Applications of Exhaled Breath Condensate Analysis for Drug Monitoring and Bioequivalence Study of Inhaled Drugs

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ABSTRACT – The exhaled breath condensate (EBC) presents a simple and non-invasive alternative approach for bioequivalence assessments and therapeutic drug monitoring of inhaled drugs. EBC better represents the drug at the site of action and eliminates the possibility of the contribution of a swallowed portion of the dose when systemic bioavailability is used for assessment. This review summarizes the recently reported analytical methods for the quantification of drugs in EBC. It also discusses the difficulties in the bioequivalence evaluation criteria of generic orally inhaled drug products suggested by various regulatory agencies that may be eliminated using the EBC analysis approach.

INTRODUCTION

Exhaled breath condensate (EBC) is the mixture of water vapor contained in breath and tiny droplets of lung lining fluid. EBC is a highly diluted and low-protein aqueous matrix. It is a unique matrix for the analysis of biomarkers which enables the study of the early outcomes of different diseases and exposures to drugs/chemicals on the lung and/or upper airways (1, 2). EBC analysis-based methods are promising, simple, non-invasive, and diagnostic methods for studying the composition of airway lining fluid and have the potential for assessing lung inflammation, exacerbations, disease severity, and the effectiveness of treatment regimens. Recent investigations reveal the potential applications of EBC analysis in various longitudinal studies (3). Measuring pediatric asthma grades and evaluating an infant's EBC instead of blood or urine sampling are some of its clinical applications (4, 5). Besides, it can be used to assess the multi markers in respiratory diseases, such as cytokines, leukotrienes, prostaglandins, adenosine, hydrogen peroxide, nitric oxide-derived products, and hydrogen ions. Moreover, it has been used for drug monitoring, diagnosis, and environmental health, and it could be considered an alternative sample type for pharmacokinetic (PK) and pharmacodynamic (PD) studies (2, 6).

This review highlights the applications of EBC analysis in drug concentration monitoring and discusses the advantages and disadvantages of these studies. Afterward, the *in vitro/in vivo* bioequivalence parameters of oral inhalers in various available guidelines were compared. Following that, the potential capability of EBC as a promising sample in pharmaceutical inhaler assessments is investigated based on features of EBC analysis and parameters that are required for evaluating the bioequivalence of inhaled drugs.

APPLICATION OF EBC IN DRUG CONCENTRATION MONITORING

Therapeutic drug monitoring (TDM) enhances therapeutic outcomes of drugs. Various biological samples such as plasma, urine, and saliva were assessed for TDM purposes (7). EBC can also be considered as an informative specimen as well due to the fact that an equilibrium exists between plasma, lung lining fluid, and EBC; therefore, therapeutic agents in the blood should also be present in EBC. So that the alveolar gas (*i.e.*, the breath rising from deep within the lungs) is in equilibrium with the blood through the airway lining fluid. The blood comes from all tissues to the ventricle and is pumped up to the lungs' alveolar capillaries. During this time, the blood is equilibrated with alveolar air using a thin layer of diffusion membrane covering its surface

with the airway lining fluid (8). The diffusion mechanism for non-gaseous therapeutic agents or biomarkers in EBC is related to the aerosolizing of the airway lining fluid arising from the turbulence in the airways (9), or the sudden opening of closed respiratory bronchioles and alveoli in the absence of bulk airflow (10), which Johnson and Morawska have suggested as a bronchiole fluid film burst model (11). Therefore, the concentrations of molecules, drugs, or biomarkers in the breath or EBC may be correlated with those of their free concentrations in the blood based on the above-mentioned mechanism. So, EBC has been performed as an alternative biological sample due to its non-invasive sampling procedure over blood samples (12-32).

