since the in silico permeability in our data set ranged from $0.1 \cdot 10^{-6}$ cm/sec (amikacin) to $61.9 \cdot 10^{-6}$ cm/sec (esketamin), it can be considered that the data set provide a good representability of solute permeability. For ionized compounds, Papp was computed for the ionized form of the active moiety and not for the salt. However, there was usually a small difference between the values estimated (e.g., Papp of ipratropium were 14.8 and 11.4 for ipratropium [CC(C)[N+](C2CCC1CC(C2)OC(=O)C(CO)C3=CC=CC=C3)C] and for ipratropium bromide, respectively). For the four other quaternary quaternised muscarinic M3 receptor antagonists the differences in Papp were lower (2.4, 0.5, 0.4 and 1.8 for acclidinium, oxytropium, tiotropium and umeclidinium, respectively).

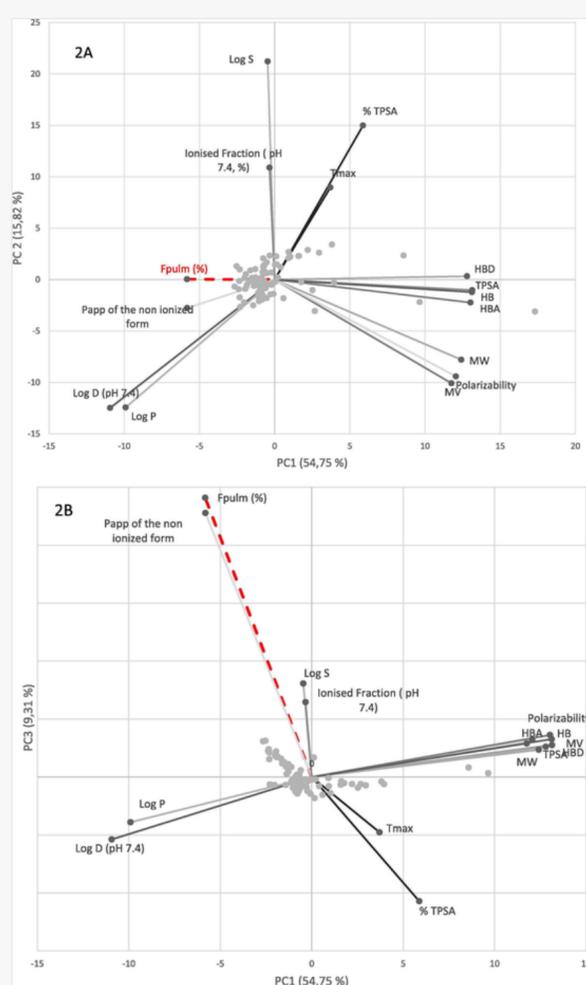
The pulmonary bioavailability of the compounds ranged from nearly zero to 100%. Not surprisingly, compounds used for a systemic delivery had a higher pulmonary bioavailability than those intended for a local delivery ($49.1 \pm 33.2\%$ vs $22.0 \pm 20.8\%$). The distribution of F_{pulm} was clearly asymmetric with an over-representation of drugs with low pulmonary bioavailability values (Fig. 1 top) and logit F_{pulm} showed a symmetric distribution (Fig. 1 bottom) so that logit F_{pulm} could be more suitable for regression models.



3.2 - principal component analysis

The principal components F1 and F2 explained 70.6% of the total data variance in descriptors from the original data set while F1 and F3 explained 64.1% of the total variance (Fig. 2A and Fig. 2B).





Ordination biplots of principal component analysis (PCA) outputs of the physico-chemical space. (A) corresponds to the first principal component (PC1) that captures 54.75% of the variability versus the second compound PC2 (15.82%). (B) corresponds to the plot of the third principal component (PC3) (9.31%) versus PC1.

Fig. 2B showed that the variables most correlated to F1 were HB (HBA + HBD), MW, MV (cm^3), TPSA, and polarizability. Conversely, log D and log P were negatively correlated to F1. Concerning the second component (F3), we observed that Fpulm was positively correlated with Papp and negatively correlated with %TPSA.

The PCA analysis showed that the first dimension (F1, x-axis) is related to size (MW, MV), lipophilicity (LogD, LogP), and polarity (TPSA, polarizability and HB features) with a quite similar contribution of these variables (around 10% each) (Table 4), and an absolute value of coefficient of correlation > 0.70 (excepted for %TPSA coefficient of correlation of 0.45). This is shown on the score-plot where these parameters are plotted to the borders of the plot.

Table 3

Performance of the different models used to predict the pulmonary bioavailability (Fpulm) ranked according to RMSE value. Since the distribution of the pulmonary bioavailability may not be symmetric both models were used to predict Fpulm either as percentage or as its logit (excepted for beta regression that can only be used for percentages). For models trained with logit Fpulm, predictions are back-transformed by logit inverse to return to Fpulm as percentage.

Type of algorithm	Variable to be predicted	PCC	R ²	RMSE	MAE
Random forest	Fpulm (%)	0.624	0.585	19.4	15.8
Random forest	logit Fpulm	0.647	0.557	19.6	15.7
Gradient boosting	logit Fpulm	0.621	0.535	20.3	16.1
Support vector machine	logit Fpulm	0.670	0.588	20.7	16.6
Multiple linear regression	Fpulm (%)	0.677	0.536	20.7	16.9
Support vector machine	Fpulm (%)	0.627	0.525	20.8	16.7
Multiple linear regression	logit Fpulm	0.679	0.527	22.2	17.5
Beta regression	Fpulm (%)	0.557	0.500	22.1	16.7
Gradient boosting	Fpulm (%)	0.576	0.550	22.4	18.9

PCC (pearson's correlation coefficient), R² (coefficient of determination), RMSE (root mean square error) and MAE (mean absolute error)
 logit Fpulm = $\log(\text{Fpulm}/(1 - \text{Fpulm}))$

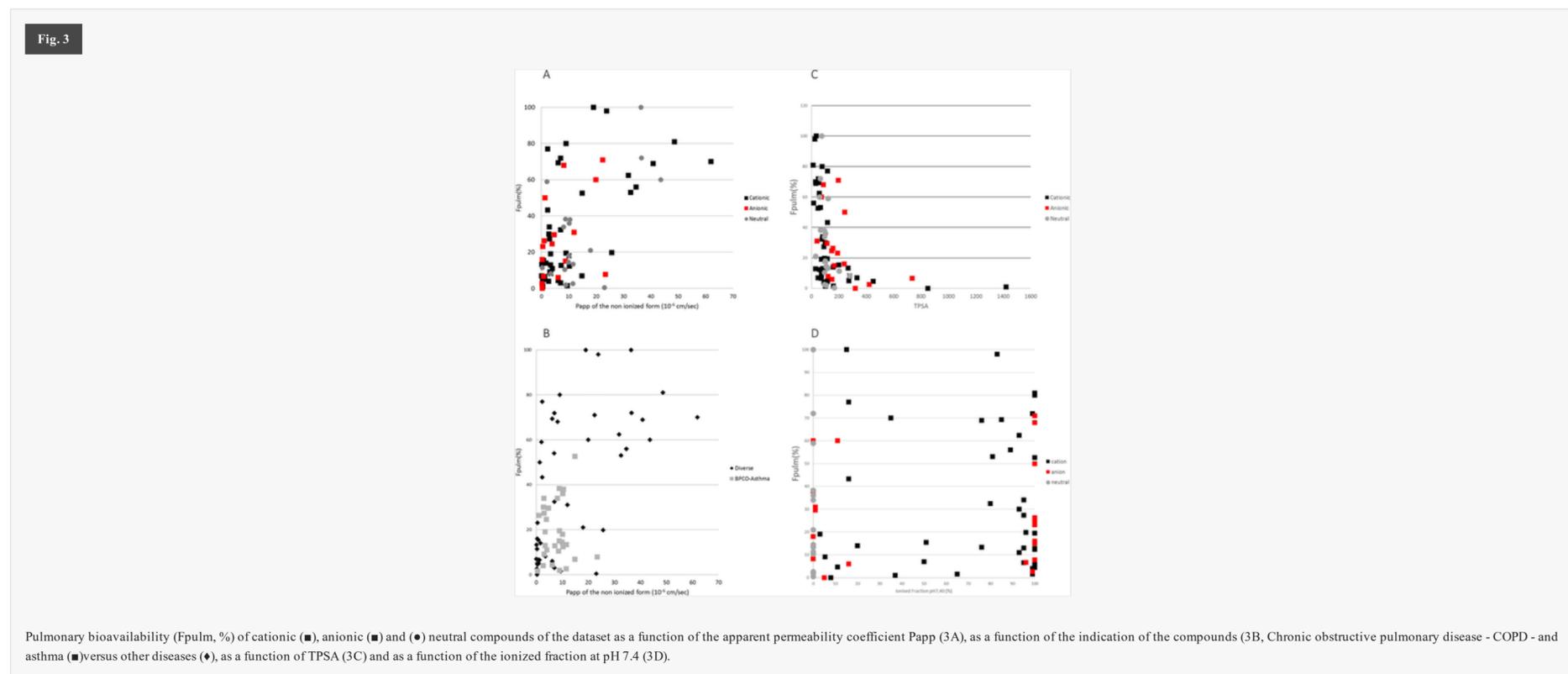
Table 4

Characteristics of principal component analysis (PCA): projections onto the plane defined by PC1 (capturing 54.75% of the variability) and PC3 (capturing 9.31% of the variability). The section on the left side of the table presents the relative contributions of the different variables to the main components (PC1 and PC3). The section on the right shows the corresponding absolute values of coefficient of correlation indicating the strength of correlation.