Various studies have been performed on EBC with the aim of tracing drug concentrations (12-32). Table 1 summarizes the therapeutic concentration of the drugs in plasma and their concentrations in EBC samples of patients receiving the drug measured using various analytical methods. Esther *et al.* confirmed the potential of EBC analysis in the Pharmacokinetic (PK) evaluation of inhaled drugs (33). Besides, Miekisch *et al.* have confirmed a reasonable plasma-EBC correlation with solid-phase microextraction followed by gas chromatography-mass spectrometry analysis of propofol (34). Furthermore, Kruizinga *et al.* have performed a study to evaluate the probable capability of EBC for PK analysis of inhaled and intravenous tobramycin and salbutamol. The results have revealed that salbutamol and tobramycin are detectable in EBS after inhalation; however, they could not be detected after intravenous injection (13). The molecular weight, protein binding, and polarization of tobramycin and salbutamol are potential factors influencing this issue. Further evidence on this application of EBC could be found in the literature (13, 35-38).

Hamidi *et al.* noted the poor correlations between methadone concentration in EBC and its total concentration in serum samples. Their study demonstrates the critical factors that should be considered in an EBC measurement, such as the humidity and temperature of the EBC sample collection area, the presence of potential contaminants (such as saliva), the rate of breathing, pH, droplet dilution, expiration, personal habits (such as smoking), concurrent drug use, trapping mechanisms, sample collection duration, and the use of EBC dilution correction factors (16).

Dincer *et al.* developed a polymer-based microfluidic sensor for the ß-lactam assay. They established the effectiveness of this platform in an *in vivo* study of Landrace pigs treated with piperacillin/tazobactam. Furthermore, their study attempts to investigate the piperacillin/tazobactam concentrations in EBC and their relationship to plasma levels (39).

Borras *et al.* reported correlation coefficients between breath and serum concentrations of morphine and hydromorphone. They indicated that the tiny EBC sample size affects the investigation of various factors' impact on correlation, including sex, weight, basal metabolic rate, etc. (14). Due to these reports, analyzing drugs/biomarkers in EBC could be a promising solution for the early diagnosis of diseases or exposure to substances with an exogenous origin, which places EBC as a good potential alternative for other accepted samples for TDM.

There are a number of parameters in the field of EBC analysis which should be addressed in future works. For example, different research groups reported the data as mass/volume concentration and/or mass/filter (*i.e.*, pg/filter, pg/min, mg/L, and parts per million by volume) and also the very wide variations in some of the reported data using a single concentration expression (*e.g.*, 4.8–29,800 pg/min for methadone (58)). Khoubnasabjafari *et al.* (2) suggested several considerations for more concise drug determination, such as standardization of collection temperature, materials used inside the collection assembly, flow design, and other parameters, including ventilation pattern, respiratory rate, and exhaled particles (2). Besides, EBC's sampling requires a specific exhalation percentage, well-defined exclusion criteria, volunteers' training to collect the sample, and highly standardized conditions for inclusion and sampling time (59). Figure 1 summarizes the advantages and disadvantages of EBC in drug monitoring. Therefore, several factors can influence the response when using this sample, and they need to be resolved prior to further studies.

CRITERIA FOR BIOEQUIVALENCE OF INHALED FORMULATIONS

Bioequivalence is described as "the equivalent rate and extent of the active moiety in contact with the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study" (60, 61). Various approaches exist to evaluate the dosage-form bioequivalence between two products during

Table 1. An overview of drug concentrations in EBC and plasma samples, along with some details of the determination procedures

Drug	Plasma Conc.	EBC Conc.	Analytical platform for EBC samples	LR[1]/LOD[2] for EBC samples	Significant feature	References
Alprazolam	NR[3]	0.005 - 0.02ppm	LC[4]–MS[5]	2 - 18 / 1 pg. filter[1]	Sensitive	(30, 40)
Amikacin	$(0.42 – 0.68) \times 10^{-3}$ ppm	1.91 – 2.81 ppm	HPLC[6] – MS/MS	$0.21 – 3000 / 0.06 \times 10^{-3}$ ppm	Quick and efficient	(43)
Amphetamine	NR[3]	0.02 - 0.15 ppm	LC–MS	2 - 18 / 3 pg. filter[1]	Sensitive	(30, 40)
Aspirin	23.2 - 24.9 ppm	150 - 300 ppm	Colorimetry	10 - 250 / 4.1 ppm	High reliability	(41, 42)
Benzoylecgonine	NR[3]	0.018 - 0.14 ppm	LC–MS	2 - 18 / 0.5 pg. filter[-1]	Sensitive	(30, 40)
Buprenorphine	NR[3]	0.001 - 0.005 ppm	LC-MS/MS	$NR^3 / 2.5 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Buprenorphine	NR[3]	0.001 -0.005 ppm	LC–MS	2 - 18 / 2 pg. filter[-1]	Sensitive	(30, 40)
Carbamazepine	0.3 - 0.5 ppm	2 - 12 ppm	Spectroflourimetry	0.2 - 20 / 0.08 ppm	Sensitive	(15, 40
Cocaine	NR[3]	0.1 - 0.3 ppm	LC–MS	2 - 18 / 2 pg. filter[-1]	Sensitive	(30, 40)
Codeine	NR[3]	0.025 - 0.25 ppm	LC-MS/MS	$NR^3 / 0.1 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Daclatasvir	0.048 - 0.992 ppm	0.052 - 0.852 ppm	Plasmon resonance	0.01 - 1.0 /0.008 ppm	Low LOD, low cost, sensitive	(44, 45)
Daclatasvir	NR[3]	0.052 - 0.852 ppm	Spectroflourimetry	$0.5\text{-}15 \times 10^{-3} / 0.12 \times 10^{-3}$ ppm	Simple, fast and sensitive	(46, 45)
Deferiprone	0.06 - 0.17 ppm	5 - 25 ppm	Spectroflourimetry	0.06 - 1.50 / 0.06 ppm	Simple, low EBC volume	(21, 47)
Diazepam	NR[3]	0.2 - 2 ppm	LC–MS	2 - 18 / 1 pg. filter[-1]	Sensitive	(30, 40)
Doxorubicin	$(48.9\text{-} 203) \times 10^{-3}$ ppm	0.006 - 0.09 ppm	Spectrophotometric	0.02 - 0.2 / 0.00416 ppm	Simple, sensitive and reliable	(24, 40)
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	NR[3]	NR[3]	LC-MS/MS	$NR^3 / 0.01 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Fentanyl	NR[3]	0.005- 0.3 ppm	LC-MS/MS	$NR^3 / 0.05 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Hydromorphone	NR[3]	0.001 - 0.03 ppm	LC-MS/MS	$NR^3 / 1 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Hydrocodone	NR[3]	0.01 - 0.1 ppm	LC-MS/MS	$NR^3 / 0.5 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Lamotrigine	0.592 - 0.771 ppm	3 - 15 ppm	Spectrophotometric	NR^3 / 0.005 ppm	Quick visual detection	(28, 40)
Lamotrigine	0.55 - 1.19 ppm	3 - 15 ppm	Spectroflourimetry	0.05 - 2.0 / 0.011 ppm	Sensitive and fast	(48, 40)