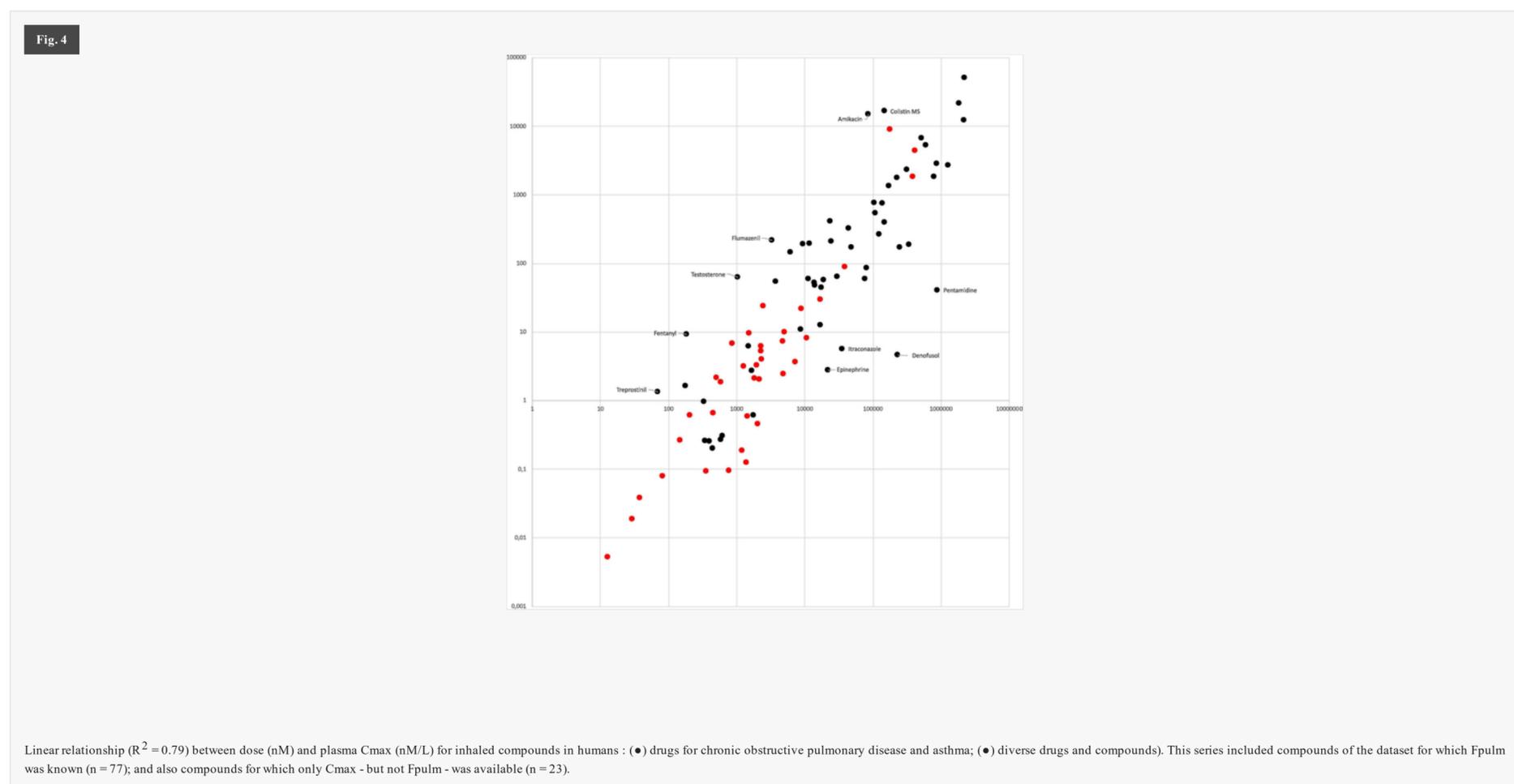
	Contribution of variables (%)		Absolute value of coefficient of correlation	
	PC1	PC3	PC1	PC3
Fpulm (%)	2.3	40.1	-0.438	0.748
Tmax (min)	0.9	1.6	0.279	-0.148
Papp (10^{-6} cm/sec)	2.3	35.8	-0.438	0.707
MW (g/mol)	10.6	0.9	0.934	0.073
MV (cm^3)	9.5	0.6	0.885	0.090
Log D (pH 7.4)	8.2	2.0	-0.823	-0.167
Log P	6.8	1.1	-0.746	-0.121
TPSA (Å^2)	11.9	0.5	0.989	0.086
% TPSA	2.4	7.9	0.442	-0.332
Polarisability (10^{-24} cm^3)	10.0	0.7	0.907	0.100
HBD	11.3	0.5	0.963	0.081
HBA	11.7	0.9	0.980	0.113
HB	11.9	0.7	0.988	0.101
Log S (mol/L)	0.02	4.5	-0.035	0.250
Ionised Fraction (pH 7.4, %)	<0.01	2.9	-0.025	0.201

3.3 Determinants of Fpulm and Cmax

Data in Fig. 3 showed a significant variability in the relationship between Fpulm and Papp (Fig. 3A), with lower Papp coefficients drug used for either asthma and/or COPD (Fig. 3B). Fig. 3C showed a decrease in Fpulm when TPSA increased for both cationic, anionic and neutral compounds. Data in Fig. 3D showed that the ionization fraction did not apparently play a role in the extent of pulmonary bioavailability.



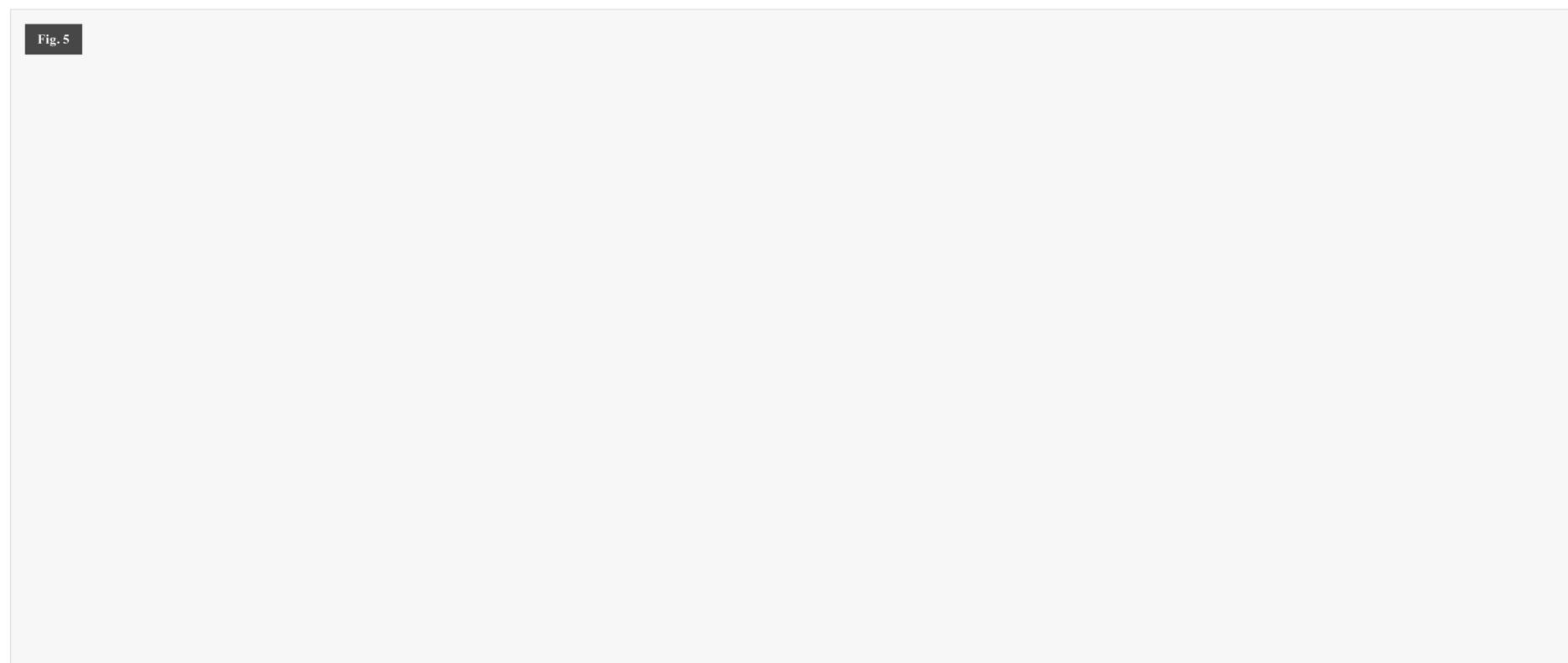
The Fig. 4 displays a linear relationship between the dose in humans and Cmax in plasma for a range of diverse drugs (including those for which F pulm was known - n = 77; and some compounds for which only Cmax was available, n = 33).

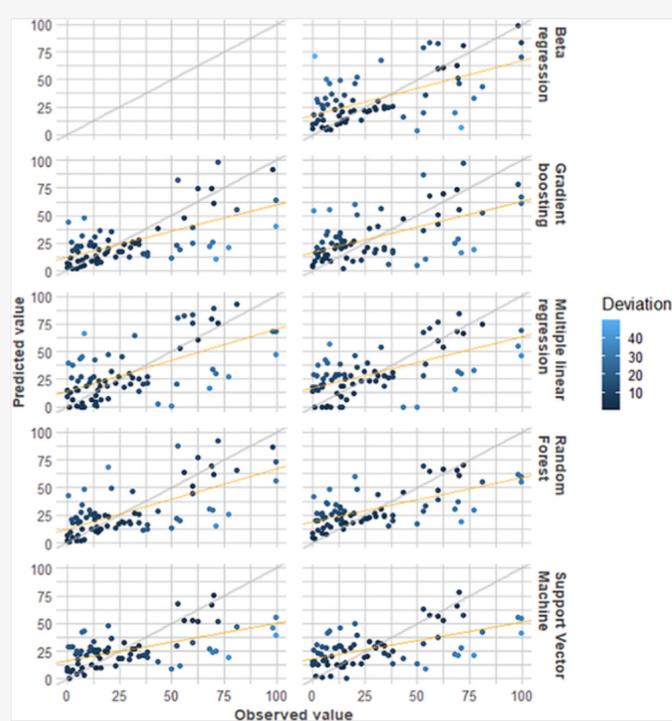


3.4 Model evaluation

Data in Table 3 shows the performance indicators of the different models ranked as a function of RMSE. Random forest algorithm (based on Fpulm or logit Fpulm) displayed the best performance on both indicators with an apparent advantage for the model based on Fpulm.