Meperidine	NR[3]	0.1 - 0.8 ppm	LC-MS/MS	$NR^3 / 0.05 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Meropenem	Not detectable	25.5 ppm	UHPLCHR-MS	21,168 pg. filter[-1] / NR[3]	Non-invasive	(55, 56)
Methadone	NR[3]	0.05 - 0.5 ppm	LC-MS/MS	$NR^3 / 0.5 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Methadone	0.16 - 1.06 ppm	0.05 - 0.5 ppm	Capillary electrophoresis	0.15 - 5 ppm / 0.15 ppm	Simple, sensitive and accurate	(16, 40)
Methadone	23.6 - 275 pg.min[-1]	0.05 - 0.5 ppm	LC–MS–MS	100 - 2000 / 3 pg/sample	Feasible	(12, 40)
Methadone	$(0.34 - 1.31) \times 10^{-3}$ ppm	0.05 - 0.5 ppm	LC	$0.5 - 10 \times 10^{-3} / 0.5 \times 10^{-3}$ ppm	Simple and low cost	(23, 40)
Methadone	0.7 - 0.48 ppm	0.05 - 0.5 ppm	Capillary electrophoresis	0.3 - 5 / 0.3 ppm	Simple and fast	(26, 40)
Methadone	NR[3]	0.05 - 0.5 ppm	LC–MS	2 - 18 / 0.5 pg. filter[-1]	Sensitive	(30, 40)
Methamphetamine	NR[3]	0.01 - 0.05 ppm	LC–MS	2 - 18 / 1 pg. filter[-1]	Sensitive	(30, 40)
Methotrexate	$(45.4\text{-}140.8) \times 10^{-3}$ ppm	2.27 ppm	Spectrofluorimetry	$20 – 998.8 \times 10^{-3} / 15.9 \times 10^{-3}$ ppm	Simple, fast and accurate	(40, 49)
Metoprolol	NR[3]	0.02 - 0.5 ppm	Spectrofluorimetry	$5 - 100 \times 10^{-3} / 2.1 – 3.4 \times 10^{-3}$ ppm	Simple, low-cost	(40, 50)
6-Acetyl morphine	NR[3]	0.015 – 0.10 ppm	LC–MS	2 - 18 / 1 pg. filter[-1]	Sensitive	(30, 40)
Morphine	NR[3]	0.01 - 0.15 ppm	LC–MS	2 - 18 / 1 pg. filter[-1]	Sensitive	(30, 40)
Morphine	$(0.10 - 5.48) \times 10^{-3}$ ppm	0.01-0.15 ppm	LC-MS/MS	$NR^3 / 0.1 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Morphine	$(89 – 173) \times 10^{-3}$ ppm	0.01 - 0.15 ppm	GC[7]-MS	$NR^3 / 2.1 \times 10^{-3}$ ppm	Repeatable and stable	(20, 40)
Naloxone	NR[3]	0.01 - 0.03 ppm	LC-MS/MS	$NR^3 / 0.25 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Naltrexone	NR[3]	0.005 - 0.03 ppm	LC-MS/MS	$NR^3 / 0.5 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Oxazepam	NR[3]	0.2 -1.5 ppm	LC–MS	2 - 18 / 1 pg. filter[-1]	Sensitive	(30, 40)
Oxycodone	NR[3]	0.02 - 0.05 ppm	LC-MS/MS	$NR^3 / 0.25 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Oxymorphone	NR[3]	NR[3]	LC-MS/MS	$NR^3 / 0.75 \times 10^{-3}$ ppm	Non-invasive and useful	(14, 40)
Oxymorphone	$(29 – 82) \times 10^{-3}$ ppm	NR[3]	GC-MS	$NR^3 / 1.5 \times 10^{-3}$ ppm	Repeatable, and stable	(20, 40)
Paracetamol	1.12 - 4.68 ppm	2.5 - 25 ppm	Colorimetry	0.2 - 10.0 / 0.49 ppm	Specific and simple	(17, 40)
Phenobarbital	0.21 - 1.65 ppm	1 - 5 ppm	Spectrofluorimetry	0.1 - 10.0 / 0.024 ppm	Feasible, efficient and simple	(40, 51)