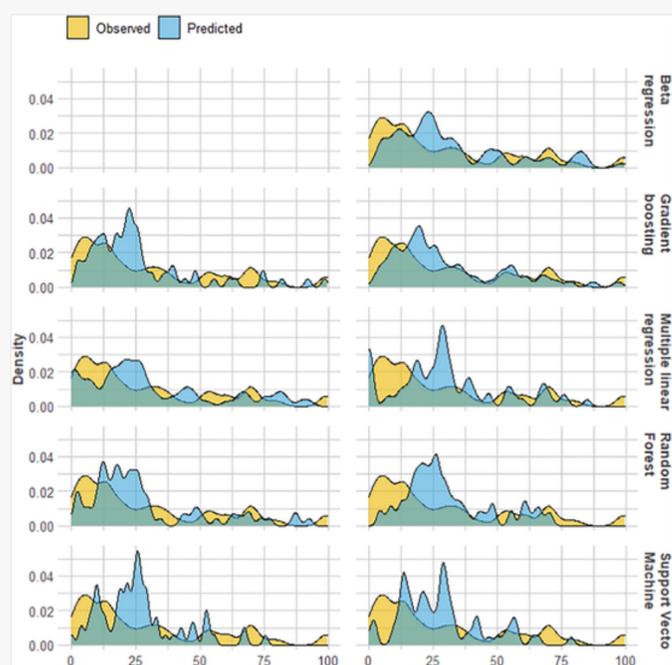
The results of the prediction of Fpulm with the different models are illustrated in Fig. 5. As a whole, all the models trained on the pulmonary bioavailability (Fpulm) are poorly calibrated, with the exception of the beta regression model. The comparative distribution of predicted and observed values of Fpulm is depicted in Fig. 6.





Graphical representation of the predicted pulmonary bioavailability F_{pulm} (y axis, in %, and regression in yellow line) as a function of the observed F_{pulm} value (x-axis, in %) by the different models (Beta regression, Gradient boosting trees, Multiple linear regression, Random forest algorithm and Support vector machine regression). Both these models were used to predict F_{pulm} either as percentage or as its logit (excepted for beta regression that can only be used for percentages). Left column : models trained on logit F_{pulm} , and right column : models trained on F_{pulm} . An iterative procedure with a 10-fold cross validation procedure was used. At each step, 9 blocks (90% of the observations) were used to construct the model, and the 10th block was used to validate the model. This procedure was runned 10-times iteratively so that the 10 blocks were successively used as test data-sets. This cross-validation procedure was repeated 5-times to avoid that the results are linked to the block division. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6



Comparison of the distribution of predicted and observed values of systemic pulmonary bioavailability (F_{pulm}) by the different models. Left column: models trained on logit F_{pulm} , and right column : models trained on F_{pulm} .

4 Discussion

The barriers to systemic delivery through (via) the lungs vary according to the site where the drug is deposited (i.e., bronchi, terminal bronchioles or alveoli). In humans, the epithelial surface is a few meters square at the airways, and more than 100 m^2 at the alveolar surface. Beyond surface features, the lung epithelium and the liquid layer become thinner as drugs molecules penetrate deeper in the lung [3]. This suggests that the best deposition area for drug molecules to obtain a high absorption is the alveolar area. However, alveolar deposition is quite challenging because it requires aerosol particles with an aerodynamic diameter of about $1\text{--}2 \mu\text{m}$, with a slow and deep inhalation. Thus, absorption at the bronchiole area (a less challenging deposition area) is likely to participate to the systemic delivery.

In an excellent review on the systemic delivery of drugs through the lung, Patton and Byron (2007) indicated that small hydrophobic molecules are absorbed into the systemic circulation by passive diffusion through the epithelium while small hydrophilic molecules are absorbed paracellularly via aqueous pores in the tight junction or via transporters [141]. Interestingly, given the higher density in tight junctions in the distal airways compared to the alveoli, absorption in the bronchioles in the distal airways (right before the alveoli) could be an optimal site for absorption of hydrophilic molecules, especially for small peptides. Hence, the rate of absorption of hydrophilic molecules should be influenced by a combination of molecular weight (or molecular volume) and degree of ionization.

Complexity in drug absorption may also come from the interplay between permeability and dissolution in the liquid layers covering the epithelia that have different qualitative and quantitative compositions and volumes depending on the site of absorption (alveolar space or conducting airways).

The drivers of systemic pulmonary absorption have only been studied *ex vivo* and *in vivo* in animal models without significant findings on the determinants of the extent of absorption [8,9]. To fill this gap, and avoid the question of translatability of findings in preclinical species to humans, we analyzed the literature reporting absolute systemic bioavailability after pulmonary administration (F_{pulm}) or reporting data allowing to calculate F_{pulm} . This allowed us to search for correlations with permeability and physico-chemical descriptors, and to set up models for predicting pulmonary absorption. These *in silico* models are built on a rather large and diverse data set, and the current study is the only one available to date with a human data set.

4.1 Determinants of F_{pulm}

PCA is a usual tool to extract and rationalize the information from a biological system with a multivariate description by complexity reduction and data simplification. F1 and F2 did not fully represent our variable of interest F_{pulm} , so that principal components F1 and F3 (Fig. 2) were considered to better describe the most important descriptors of the pulmonary bioavailability (F_{pulm}).

For the F3 component, the graph suggests that Fpulm is positively correlated with Papp permeability (with a contribution of about 40% and a correlation coefficient > 0.70, Table 4) and negatively correlated with %TPSA (to a lesser extent, about 10%, with a correlation coefficient of -0.35).

Intestinal absorption has been shown to be influenced by Papp in a sigmoidal relationship, and drugs almost completely absorbed have Papp coefficient $> 1 \times 10^{-6}$ cm/sec, while drugs absorbed to < 1% have Papp coefficients of $\leq 1 \times 10^{-6}$ cm/sec [142]. Although there was a significant variability in the relationship between Fpulm and Papp, it appeared that the neutral and cationic compounds with Papp coefficients $> 30 \times 10^{-6}$ cm/sec have Fpulm > 50% (Fig. 3). Compared to the oral route, it appeared that higher Papp coefficient values are necessary to allow a significant (>50%) extent in pulmonary systemic bioavailability.

It also appeared that drug used for either asthma and/or COPD have Papp coefficients lower $< 30 \times 10^{-6}$ cm/sec with a pulmonary systemic bioavailability as a whole lower than 40% (Fig. 3B). Such a systemic bioavailability is far from being insignificant for drugs intended to have only a local action, and may likely led to systemic adverse effects. However, systemic side effects depend not only from the magnitude of the amount reaching the blood (via pulmonary route and via oral route for the swallowed fraction) but also from the magnitude of the systemic tissue distribution and blood clearance.

The fact that Fpulm is negatively correlated with %TPSA and not with TPSA (Fig. 1A) is not surprising. Indeed, %TPSA should be more relevant than TPSA when comparisons are made on a series of compounds in a wide range of MW (149–2,015 g/mol in our dataset). Stronger correlations with %TPSA (compared to TPSA) and pulmonary biopharmaceutical parameters (F and ka) have been found between in a previous study in rats carried out with a data set of 10 compounds [8]. Moreover, considering intestinal absorption of drugs in humans, TPSA criterion has been considered as not reliable to identify poor or good drug absorption [143]. Data in Fig. 3C showed a decrease in Fpulm when TPSA increased suggesting that such parameter need to be considered for the development of a drug to be administered via pulmonary route for a systematic action. This could be quite challenging for the systemic delivery via pulmonary route of polar drugs such as peptides for which pulmonary administration may be of interest compared to oral administration where the environment is rather harsh [144]. However, pulmonary metabolism should be considered although it is still much less intense than via oral route [145,146].

It also appeared that the solubility (estimated by Log S) did not play a significant role in the extent of pulmonary bioavailability. However, in silico solubility in water at pH 7.40 may not be a relevant parameter to be considered since inhaled drugs come in contact either to mucus layer at the upper airways or to lung surfactant layer in the lower airways and alveoli. Dissolution fluids used for the assessment of orally inhaled compounds are usually based on water, salt solutions, which composition is similar to the aqueous phase of the surfactant, or on PBS pH 7.4 [147]. However, it should be emphasized that the estimation of the solubility in a solution at pH 7.40 is a somewhat approximation of the actual solubility at the epithelial interface. Indeed, the pH at the lung level is considered slightly acidic [148] and has been shown to vary quite substantially [149]. If normal pH is regulated around neutral, variations from pH of 4.5 to 8.5 have been reported for a number of airway conditions [150]. Besides the pH, the composition of the epithelial lining fluid may influence the dissolution with differences in composition at the conducting airways (mainly represented by a mucus layer) and at the respiratory airways (surfactant layer with aqueous subphase). Studies have suggested biorelevant synthetic lung fluid suitable to study biopharmaceutical properties of inhalation drugs [151].

Moreover, in a recent paper [152], the role of mucus layer has been studied as well as the interplay between dissolution and absorption. They showed that low solubility in water is not per se a limiting factor to lung absorption of drugs as encountered at the gastro-intestinal level. Furthermore, the mucus may have solubility enhancing properties that may favor drug solubilization in the upper airways. At the lower airways and alveoli levels, the presence of surfactants composed of around 90%-10% lipid and mixture of proteins is thought to increase the solubilization of poorly water soluble drugs and as such is considered as a way to increase dissolution [153]. Hence, biorelevant in vitro dissolution methods are needed to provide accurate estimation of drug solubility at the pulmonary level. However, dissolution rate may be more relevant than solubility in the prediction of pulmonary absorption, at least in the upper regions of the lung, because there is a kinetic competition between systemic absorption and non-absorptive elimination by mucociliary clearance [154]. Evaluation of dissolution rate may require dedicated techniques enabling a kinetic assessment for defined-size and respirable aerosol drug particles generated from inhaler products [155]. Different formulations of the compounds (solution or solid particles: suspension or dry powders) have been used in the studies, and we did not consider the formulation per se as a parameter. Indeed, soluble compounds are usually used as solutions, but their administration as solid particles should not influence the absorption; neither rate nor extent. However, administration of compounds with very low solubility as solid particles (suspension or dry powders) or as a solution might influence the systemic bioavailability. However, for these drugs, the use of solid particles should preferentially alter the rate of absorption with a prolonged systemic absorption rather than the extent of absorption.

Data in Fig. 3D and in Table 2 (ionization fraction, %) showed that the ionization fraction, either for anionic or for cationic compounds, did not play a role in the extent of pulmonary bioavailability. Indeed, anionic and cationic solutes with low (<20%) and high (>80%) ionization fractions has variable Fpulm ranging from zero up to almost 100%.

As a whole, the current study showed that the extent of systemic absorption was essentially dependent on permeability and on the percentage of topological polar surface area without significant influence of solubility and ionization fraction, and no apparent link with lipophilicity and drug size parameters.

4.2 Absorption rate

Eventhough Tmax is not *per se* the best parameter to estimate the absorption rate, our data showed that hydrophilic drugs (logP < 0) had a 5-fold higher Tmax that lipophilic drugs (median Tmax : 60 min versus 12 min, $p < 0.005$, Mann–Whitney *U* test) suggesting a significant positive influence of lipophilicity on absorption rate. This difference was not biased by differences in the size of the compounds because their median MW and MV were close (462 versus 435 g/mol, and 324 versus 325 cm³ for hydrophilic and lipophilic compounds, respectively). These observations are in agreement with a previous in vivo experiment performed in rats with a small series of compounds where log ka was correlated with Papp and inversely correlated with HBD and %PSA [8].

The charge may apparently have a small influence the absorption rate since median Tmax of anionic (20 min), cationic (15 min) and neutral (12 min) compounds were very close. However, the influence of charge on absorption may be quite complex especially for cationic drug, and should take in consideration other factors related to mucus and to tight junction. A recent in vitro study has suggested that drugs with a positive charge at neutral pH may bind by electrostatic interactions to negatively charged mucins with a subsequent negative impact on transport through membranes. This has been shown with salbutamol sulfate, a highly soluble drug, whose transport through ALI Calu-3 layer was hindered when mucus was added [152]. Systemic pulmonary absorption results not only from passive diffusion through the epithelium but also from paracellular diffusion via aqueous pores in the tight junctions. Tight junctions contribute to barrier function, and restrict the diffusion of solutes with size and charge selectivity. At the pulmonary level, channel-forming claudins have a cation channel selectivity [156] favoring the diffusion of polar cationic solutes.

Drug trafficking of cationic drugs in the lung may also be influenced by trapping in the lysosomal sub-compartment that has been identified as a mechanism involved in local retention [157]. Indeed, accumulation in the lysosome, as a result of a difference between influx and efflux rate, occurs from drugs that are predominantly protonated with the lysosome (i.e., basic drugs such as β -agonists). In an in vitro study performed with lung slices, salbutamol which is a short-acting antagonist was not trapped in lysosomes while salmeterol and indacaterol which are a long-acting antagonists accumulated in the lysosome retention [157]. If trapping in the lysosome should influence the pharmacodynamic profile, it does not seem to influence their systemic pulmonary bioavailability and their absorption rate that were quite close for these drugs (Table 2). This also apparently holds true for olodaterol (Table 2) although the hypothesis of a lysosomal trapping has been put forward to explain that a fraction of the olodaterol deposited in the lung was slowly absorbed in the systemic circulation [112].

Cmax is a critical component of the systemic exposure, especially for administration routes where a rapid absorption may occur such as the pulmonary route. Moreover, high systemic levels may result in adverse effects. A significant linear relationship between Cmax and dose ($R^2 = 0.79$, Fig. 4) suggests that Cmax is primarily dependent on dose and absorption rate [158]. The effect of systemic clearance on Cmax should not be considered as significant given that the rate of systemic absorption from the lungs is usually rapid compared to the rate of elimination from plasma. Such a correlation may be used to estimate Cmax in humans for new inhaled drugs.

4.3 Model evaluation

The performance indicators of the different models showed that random forest algorithm showed the best performance with an apparent advantage for the model predicting raw Fpulm. Support vector machine led to performance quite close to random forest algorithm, but gradient boosting algorithm surprisingly led to disappointing performances even though it is state-of-the-art algorithm found to outperform other algorithm in many domains. it was supposed to perform better. This may come either from the hyperparameters tested that did not allow to find the suitable combination to resolve the solution or to a problem of overfitting as a result of a too small data set.

Overall, the models presented a prediction error of less than 20% and captured approximately 50–60% of the variability in systemic pulmonary bioavailability. These performances are relatively average but not surprising given the limited size of the data set. Moreover, it should be noticed that drug trafficking at the lung level is quite complex and that other potentially relevant parameters should have been considered and helped improving the predictive value of the model. However, it should be noticed that the choice of the algorithm is still important since random forest algorithms capture almost 10% more variability compared to a linear regression.

As a whole, all the models trained on the pulmonary bioavailability (Fpulm) are poorly calibrated (Fig. 5), with the exception of beta regression. A model is well calibrated when it provides predictions which do not underestimate or overestimate the real values throughout the range of possible values. As a result of the underrepresentation of high Fpulm values (Fig. 1 Top), the models tend to overestimate the low values of Fpulm and to underestimate the high values. This problem is relatively well corrected by the use of the logit transformation of the variable to predict (logit Fpulm), even if the overall performance of random forest and support vector machine algorithms run on logit Fpulm was slightly lower.

The comparative distribution of predicted and observed values of Fpulm (Fig. 6) showed that multiple linear regression run on logit Fpulm led to the apparent better superposition. However, this does not indicate that for a specific drug the prediction will be close to the observed value.

As a whole, the most interesting model was the random forest algorithm model run on logit Fpulm. The statistics clearly indicate that the models are not highly predictive but may be interesting for a semi-quantitative ranking.

The first limitation resides in the size of the data set combined with the diversity of the compounds that precludes the development of models with high performance and prediction capabilities as shown for the intestinal absorption where huge data sets are available. Limitation in our model also arises from the fact that all relevant parameters involved in drug trafficking and permeation at the lung level have not been taken into consideration. Indeed, permeability in respiratory cell culture models, impact of transporters, mucus-drug interactions, solubility in situ for our drug sample set would enhance the quality of the prediction of our model. Furthermore, extrapolation to patients with pulmonary diseases should be made with caution because less efficient pulmonary drug deposition may occur, especially for deep lung targeting [159].

Since there is no large, single-source Caco-2 (or lung cell lines) permeability datasets where we could find Papp values for our dataset, we decided to use in silico Papp values. The potential inclusion of discrepant Caco-2 permeability data during in silico model development can lead to misleading information and may also constitute a limitation to be considered. Indeed, identification of the types of compounds for which Caco-2 cells may be not relevant in accurately predicting their human intestinal permeabilities has to be considered. Substrates of transporters highly expressed in small intestinal cells may be such compounds. Hydrophilic compounds usually believed to use the paracellular pathway rather than transcellular pathway as a result of too small lipophilic properties to cross intestinal membranes may also be concerned. These potential discrepancies may question the relevance of the use of Caco-2 cell-based Papp in estimating lung permeability eventhough Caco-2 cell-related permeability measurements have been shown to give good predictions of the passive transcellular permeability of lung epithelium to drugs [26]. As mentioned above, the Papp of some specific types of compounds may not be adequately estimated from in silico intestinal Papp models, and quaternised compounds may be such drugs (and an over-estimation of their Papp is likely). Substrates of transporters expressed at the lung level is an other issue that should be taken into account. Hence for a better understanding of lung biopharmaceutics, large, single-source lung cell lines permeability datasets are warranted.

These limitations may have decreased the predictability of our model but we have developed specific algorithms to improve the model performance, and the current data set is to our knowledge the only human-based data set on systemic pulmonary data.

5 Conclusions

The extent of absorption in humans was found to correlate positively with Papp and negatively with %TPSA without a significant influence of other classical physico-chemical descriptors. Compared to the oral route, higher Papp coefficient values should be required to allow a significant (>50%) extent in systemic bioavailability. With regard to absorption rate, estimated by Tmax, lipophilicity, polarity descriptors and Papp may have a more preminent influence on absorption rate via passive diffusion compared to charge.

The current investigation provides new in vivo biopharmaceutic data on the systemic absorption after oral inhalation in humans, and information on the main drivers of extent and rate of systemic absorption. However, due to the complexity of the environment and of the drug trafficking that a drug encounters throughout the pulmonary tree, larger databases of high quality will be necessary to adequately model and predict human systemic bioavailability after oral inhalation, and to set up a