Table 1. continues...

Drug	Plasma Conc.	EBC Conc.	Analytical platform for EBC samples	LR[1]/LOD[2] for EBC samples	Significant feature	References
Phenytoin	0.013 - 0.13 ppm	5 - 20 ppm	Capillary electrophoresis	0.001 - 0.10 / 0.001 ppm	Selectivity	(40, 53)
Piperacillin	90×10^{-3} ppm	5 - 20 ppm	Microfluidic sensor	NR[3] / 56×10^{-3} ppm	Versatile and low LOD	(39, 40, 54)
Piperacillin	45 pg	5 - 20 ppm	UHPLCHR[8]-MS	988 - 203,895 / 3,083 pg. filter^{-1}	Non-invasive	(55, 56)
Propranolol	0.030 ppm	0.02 - 0.3 ppm	LC-MS/MS	5.6 - 224×10^{-3} ppm / NR[3]	Simple, cheap and feasible	(31, 40)
Tazobactam	90×10^{-3} ppm	7.7 - 13.7 ppm	Microfluidic sensor	NR[3] / 56×10^{-3} ppm	Versatile and low LOD	(39, 40, 54)
Tazobactam	45 pg	7.7 - 13.7 ppm	UHPLCHR[8]-MS	988 - 203,895 / 3,083 pg. filter^{-1}	Non-invasive	(55, 56)
Tetrahydrocannabinol	NR[3]	0.001 - 0.007 ppm	LC–MS	2 - 18 / 3 pg. filter[1]	Sensitive	(30, 40)
Tobramycin	$(13.7 - 32.2) \times 10^{-3}$ ppm	5 - 10 ppm	Colorimetry	1.0 - 50.0×10^{-3} / 0.5×10^{-3} ppm	Repeatable and low LOD	(18, 40)
Tobramycin	$(21.4 - 41.6) \times 10^{-3}$ ppm	5 - 10 ppm	UV spectroscopy	1.0 - 50.0×10^{-3} / $(0.5 \times 10^{-3}$ ppm	Sensitive	(13, 40)
Tobramycin	$(2.4 - 17.0) \times 10^{-6}$ ppm	5 - 10 ppm	LC–MS	NR[3]	Wide LR	(32, 40)
Tramadol HCl	NR[3]	0.1 – 1 ppm	LC-MS/MS	NR[3] / 0.5×10^{-3} ppm	Non-invasive and useful	(14, 40)
Salbutamol	$(32.2 - 645.0) \times 10^{-6}$ ppm	<0.01 - 0.02 ppm	LC–MS	NR[3]	Wide LR	(32, 40)
Salbutamol sulfate	$(89 – 173) \times 10^{-3}$ ppm	<0.01 - 0.02 ppm	GC-MS	0.615 – 5 / 370 ppm	Wide LR and low LOD	(40, 57)
Valproic acid	$(0.13 – 500) \times 10^{-3}$ ppm	40 - 100 ppm	GC-MS	1.0 - 5.0×10^{-3} / 0.08×10^{-3} ppm	Repeatable, wide LR	(27, 40)
Vancomycin	0.36 - 1.87 ppm	5 - 40 ppm	Spectrofluorimetry	0.1 - 8 / 0.06 ppm	Sensitive and low cost	(19, 40)
Verapamil	0.059 - 0.067 ppm	0.05 - 0.25 ppm	Spectrofluorimetry	0.02 - 12.0 / 0.008 ppm	Suitable and accurate	(29, 40)

LR[1]: Linear range; LOD[2]: Limit of detection; NR[3]: Not reported; LC[4]: Liquid chromatography; MS[5]: Mass spectrometry; HPLC[6]: High-performance liquid chromatography; GC[7]: Gas chromatography; UHPLCHR[8]: Ultra-high-pressure liquid chromatography high-resolution mass spectrometry.

during drug development and product life-cycle management. Methodologies to determine bioequivalence are well established for systemically acting formulations, comprising: i) qualitative and quantitative sameness of the active pharmaceutical ingredient and excipients; ii) *in vitro* dissolution testing; and iii) a human PK study. This approach, however, is not quite suitable for inhaled drugs because a drug's concentration in the systemic circulation does not always correspond to the drug's concentration at its (topical) site(s) of action (62, 63).