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Bäckman P, Adelman H., Petersson G., Jones C.B., Advances in Inhaled Technologies: Understanding the Therapeutic Challenge, Predicting Clinical Performance, and Designing the Optimal Inhaled Product, *Clin. Pharmacol. Ther.* 95 (5) (2014) 509–520, doi:10.1038/clpt.2014.27.
- [2] Strong P, Ito K., Murray J., Rapeport G., Current approaches to the discovery of novel inhaled medicines, *Drug Discov. Today* 23 (10) (2018) 1705–1717.
- [3] Patton J.S., Byron P.R., Inhaling medicines: delivering drugs to the body through the lungs, *Nat. Rev. Drug. Discov.* 6 (1) (2007) 67–74.
- [4] Ehrhardt C., Bäckman P., Couet W., Edwards C., Forbes B., Fridén M., et al., Current Progress Toward a Better Understanding of Drug Disposition Within the Lungs: Summary Proceedings of the First Workshop on Drug Transporters in the Lungs, *J. Pharm. Sci.* 106 (9) (2017) 2234–2244.
- [5] Nickel S., Clerkin C.G., Selo M.A., Ehrhardt C., Transport mechanisms at the pulmonary mucosa: implications for drug delivery, *Expert Opin. Drug Deliv.* 13 (5) (2016) 667–690.
- [6] Gumbleton M., Al-Jayyousi G., Crandon-Lewis A., Francombe D., Kreitmeyr K., Morris C.J., et al., Spatial expression and functionality of drug transporters in the intact lung: objectives for further research, *Adv. Drug Deliv. Rev.* 63 (1–2) (2011) 110–118.
- [7] Tronde A., Nördén B., Jeppsson A.-B., Brunmark P., Nilsson E., Lennernäs H., et al., Drug absorption from the isolated perfused rat lung—correlations with drug physicochemical properties and epithelial permeability, *J. Drug Target.* 11 (1) (2003) 61–74.
- [8] Tronde A., Nördén B., Marchner H., Wendel A.-K., Lennernäs H., Bengtsson U.H., Pulmonary absorption rate and bioavailability of drugs in vivo in rats: structure-absorption relationships and physicochemical profiling of inhaled drugs, *J. Pharm. Sci.* 92 (6) (2003) 1216–1233.
- [9] Edwards C.D., Luscombe C., Eddershaw P., Hessel E.M., Development of a Novel Quantitative Structure-Activity Relationship Model to Accurately Predict Pulmonary Absorption and Replace Routine Use of the Isolated Perfused Respiring Rat Lung Model, *Pharm. Res.* 33 (11) (2016) 2604–2616.
- [10] Sakagami M., In vitro, ex vivo and in vivo methods of lung absorption for inhaled drugs, *Adv. Drug Deliv. Rev.* (2020).
- [11] Liu X., Jin L., Upham J.W., Roberts M.S., The development of models for the evaluation of pulmonary drug disposition, *Expert Opin. Drug Metab. Toxicol.* 9 (4) (2013) 487–505.
- [12] Bosquillon C., Madlova M., Patel N., Clear N., Forbes B., A Comparison of Drug Transport in Pulmonary Absorption Models: Isolated Perfused rat Lungs, Respiratory Epithelial Cell Lines and Primary Cell Culture, *Pharm. Res.* 34 (12) (2017) 2532–2540.
- [13] Fernandes C.A., Vanbever R., Preclinical models for pulmonary drug delivery, *Expert Opin Drug Deliv.* 6 (11) (2009) 1231–1245.
- [14] Borghardt J.M., Weber B., Staab A., Kloft C., Pharmacometric Models for Characterizing the Pharmacokinetics of Orally Inhaled Drugs, *AAPS J.* 17 (4) (2015) 853–870.
- [15] Bhagwat S., Schilling U., Chen M.-J., Wei X., Delvadia R., Absar M., et al., Predicting Pulmonary Pharmacokinetics from In Vitro Properties of Dry Powder Inhalers, *Pharm. Res.* 34 (12) (2017) 2541–2556.
- [16] Hochhaus G., Horhota S., Hendeles L., Suarez S., Rebello J., Pharmacokinetics of Orally Inhaled Drug Products, *AAPS J.* 17 (3) (2015) 769–775.
- [17] Fröhlich E., Biological Obstacles for Identifying In Vitro-In Vivo Correlations of Orally Inhaled Formulations, *Pharmaceutics* 11 (7) (2019).
- [18] Barros A.S., Costa A., Sarmiento B., Building three-dimensional lung models for studying pharmacokinetics of inhaled drugs, *Adv. Drug Deliv. Rev.* (2020).
- [19] Eriksson J., Thörn H., Lennernäs H., Sjögren E., Pulmonary drug absorption and systemic exposure in human: Predictions using physiologically based biopharmaceutics modeling, *Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft Pharm Verfahrenstechnik EV.* 156 (2020) 191–202.
- [20] Ehrhardt C., Inhalation Biopharmaceutics: Progress Towards Comprehending the Fate of Inhaled Medicines, *Pharm. Res.* 34 (12) (2017) 2451–2453.
- [21] Ericsson T., Fridén M., Kärman-Mårdh C., Dainty I., Grime K., Benchmarking of Human Dose Prediction for Inhaled Medicines from Preclinical In Vivo Data, *Pharm. Res.* 34 (12) (2017) 2557–2567.
- [22] Hendrickx R., Lamm Bergström E., Janzén D.L.I., Fridén M., Eriksson U., Grime K., et al., Translational model to predict pulmonary pharmacokinetics and efficacy in man for inhaled bronchodilators, *CPT Pharmacomet. Syst. Pharmacol.* 7 (3) (2018) 147–157.
- [23] Cipolla D., Chan H.-K., Current and Emerging Inhaled Therapies of Repositioned Drugs, *Adv. Drug Deliv. Rev.* 133 (2018) 1–4.
- [24] Thorsson L., Edsbäcker S., Källén A., Löfdahl C.G., Pharmacokinetics and systemic activity of fluticasone via Diskus and pMDI, and of budesonide via Turbuhaler, *Br. J. Clin. Pharmacol.* 52 (5) (2001) 529–538.
- [25] Larregieu C.A., Benet L.Z., Drug discovery and regulatory considerations for improving in silico and in vitro predictions that use Caco-2 as a surrogate for human intestinal permeability measurements, *AAPS J.* 15 (2) (2013) 483–497.
- [26] Eriksson J., Sjögren E., Thörn H., Rubin K., Bäckman P., Lennernäs H., Pulmonary absorption - estimation of effective pulmonary permeability and tissue retention of ten drugs using an ex vivo rat model and computational analysis, *Eur. J. Pharm. Biopharm.* 124 (2018) 1–12.
- [27] Dong J., Wang N.-N., Yao Z.-J., Zhang L., Cheng Y., Ouyang D., et al., ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database, *J. Cheminform.* 10 (1) (2018) 29.
- [28] Wang N.-N., Dong J., Deng Y.-H., Zhu M.-F., Wen M., Yao Z.-J., et al., ADME Properties Evaluation in Drug Discovery: Prediction of Caco-2 Cell Permeability Using a Combination of NSGA-II and Boosting, *J. Chem. Inf. Model.* 56 (4) (2016) 763–773.
- [29] Mauri A., Consonni V., Pavan M., Todeschini R., DRAGON software: An easy approach to molecular descriptor calculations, *MATCH Commun. Math. Comput. Chem.* 1 (56) (2006) 237–248.
- [30] Espinheira P.L., Santos E.G., Cribari-Neto F., On nonlinear beta regression residuals, *Biom. J. Biom. Z.* 59 (3) (2017) 445–461.
- [31] Veber D.F., Johnson S.R., Cheng H.-Y., Smith B.R., Ward K.W., Kopple K.D., Molecular properties that influence the oral bioavailability of drug candidates, *J. Med. Chem.* 45 (12) (2002) 2615–2623.
- [32] Beier J., Pujol H., Seoane B., Jimenez E., Astbury C., Massana E., et al., Abediterol, a novel long-acting β_2 -agonist: bronchodilation, safety, tolerability and pharmacokinetic results from a single-dose, dose-ranging, active-comparator study in patients with COPD, *BMC Pulm. Med.* 16 (1) (2016) 102.
- [33] Timmer W., Massana E., Jimenez E., Seoane B., de Miquel G., Ruiz S., First-in-human study of the safety, tolerability, pharmacokinetics and pharmacodynamics of abediterol (LAS100977), a novel long-acting B₂ -agonist, *J. Clin. Pharmacol.* 54 (12) (2014) 1347–1353.
- [34] Ortiz S., Flach S., Caracta C., Gil E.G., Jansat J.M., Safety and tolerability of acridinium administered intravenously and absolute bioavailability of inhaled acridinium in healthy male participants, *J. Clin. Pharmacol.* 52 (6) (2012) 819–827.
- [35] Ortiz S., Flach S., Ho J., Li F., Caracta C.F., Gil E.G., et al., Mass balance and metabolism of acridinium bromide following intravenous administration of [¹⁴C]-acridinium bromide in healthy subjects, *Biopharm. Drug Dispos.* 33 (1) (2012) 39–45.
- [36] Zheng Y., Marsh K.C., Bertz R.J., El-Shourbagy T., Adjei A.L., Pulmonary delivery of a dopamine D-1 agonist, ABT-431, in dogs and humans, *Int. J. Pharm.* 191 (2) (1999) 131–140.
- [37] Ehrmann S., Mercier E., Vecellio L., Temant D., Paintaud G., Dequin P.-F., Pharmacokinetics of high-dose nebulized amikacin in mechanically ventilated healthy subjects, *Intensive Care Med.* 34 (4) (2008) 755–762.
- [38] Petitcollin A., Dequin P.-F., Darrouzain F., Vecellio L., Boulain T., Garot D., et al., Pharmacokinetics of high-dose nebulized amikacin in ventilated critically ill patients, *J. Antimicrob. Chemother.* 71 (12) (2016) 3482–3486.
- [39] Noone P.G., Regnis J.A., Liu X., Brouwer K.L., Robinson M., Edwards L., et al., Airway deposition and clearance and systemic pharmacokinetics of amiloride following aerosolization with an ultrasonic nebulizer to normal airways, *Chest* 112 (5) (1997) 1283–1290.
- [40] Husain S., Capitano B., Corcoran T., Studer S.M., Crespo M., Johnson B., et al., Intrapulmonary disposition of amphotericin B after aerosolized delivery of amphotericin B lipid complex (Abelcet; ABLC) in lung transplant recipients, *Transplantation* 90 (11) (2010) 1215–1219.
- [41] Monforte V., Ussetti P., López R., Gavaldà J., Bravo C., de Pablo A., et al., Nebulized liposomal amphotericin B prophylaxis for Aspergillus infection in lung transplantation: pharmacokinetics and safety, *J. Heart Lung Transplant. Off. Publ. Int. Soc. Heart Transplant.* 28 (2) (2009) 170–175.

- [42] Harrison L.I., Smallridge R.C., Lassetter K.C., Goldlust M.B., Shamblen E.C., Gam V.W., et al., Comparative absorption of inhaled and intramuscularly administered atropine, *Am. Rev. Respir. Dis.* 134 (2) (1986) 254–257.
- [43] Corcoran T.E., Venkataraman R., Hoffman R.M., George M.P., Petrov A., Richards T., et al., Systemic delivery of atropine sulfate by the MicroDose Dry-Powder Inhaler, *J. Aerosol. Med. Pulm. Drug Deliv.* 26 (1) (2013) 46–55.
- [44] Hutchinson D., Barclay M., Prescott W.A., Brown J., Inhaled aztreonam lysine: an evidence-based review, *Expert Opin. Pharmacother.* 14 (15) (2013) 2115–2124.
- [45] Ambery C., Young G., Fuller T., Lazaar A.L., Pereira A., Hughes A., et al., Pharmacokinetics, Excretion, and Mass Balance of [14 C]-Batefenterol Following a Single Microtracer Intravenous Dose (Concomitant to an Inhaled Dose) or Oral Dose of Batefenterol in Healthy Men, *Clin. Pharmacol. Drug Dev.* 7 (8) (2018) 901–910.
- [46] Daley-Yates P.T., Price A.C., Sisson J.R., Pereira A., Dallow N., Beclomethasone dipropionate: absolute bioavailability, pharmacokinetics and metabolism following intravenous, oral, intranasal and inhaled administration in man, *Br. J. Clin. Pharmacol.* 51 (5) (2001) 400–409.
- [47] Ratner P.H., Melchior A., Dunbar S.A., Tantry S.K., Dorinsky P.M., Pharmacokinetic profile of beclomethasone dipropionate hydrofluoroalkane after intranasal administration versus oral inhalation in healthy subjects: results of a single-dose, randomized, open-label, 3-period crossover study, *Clin. Ther.* 34 (6) (2012) 1422–1431.
- [48] Meyer M., Beeh K.-M., Beier J., Beyer D., Aydt E., Zahlten R., et al., Tolerability and pharmacokinetics of inhaled bimosiamose disodium in healthy males, *Br. J. Clin. Pharmacol.* 63 (4) (2007) 451–458.
- [49] Minto C., Li B., Tattam B., Brown K., Seale J.P., Donnelly R., Pharmacokinetics of epimeric budesonide and fluticasone propionate after repeat dose inhalation—intersubject variability in systemic absorption from the lung, *Br. J. Clin. Pharmacol.* 50 (2) (2000) 116–124.
- [50] Zandvliet A.S., Huitema A.D.R., de Jonge M.E., den Hoed R., Sparidans R.W., Hendriks V.M., et al., Population pharmacokinetics of caffeine and its metabolites theobromine, paraxanthine and theophylline after inhalation in combination with diacetylmorphine, *Basic Clin. Pharmacol. Toxicol.* 96 (1) (2005) 71–79.
- [51] Millar S.A., Stone N.L., Yates A.S., O’Sullivan S.E., A Systematic Review on the Pharmacokinetics of Cannabidiol in Humans, *Front. Pharmacol.* 9 (2018) 1365.
- [52] Bressolle F., de la Coussaye J.E., Ayoub R., Fabre D., Gomeni R., Saissi G., et al., Endotracheal and aerosol administrations of ceftazidime in patients with nosocomial pneumonia: pharmacokinetics and absolute bioavailability, *Antimicrob. Agents Chemother.* 36 (7) (1992) 1404–1411.
- [53] Nave R., Bethke T., Seiberling M., Steinijans V., Zech K., Pharmacokinetics and pharmacodynamics of ciclesonide and its active principle after inhalative and intravenous administration in healthy subjects, In (2002) 749.
- [54] Nave R., Clinical pharmacokinetic and pharmacodynamic profile of inhaled ciclesonide, *Clin. Pharmacokinet.* 48 (4) (2009) 243–252.
- [55] Burkart G.J., Smaldone G.C., Eldon M.A., Venkataraman R., Dauber J., Zeevi A., et al., Lung deposition and pharmacokinetics of cyclosporine after aerosolization in lung transplant patients, *Pharm. Res.* 20 (2) (2003) 252–256.
- [56] Rohatagi S., Calic F., Harding N., Ozoux M.L., Bouriot J.P., Kirkesseli S., et al., Pharmacokinetics, pharmacodynamics, and safety of inhaled cyclosporin A (ADI628) after single and repeated administration in healthy male and female subjects and asthmatic patients, *J. Clin. Pharmacol.* 40 (11) (2000) 1211–1226.
- [57] Stass H., Delesen H., Nagelschmitz J., Staab D., Safety and pharmacokinetics of ciprofloxacin dry powder for inhalation in cystic fibrosis: a phase I, randomized, single-dose, dose-escalation study, *J. Aerosol. Med. Pulm. Drug Deliv.* 28 (2) (2015) 106–115.
- [58] Cone E.J., Pharmacokinetics and pharmacodynamics of cocaine, *J. Anal. Toxicol.* 19 (6) (1995) 459–478.
- [59] Cone E.J., Oyler J., Darwin W.D., Cocaine disposition in saliva following intravenous, intranasal, and smoked administration, *J. Anal. Toxicol.* 21 (6) (1997) 465–475.
- [60] Yapa S., Li J., Patel K., Wilson J.W., Dooley M.J., George J., et al., Pulmonary and systemic pharmacokinetics of inhaled and intravenous colistin methanesulfonate in cystic fibrosis patients: targeting advantage of inhalational administration, *Antimicrob. Agents Chemother.* 58 (5) (2014) 2570–2579.
- [61] Neale M.G., Brown K., Foulds R.A., Lal S., Morris D.A., Thomas D., The pharmacokinetics of nedocromil sodium, a new drug for the treatment of reversible obstructive airways disease, in human volunteers and patients with reversible obstructive airways disease, *Br. J. Clin. Pharmacol.* 24 (4) (1987) 493–501.
- [62] Richards R., Dickson C.R., Renwick A.G., Lewis R.A., Holgate S.T., Absorption and disposition kinetics of cromolyn sodium and the influence of inhalation technique, *J. Pharmacol. Exp. Ther.* 241 (3) (1987) 1028–1032.
- [63] Kellerman D., Rossi Mospan A., Engels J., Schaberg A., Gorden J., Smiley L., Denufosol: a review of studies with inhaled P2Y₂ agonists that led to Phase 3, *Pulm. Pharmacol. Ther.* 21 (4) (2008) 600–607.
- [64] Shrewsbury S.B., Cook R.O., Taylor G., Edwards C., Ramadan N.M., Safety and pharmacokinetics of dihydroergotamine mesylate administered via a Novel (Tempo) inhaler, *Headache* 48 (3) (2008) 355–367.
- [65] Tepper S.J., Orally inhaled dihydroergotamine: a review, *Headache* 53 (Suppl 2) (2013) 43–53.
- [66] Bondesson E., Bengtsson T., Nilsson L.-E., Wollmer P., Site of deposition and absorption of an inhaled hydrophilic solute, *Br. J. Clin. Pharmacol.* 63 (6) (2007) 722–731.
- [67] Steiner I., Errhalt P., Kubesch K., Hubner M., Holy M., Bauer M., et al., Pulmonary pharmacokinetics and safety of nebulized duramycin in healthy male volunteers, *Naunyn. Schmiedeberg Arch. Pharmacol.* 378 (3) (2008) 323–333.
- [68] Breuer C., Wachall B., Gerbeth K., Abdel-Tawab M., Fuhr U., Pharmacokinetics and pharmacodynamics of moist inhalation epinephrine using a mobile inhaler, *Eur. J. Clin. Pharmacol.* 69 (6) (2013) 1303–1310.
- [69] Frechen S., Suleiman A.A., Mohammad Nejad Sigaroudi A., Wachall B., Fuhr U., Population pharmacokinetic and pharmacodynamic modeling of epinephrine administered using a mobile inhaler, *Drug Metab. Pharmacokinet.* 30 (6) (2015) 391–399.
- [70] Armer T.A., Shrewsbury S.B., Newman S.P., Pitcairn G., Ramadan N., Aerosol delivery of ergotamine tartrate via a breath-synchronized plume-control inhaler in humans, *Curr. Med. Res. Opin.* 23 (12) (2007) 3177–3187.
- [71] Jonkman K., Duma A., Olofsen E., Henthorn T., van Velzen M., Mooren R., et al., Pharmacokinetics and Bioavailability of Inhaled Esketamine in Healthy Volunteers, *Anesthesiology* 127 (4) (2017) 675–683.
- [72] Jonkman K., Duma A., Velzen M., Dahan A., Ketamine inhalation, *Br. J. Anaesth.* 118 (2) (2017) 268–269.
- [73] Fenoterol S.N., a beta₂-adrenergic agonist for use in asthma. Pharmacology, pharmacokinetics, clinical efficacy and adverse effects, *Pharmacotherapy* 5 (3) (1985) 109–126.
- [74] Mather L.E., Woodhouse A., Ward M.E., Farr S.J., Rubsamen R.A., Eltherington L.G., Pulmonary administration of aerosolised fentanyl: pharmacokinetic analysis of systemic delivery, *Br. J. Clin. Pharmacol.* 46 (1) (1998) 37–43.
- [75] Macleod D.B., Habib A.S., Ikeda K., Spyker D.A., Cassella J.V., Ho K.Y., et al., Inhaled fentanyl aerosol in healthy volunteers: pharmacokinetics and pharmacodynamics, *Anesth. Analg.* 115 (5) (2012) 1071–1077.
- [76] Palmer R.B., Mautz D.S., Cox K., Kharasch E.D., Endotracheal flumazenil: a new route of administration for benzodiazepine antagonism, *Am. J. Emerg. Med.* 16 (2) (1998) 170–172.
- [77] Dickens G.R., Wermeling D.P., Matheny C.J., John W., Abramowitz W., Sista S.M., et al., Pharmacokinetics of flunisolide administered via metered dose inhaler with and without a spacer device and following oral administration, *Ann. Allergy Asthma Immunol. Off. Publ. Am. Coll. Allergy Asthma Immunol.* 84 (5) (2000) 528–532.
- [78] Allen A., The relationship between fluticasone furoate systemic exposure and cortisol suppression, *Clin. Pharmacokinet.* 52 (10) (2013) 885–896.
- [79] Allen A., Bareille P.J., Rousell V.M., Fluticasone furoate, a novel inhaled corticosteroid, demonstrates prolonged lung absorption kinetics in man compared with inhaled fluticasone propionate, *Clin. Pharmacokinet.* 52 (1) (2013) 37–42.
- [80] Thorsson L., Dahlström K., Edsbäcker S., Källén A., Paulson J., Wirén J.E., Pharmacokinetics and systemic effects of inhaled fluticasone propionate in healthy subjects, *Br. J. Clin. Pharmacol.* 43 (2) (1997) 155–161.
- [81] Brutsche M.H., Brutsche I.C., Munawar M., Langley S.J., Masterson C.M., Daley-Yates P.T., et al., Comparison of pharmacokinetics and systemic effects of inhaled fluticasone propionate in patients with asthma and healthy volunteers: a randomised crossover study, *Lancet Lond. Engl.* 356 (9229) (2000) 556–561.
- [82] Potocka E., Cassidy J.P., Haworth P., Heuman D., van Marle S., Baughman R.A., Pharmacokinetic characterization of the novel pulmonary delivery excipient fumaryl diketopiperazine, *J. Diabetes Sci. Technol.* 4 (5) (2010) 1164–1173.
- [83] Al-Amoud A.I., Clark B.J., Assi K.A., Chrystyn H., Determination of the bioavailability of gentamicin to the lungs following inhalation from two jet nebulizers, *Br. J. Clin. Pharmacol.* 59 (5) (2005) 542–545.
- [84] Bartels C., Looby M., Sechaud R., Kaiser G., Determination of the pharmacokinetics of glycopyrronium in the lung using a population pharmacokinetic modelling approach, *Br. J. Clin. Pharmacol.* 76 (6) (2013) 868–879.
- [85] Prakash A., Babu K.S., Morjaria J.B., Profile of inhaled glycopyrronium bromide as monotherapy and in fixed-dose combination with indacaterol maleate for the treatment of COPD, *Int. J. Chron. Obstruct. Pulmon. Dis.* 10 (2015) 111–123.
- [86] Hendriks V.M., van den Brink W., Blanken P., Bosman I.J., van Ree J.M., Heroin self-administration by means of “chasing the dragon”: pharmacodynamics and bioavailability of inhaled heroin, *Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol.* 11 (3) (2001) 241–252.