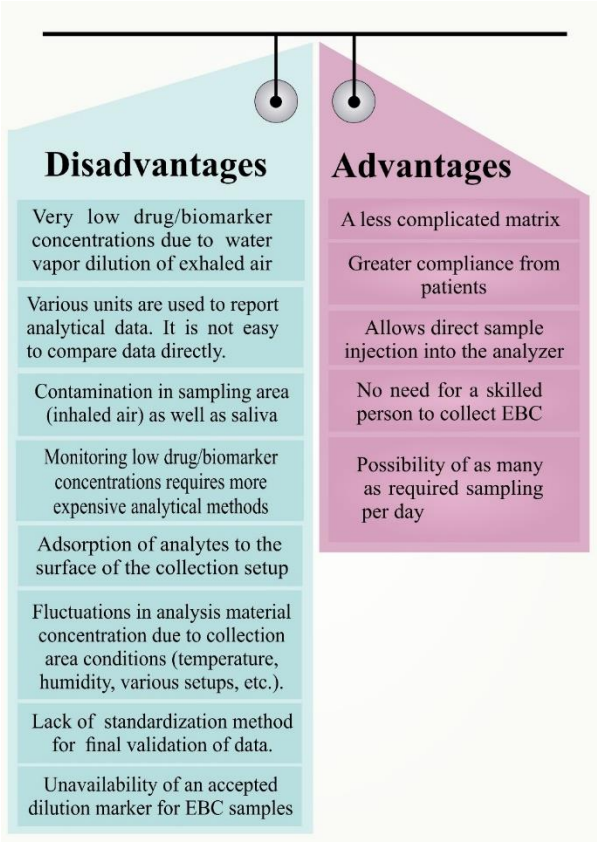

Figure 1. The advantages and disadvantages of EBC analysis

Hence, other approaches must be considered, and establishing bioequivalence may take as many as five steps where data may be required: i) qualitative and quantitative similarity of the active pharmaceutical ingredient and excipients; ii) device similarity to ensure the product performance and the patient-device interaction are unchanged; iii) *in vitro* device performance testing, including emitted fine particle mass (FPM) dose and particle-size profiling; iv) *in vivo* product performance, including lung deposition and systemic PK characteristics; and v) confirmation of equivalent topical efficacy (64). According to the international guidelines, the main variables for testing the bioequivalence of two formulations are the area under the plasma concentration-time curve (AUC) and the peak concentration (C_{max}) (65, 66). For the inhaled drugs, however, many factors, including age, sex, education, duration of the disease, type of inhaler used, correct inhalation technique, or use of several inhalers, can influence AUC and C_{max} effectiveness of drugs for inhalation (67-69). As a result, establishing a link between an inhaled product's *in vitro* performance, and its *in vivo* activity has proven difficult (70).

Similarly, *in vitro* assessments of the emitted doses of inhalers are not universally predictive of PK and PD outcomes in clinical studies. In addition, systemic PK data does not always correlate well with *in vitro* or clinical efficacy data. All these render the assessment of the bioequivalence of inhalers problematic.

Various worldwide regulatory authorities have published guidelines to evaluate the bioequivalence of inhalation products. These approaches had several similarities and differences. Their discrepancy involves many considerations, including cultural, economic, and historical differences (71). Australia's Therapeutic Goods Administration (TGA), Canada's Health Canada (HC), Europe's Medicines Agency (EMA), and the FDA each have their own guidelines.

For example, while the agencies represent similar assessment steps for *in vitro* testing, *in vivo* PK studies, *in vivo* PD studies, and clinical studies on bronchodilators, they consider specific issues (72, 73). Lu *et al.* outlined the details of their comparisons (72).

FDA regulatory agencies suggest bioequivalence based on the therapeutic agent's concentration in a relevant biological fluid (*e.g.*, plasma or blood). As these fluids primarily denote the concentration of medicinally active ingredients that entered the systemic circulation and not the lungs (as the site of action), this could be challenging for locally acting components (74). The FDA established several points in *in vitro* studies, PK studies, and PD or clinical studies and names an aggregate weight-of-evidence method to deal with this issue. This technique has been developed for dry powder inhalers containing long-acting β-agonists and corticosteroids (75). Single actuation content (SAC) and aerodynamic particle size distribution (APSD) are two notable tests utilized in *in vitro* studies. These tests should be performed considering the patient's inspiratory flow rate range and several

steps of the drug's lifetime. From the viewpoint of the flow rates, a minimum of three flow rates of 30, 60, and 90 L/min should be performed to pass this stage. Multiple steps of drug life include the first inhalation(s), reaching 50% of the labeled number of inhalations, and attaining the last inhalation(s) of the tagged number of inhalations (75). Despite these precautions, the FDA protocol was unable to determine the range of drugs administered locally to the lungs.