- [87] Rook E.J., Huitema A.D.R., van den Brink W., van Ree J.M., Beijnen J.H., Population pharmacokinetics of heroin and its major metabolites, *Clin. Pharmacokinet.* 45 (4) (2006) 401–417.
- [88] Rook E.J., Huitema A.D.R., van den Brink W., van Ree J.M., Beijnen J.H., Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature, *Curr. Clin. Pharmacol.* 1 (1) (2006) 109–118.
- [89] Hsu H.H., Rubin L.J., Iloprost inhalation solution for the treatment of pulmonary arterial hypertension, *Expert Opin. Pharmacother.* 6 (11) (2005) 1921–1930.
- [90] Cazzola M., Calzetta L., Page C.P., Matera M.G., Use of indacaterol for the treatment of COPD: a pharmacokinetic evaluation, *Expert Opin. Drug Metab. Toxicol.* 10 (1) (2014) 129–137.
- [91] Ensing K., de Zeeuw R.A., Nossent G.D., Koëter G.H., Cornelissen P.J., Pharmacokinetics of ipratropium bromide after single dose inhalation and oral and intravenous administration, *Eur. J. Clin. Pharmacol.* 36 (2) (1989) 189–194.
- [92] Hava D.L., Tan L., Johnson P., Curran A.K., Perry J., Kramer S., et al., A phase 1/1b study of PUR1900, an inhaled formulation of itraconazole, in healthy volunteers and asthmatics to study safety, tolerability and pharmacokinetics, *Br. J. Clin. Pharmacol.* 86 (4) (2020) 723–733.
- [93] Stockmann C., Sherwin C.M.T., Ampofo K., Spigarelli M.G., Development of levofloxacin inhalation solution to treat *Pseudomonas aeruginosa* in patients with cystic fibrosis, *Ther. Adv. Respir. Dis.* 8 (1) (2014) 13–21.
- [94] Geller D.E., Flume P.A., Griffith D.C., Morgan E., White D., Loutit J.S., et al., Pharmacokinetics and safety of MP-376 (levofloxacin inhalation solution) in cystic fibrosis subjects, *Antimicrob. Agents Chemother.* 55 (6) (2011) 2636–2640.
- [95] Azoicai D., Antoniu S.A., MP-376 (Aeroquin) for chronic *Pseudomonas aeruginosa* infections, *Expert Opin. Invest. Drugs* 22 (2) (2013) 267–276.
- [96] Takaenoki Y., Masui K., Oda Y., Kazama T., The Pharmacokinetics of Atomized Lidocaine Administered via the Trachea: A Randomized Trial, *Anesth. Analg.* 123 (1) (2016) 74–81.
- [97] Burness C.B., Keating G.M., Mannitol dry powder for inhalation: in patients with cystic fibrosis, *Drugs* 72 (10) (2012) 1411–1421.
- [98] Cook C.E., Jeffcoat A.R., Hill J.M., Pugh D.E., Patetta P.K., Sadler B.M., et al., Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride, *Drug Metab. Dispos. Biol. Fate Chem.* 21 (4) (1993) 717–723.
- [99] Harris D.S., Boxenbaum H., Everhart E.T., Sequeira G., Mendelson J.E., Jones R.T., The bioavailability of intranasal and smoked methamphetamine, *Clin. Pharmacol. Ther.* 74 (5) (2003) 475–486.
- [100] Vaidya S.S., Khindri S., Maahs S., Machineni S., Hara H., Juan A., et al., Lung Delivery of Indacaterol and Mometasone Furoate Following Inhalation of QMF149 in Healthy Volunteers, *Clin. Pharmacol. Drug Dev.* 5 (4) (2016) 285–295.
- [101] Affrime M.B., Cuss F., Padhi D., Wirth M., Pai S., Clement R.P., et al., Bioavailability and metabolism of mometasone furoate following administration by metered-dose and dry-powder inhalers in healthy human volunteers, *J. Clin. Pharmacol.* 40 (11) (2000) 1227–1236.
- [102] Penson R.T., Joel S.P., Roberts M., Gloyne A., Beckwith S., Slevin M.L., The bioavailability and pharmacokinetics of subcutaneous, nebulized and oral morphine-6-glucuronide, *Br. J. Clin. Pharmacol.* 53 (4) (2002) 347–354.
- [103] Dershwitz M., Walsh J.L., Morishige R.J., Connors P.M., Rubsamen R.M., Shafer S.L., et al., Pharmacokinetics and pharmacodynamics of inhaled versus intravenous morphine in healthy volunteers, *Anesthesiology* 93 (3) (2000) 619–628.
- [104] Ward M.E., Woodhouse A., Mather L.E., Farr S.J., Okikawa J.K., Lloyd P., et al., Morphine pharmacokinetics after pulmonary administration from a novel aerosol delivery system, *Clin. Pharmacol. Ther.* 62 (6) (1997) 596–609.
- [105] Masood A.R., Thomas S.H., Systemic absorption of nebulized morphine compared with oral morphine in healthy subjects, *Br. J. Clin. Pharmacol.* 41 (3) (1996) 250–252.
- [106] Summers Q.A., Honeywell R., Renwick A.G., Holgate S.T., The protective efficacy of inhaled, oral and intravenous nedocromil sodium against adenosine-5'-monophosphate-induced bronchoconstriction in asthmatic volunteers, *Pulm. Pharmacol.* 3 (4) (1990) 190–197.
- [107] Begg M., Edwards C.D., Hamblin J.N., Pefani E., Wilson R., Gilbert J., et al., Translation of Inhaled Drug Optimization Strategies into Clinical Pharmacokinetics and Pharmacodynamics Using GSK2292767A, a Novel Inhaled Phosphoinositide 3-Kinase δ Inhibitor, *J. Pharmacol. Exp. Ther.* 369 (3) (2019) 443–453.
- [108] Wilson R., Jarvis E., Montebault M., Hamblin J.N., Hessel E.M., Cahn A., Safety, Tolerability, and Pharmacokinetics of Single and Repeat Doses of Nemiralisib Administered via the Ellipta Dry Powder Inhaler to Healthy Subjects, *Clin. Ther.* 40 (8) (2018) 1410–1417.
- [109] Molander L., Lunell E., Andersson S.B., Kuylenstierna F., Dose released and absolute bioavailability of nicotine from a nicotine vapor inhaler, *Clin. Pharmacol. Ther.* 59 (4) (1996) 394–400.
- [110] Curry S.H., Lopez L.M., Lambert C.R., Kwon H.R., Stack R.K., Plasma concentrations and hemodynamic effects of intravenous, sublingual, and aerosolized nitroglycerin in patients undergoing cardiac catheterization, *Biopharm. Drug Dispos.* 14 (2) (1993) 107–118.
- [111] Borghardt J.M., Weber B., Staab A., Kunz C., Kloft C., Model-based evaluation of pulmonary pharmacokinetics in asthmatic and COPD patients after oral olodaterol inhalation, *Br. J. Clin. Pharmacol.* 82 (3) (2016) 739–753.
- [112] Borghardt J.M., Weber B., Staab A., Kunz C., Formella S., Kloft C., Investigating pulmonary and systemic pharmacokinetics of inhaled olodaterol in healthy volunteers using a population pharmacokinetic approach, *Br. J. Clin. Pharmacol.* 81 (3) (2016) 538–552.
- [113] Fernando D., Siederer S., Singh S., Schneider I., Gupta A., Powell M., et al., Safety, Tolerability and Pharmacokinetics of Single Doses of Oxytocin Administered via an Inhaled Route in Healthy Females: Randomized, Single-blind, Phase 1 Study, *EBioMedicine.* 22 (2017) 249–255.
- [114] Conte J.E., Golden J.A., Concentrations of aerosolized pentamidine in bronchoalveolar lavage, systemic absorption, and excretion, *Antimicrob. Agents Chemother.* 32 (10) (1988) 1490–1493.
- [115] Avram M.J., Spyker D.A., Henthorn T.K., Cassella J.V., The pharmacokinetics and bioavailability of prochlorperazine delivered as a thermally generated aerosol in a single breath to volunteers, *Clin. Pharmacol. Ther.* 85 (1) (2009) 71–77.
- [116] Li F., Yang J., Revefenacin for the treatment of chronic obstructive pulmonary disease, *Expert Rev. Clin. Pharmacol.* 12 (4) (2019) 293–298.
- [117] Ward J.K., Dow J., Dallow N., Eynott P., Miller S., Ventresca G.P., Enantiomeric disposition of inhaled, intravenous and oral racemic-salbutamol in man—no evidence of enantioselective lung metabolism, *Br. J. Clin. Pharmacol.* 49 (1) (2000) 15–22.
- [118] Moore A., Riddell K., Joshi S., Chan R., Mehta R., Pharmacokinetics of Salbutamol Delivered from the Unit Dose Dry Powder Inhaler: Comparison with the Metered Dose Inhaler and Diskus Dry Powder Inhaler, *J. Aerosol. Med. Pulm. Drug Deliv.* 30 (3) (2017) 164–172.
- [119] Soulele K., Macheras P., Karalis V., Pharmacokinetic analysis of inhaled salmeterol in asthma patients: Evidence from two dry powder inhalers, *Biopharm. Drug Dispos.* 38 (7) (2017) 407–419.
- [120] Borgström L., Nilsson M., A method for determination of the absolute pulmonary bioavailability of inhaled drugs: terbutaline, *Pharm. Res.* 7 (10) (1990) 1068–1070.
- [121] Naef M., Russmann S., Petersen-Felix S., Brenneisen R., Development and pharmacokinetic characterization of pulmonary and intravenous delta-9-tetrahydrocannabinol (THC) in humans, *J. Pharm. Sci.* 93 (5) (2004) 1176–1184.
- [122] Heuberger J.A.A.C., Guan Z., Oyetao O.-O., Klumpers L., Morrison P.D., Beumer T.L., et al., Population pharmacokinetic model of THC integrates oral, intravenous, and pulmonary dosing and characterizes short- and long-term pharmacokinetics, *Clin. Pharmacokinet.* 54 (2) (2015) 209–219.
- [123] Keating G.M., Tiotropium bromide inhalation powder: a review of its use in the management of chronic obstructive pulmonary disease, *Drugs.* 72 (2) (2012) 273–300.
- [124] Parra-Guilln Z., Weber B., Freijer J., Retlich S., Borghardt J., Trocniz I., Population Pharmacokinetic Analysis of Tiotropium in Healthy Volunteers after Intravenous Administration and Inhalation, *J. Pharmacokinet. Pharmacodyn.* 41 (2014) S7–S101 2014. p. 54-T-034.
- [125] Cooney G.F., Lum B.L., Tomaselli M., Fiel S.B., Absolute bioavailability and absorption characteristics of aerosolized tobramycin in adults with cystic fibrosis, *J. Clin. Pharmacol.* 34 (3) (1994) 255–259.
- [126] Touw D.J., Jacobs F.A., Brimicombe R.W., Heijerman H.G., Bakker W., Briemer D.D., Pharmacokinetics of aerosolized tobramycin in adult patients with cystic fibrosis, *Antimicrob. Agents Chemother.* 41 (1) (1997) 184–187.
- [127] Newhouse M.T., Hirst P.H., Duddu S.P., Walter Y.H., Tarara T.E., Clark A.R., et al., Inhalation of a dry powder tobramycin PulmoSphere formulation in healthy volunteers, *Chest* 124 (1) (2003) 360–366.
- [128] Kumar P., Thudium E., Laliberte K., Zaccardelli D., Nelsen A., A Comprehensive Review of Treprostinil Pharmacokinetics via Four Routes of Administration, *Clin. Pharmacokinet.* 55 (12) (2016) 1495–1505.
- [129] Argenti D., Shah B., Heald D., A pharmacokinetic study to evaluate the absolute bioavailability of triamcinolone acetonide following inhalation administration, *J. Clin. Pharmacol.* 39 (7) (1999) 695–702.
- [130] GlaxoSmithKline, A Phase 2a Study to Evaluate the Pharmacokinetic, Safety, Tolerability and Clinical Effect of Topically Applied Umeclidinium/GSK573719 in Subjects With Primary Axillary Hyperhidrosis [Internet]. clinicaltrials.gov; 2018 Sep [cited 2020 Nov 29]. Report No.: results/NCT02563899, Available from: <https://clinicaltrials.gov/ct2/show/results/NCT02563899>
- [131] Allen A., Bal J., Bianco J., Moore A., The Pharmacokinetics of Fluticasone Furoate and Vilanterol Following Single Inhaled Administration in Combination and Intravenous Administration of Individual Components in Healthy Subjects, *J. Bioequiv. Availab.* 5 (2013) 165–173.