PK parameters (C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$, $t_{1/2}$, λz) are reported to be similar between the United States, Europe, and Canada as they follow the International Council for Harmonisation's Good Clinical Practice guidelines (76). In addition, the EMA is considering a step-wise method for local inhalers as well. The first step is an *in vitro* method that determines therapeutic equivalence, the same as the FDA guideline. The only difference between the EMA and FDA approaches is the *in vitro* comparison (77). The EMA states that comparisons should be based on average bioequivalence rather than statistical population bioequivalence (77, 78). The next stage brings up PK studies to evaluate systemic safety and lung deposition. The EU has declared that the AUC of the drug in the blood may reflect lung deposition (whether absorption from the lungs is not saturated and the swallowed fraction is insignificant or a blockade of charcoal can ignore it). However, this approach could not measure the drug's elimination by mucociliary clearance, local efficacy, or safety (78). The last step includes PD or clinical studies. In this stage, the EMA performs parallel design and PD endpoints/markers such as methacholine PD20, exhaled nitric oxide (eNO), and sputum eosinophilia for inflammation determination leading to data with low statistical power (78).

When a systemic bioequivalence approach is used, orally administered charcoal is expected to prevent gastrointestinal absorption by the adsorption action. To differentiate between the contribution of absorption from the lungs and that from swallowing, therefor, the oral use of charcoal has been proposed. Recently, HC has given consideration to the importance of the gastrointestinal absorption of inhaled drugs in bioequivalence assessment by stating that "it is not necessary to use a charcoal block to reduce the contribution of gastrointestinal absorption if: i) no significant gastrointestinal absorption is expected, based on published literature, or ii) it is possible to differentiate lung absorption from gastrointestinal absorption, using the pharmacokinetic profile (79). The agency suggests <5% of the total observed AUC as "significant".

On occasions, agencies issue guidelines for individual inhalers. For example, for corticosteroids and bronchodilators, HC suggests sputum eosinophil and eNO measurements for bioequivalence studies of locally acting inhaled (79). Regarding corticosteroid efficacy, adults' single- or multiple-dose PD studies were designed to reveal these products' effect on the hypothalamic-pituitary-adrenocortical (HPA) axis. It is noteworthy to mention that TGA's and EMA's guidelines state bronchodilation and broncho protection studies for long-acting beta-2 agonist bronchodilators (LABA), short-acting beta-2 agonist bronchodilators (SABA), and anticholinergics. However, HC includes only SABA-metered dose inhalers (80).

TGA assesses aerosol particle size distribution and uses a crossover substitution design or a parallel-group design for PK studies. Besides, a gamma camera and radiolabeled drug are suggested to evaluate local efficacy for inhaled corticosteroids and bronchodilators (80).

Japan's pharmaceutical and medical devices agency (PMDA) has announced fundamental principles for the bioequivalence assessment of inhalers in 2016. This agency indicated the three-stage evaluation for developing inhalation products, including *in vitro* studies, PK reports, and clinical endpoint studies, the same as FDA steps (71, 81). It delineated the significance of the delivered dose in line with fine particle mass and at least four sets of stages at three flow rates (10th, 50th, and 90th percentiles; *e.g.*, 30 l/min, 60 l/min, and 90 l/min) in *in vitro* tests to confirm bioequivalence (81). Furthermore, the PMDA takes into account clinical population variance based on various stages of lung disease that could be resolved using *in vitro* tests (81).

It can be understood from the previous sections that the critical parameters in the bioequivalence assessment of corticosteroids in various guidelines are relative glucocorticoid receptor binding affinity, glucocorticoid receptor dissociation constant (Kd, $nmol^{-1}$), relative potency, device efficiency (delivered lung dose) (lung deposited dose/nominal dose), pulmonary residency time (lung retention time (FF)), and bioavailability (F_{oral} = oral bioavailability, F_{inh} = inhalation bioavailability), systemic clearance of the exogenous glucocorticoids (CL = total body clearance), plasma protein binding, Vd = apparent volume of distribution at steady-state, and $t_{1/2}$= plasma elimination half-life (71, 81).

The EBC approach for bioequivalence is void of many issues and obstacles involved in the use of systemic bioavailability. It is a direct measure of the drug exposure to the site of action and obviates the possibility of gastrointestinal absorption (82).

CONCLUSION

Although, thus far, EBC has not been employed for TDM and bioequivalence studies, there is ample evidence to support its use as reliable alternatives.

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