- [132] Rosen A.S., Fournié P., Darwish M., Danjou P., Troy S.M., Zaleplon pharmacokinetics and absolute bioavailability, *Biopharm. Drug Dispos.* 20 (3) (1999) 171–175.
- [133] Shelton M.J., Lovern M., Ng-Cashin J., Jones L., Gould E., Gauvin J., et al., Zanamivir pharmacokinetics and pulmonary penetration into epithelial lining fluid following intravenous or oral inhaled administration to healthy adult subjects, *Antimicrob. Agents Chemother.* 55 (11) (2011) 5178–5184.
- [134] Melin J., Prothon S., Kloft C., Cleton A., Amilon C., Jorup C., et al., Pharmacokinetics of the Inhaled Selective Glucocorticoid Receptor Modulator AZD5423 Following Inhalation Using Different Devices, *AAPS J.* 19 (3) (2017) 865–874.
- [135] Werkström V., Prothon S., Ekholm E., Jorup C., Edsbäcker S., Safety, Pharmacokinetics and Pharmacodynamics of the Selective Glucocorticoid Receptor Modulator AZD5423 after Inhalation in Healthy Volunteers, *Basic Clin. Pharmacol. Toxicol.* 119 (6) (2016) 574–581.
- [136] Prothon S., Wählby Hamrén U., Tehler U., Yoon E., Forsman H., Arfvidsson C., et al., Safety, pharmacokinetics and pharmacodynamics of the selective glucocorticoid receptor modulator AZD7594, following inhalation in healthy Japanese volunteers, *Drug Des. Devel. Ther.* 13 (2019) 3845–3853.
- [137] Marino M.T., Costello D., Baughman R., Boss A., Cassidy J., Damico C., et al., Pharmacokinetics and pharmacodynamics of inhaled GLP-1 (MKC253): proof-of-concept studies in healthy normal volunteers and in patients with type 2 diabetes, *Clin. Pharmacol. Ther.* 88 (2) (2010) 243–250.
- [138] Shah B., Jensen B.K., Zhang J., Hunt T., Rohatagi S., Effect of food on pharmacokinetics of an inhaled drug: a case study with a VLA-4 antagonist, HMR1031, *J. Clin. Pharmacol.* 43 (12) (2003) 1341–1349.
- [139] Rohatagi S., Shah B., Zhang J., Zeng Z., Kirkesseli S., Jensen B.K., Estimating mass balance for inhaled drugs in humans: an example with a VLA-4 antagonist, IVL745, *J. Clin. Pharmacol.* 44 (4) (2004) 348–358.
- [140] Research C for DE and. Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. Guidance for Industry [Internet]. U.S. Food and Drug Administration. FDA; 2019 [cited 2020 Dec 1]. Available from: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/waiver-vivo-bioavailability-and-bioequivalence-studies-immediate-release-solid-oral-dosage-forms>
- [141] Patton J.S., Fishburn C.S., Weers J.G., The lungs as a portal of entry for systemic drug delivery, *Proc. Am. Thorac. Soc.* 1 (4) (2004) 338–344.
- [142] Artursson P., Karlsson J., Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells, *Biochem. Biophys. Res. Commun.* 175 (3) (1991) 880–885.
- [143] Hou T., Wang J., Structure-ADME relationship: still a long way to go?, *Expert Opin. Drug Metab. Toxicol.* 4 (6) (2008) 759–770.
- [144] Drucker D.J., Advances in oral peptide therapeutics, *Nat. Rev. Drug Discov.* 19 (4) (2020) 277–289.
- [145] Zhang J.Y., Wang Y., Prakash C., Xenobiotic-metabolizing enzymes in human lung, *Curr. Drug Metab.* 7 (8) (2006) 939–948.
- [146] Yilmaz Y., Williams G., Walles M., Manevski N., Krähenbühl S., Camenisch G., Comparison of Rat and Human Pulmonary Metabolism Using Precision-cut Lung Slices (PCLS), *Drug Metab. Lett.* 13 (1) (2019) 53–63.
- [147] Radivojevic S., Zellnitz S., Paudel A., Fröhlich E., Searching for physiologically relevant in vitro dissolution techniques for orally inhaled drugs, *Int. J. Pharm.* 10 (556) (2019) 45–56.
- [148] Gaohua L., Wedagedera J., Small B.G., Almond L., Romero K., Hermann D., et al., Development of a Multicompartment Permeability-Limited Lung PBPK Model and Its Application in Predicting Pulmonary Pharmacokinetics of Antituberculosis Drugs, *CPT Pharmacomet. Syst. Pharmacol.* 4 (10) (2015) 605–613.
- [149] Fischer H., Function of proton channels in lung epithelia, *Wiley Interdiscip. Rev. Membr. Transp. Signal.* 1 (3) (2012) 247–258.
- [150] Fischer H., Widdicombe J.H., Mechanisms of acid and base secretion by the airway epithelium, *J. Membr. Biol.* 211 (3) (2006) 139–150.
- [151] Kumar A., Terakosolphan W., Hassoun M., Vandera K.-K., Novicky A., Harvey R., et al., A Biocompatible Synthetic Lung Fluid Based on Human Respiratory Tract Lining Fluid Composition, *Pharm. Res.* 34 (12) (2017) 2454–2465.
- [152] Cingolani E., Alqahtani S., Sadler R.C., Prime D., Stolnik S., Bosquillon C., In vitro investigation on the impact of airway mucus on drug dissolution and absorption at the air-epithelium interface in the lungs, *Eur. J. Pharm. Biopharm. Off. J. Arbeitsgemeinschaft. Pharm. Verfahrenstechnik EV.* 141 (2019) 210–220.
- [153] Das S.C., Stewart P.J., The influence of lung surfactant liquid crystalline nanostructures on respiratory drug delivery, *Int. J. Pharm.* 514 (2) (2016) 465–474.
- [154] J.E. Hastedt, P. Bäckman, A.R. Clark, W. Doub, A. Hickey, G. Hochhaus, et al. Scope and relevance of a pulmonary biopharmaceutical classification system AAPS/FDA/USP Workshop March 16-17th, 2015 in Baltimore, MD. *AAPS Open* [Internet]. 2016 [cited 2020 Dec 1]; 2(1): 1. Available from: <https://doi.org/10.1186/s41120-015-0002-x>
- [155] Arora D., Shah K.A., Halquist M.S., Sakagami M., In vitro aqueous fluid-capacity-limited dissolution testing of respirable aerosol drug particles generated from inhaler products, *Pharm. Res.* 27 (5) (2010) 786–795.
- [156] Krug S.M., Günzel D., Conrad M.P., Lee I-F.M., Amasheh S., Fromm M., et al., Charge-selective claudin channels, *Ann. N Y Acad. Sci.* 1257 (2012) 20–28.
- [157] Bäckström E., Boger E., Lundqvist A., Hammarlund-Udenaes M., Fridén M., Lung Retention by Lysosomal Trapping of Inhaled Drugs Can Be Predicted In Vitro With Lung Slices, *J. Pharm. Sci.* 105 (11) (2016) 3432–3439.
- [158] Cooper A.E., Ferguson D., Grime K., Optimisation of DMPK by the inhaled route: challenges and approaches, *Curr. Drug Metab.* 13 (4) (2012) 457–473.
- [159] Wang Y.-B., Watts A.B., Peters J.I., Williams R.O., The impact of pulmonary diseases on the fate of inhaled medicines—a review, *Int. J. Pharm.* 461 (1–2) (2014) 112–128.

Graphical abstract



Query: Decimal commas have been changed to decimal dots in Tables 2 and 4. Please check and correct if necessary.

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