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In-vitro in-vivo correlation (IVIVC) of inhaled products using Twin Stage Impinger

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Abstract

In vitro dissolution testing as a form of quality control has become a necessity in the pharmaceutical industry. As such, the need to establish a method that investigates the in vitro dissolution profile of inhaled products should be taken into account. The prime focus in this study was to examine the in-vitro in-vivo correlation utilising a modified version of the Twin Stage Impinger and to promote an in vitro dissolution model by enhancing the Fine Particle Dose (FPD) collection method for dry powder inhalers.

The Twin Impinger was modified by inserting a stainless steel membrane holder disk in the base of the lower chamber. The design, with optimum drug deposition, was adopted for the dissolution study of budesonide and salbutamol. Afterwards, the membrane holder system was placed in the bottom of the dissolution vessel. Phosphate buffer saline (PBS), simulated lung fluid (SLF, Gamble solution) and Phosphate buffer (PB) were used in the study. The paddle dissolution apparatus, containing 300 mL of the medium, was operated at 75 rpm paddle speed. Samples were collected at defined time intervals and analysed using a validated HPLC method.

The largest proportion of the budesonide dose was dissolved in PBS compared to PB and SLF. This was due to the presence of surfactant (0.2% w/v polysorbate), which enhances the wettability and the solubility of the poorly soluble drug (budesonide). The similarity factors for PBS and PB were 47.6 and 69.7, respectively, using SLF as a reference, whereas the similarity factor for salbutamol dissolution between PB and SLF was 81.3, suggesting PB is a suitable substitute. Comparison using both the predicted and actual in vivo pharmacokinetics (PK) values of the two drugs, as well as the pattern of their Concentration-Time (c-t) profiles, showed good similarity, which gave an indication of the validity of this in vitro dissolution method.

1. Introduction:

The efficacy of aerosol therapy depends on the ability of an inhaler to deliver sufficient drug of suitable-particles to appropriate sites of action in the lungs (Pauwels R, Newman S, & L., 1997). This, in turn, depends on airway anatomy and physiology, which change with age and disease status (Martin et al., 1988; Stocks, 1995; Wohl, 1998:) and therefore should be taken into account to assess both delivery and deposition. The performance of the orally inhaled drug product (OIDP) is evaluated in vitro by the total aerosolised dose delivered (TDD) to the lung and the aerodynamic particle size distribution (APSD). The APSD is the Critical Quality Attribute (CQA) of the OIDP and the guidance requires Quality Control (QC) to characterise this parameter (Labiris and Dolovich, 2003; EMA, 2006). However, the effectiveness of inhaled products relies on inhaled drug particles dissolving in the lung fluid prior to absorption to produce the desired pharmacological effect (Olsson *et al*., 2011). The dissolution is considered a significant step in the action and systemic absorption of the poorly soluble inhaled drugs, such as glucocorticoids (Davies *et al*., 2003). Hence, the rate of dissolution of OIDPs has gained increased interest within respiratory research and among regulatory agencies (Riley *et al*., 2012).

Following dissolution in the fluid layer lining of the internal lung surfaces, the soluble drug diffuses and is absorbed. The dissolution rate of the drug is proportional to the drug's solubility (Dokoumetzidis *et al*., 2006). The drug solubility is dependent on the formulation, the physical properties of the drug and the constituents of the dissolution media. The bronchial epithelial lining fluid consists of water (96%), salt, proteins, phospholipids, mucins and the surfactant (Boat and Cheng, 1980; Fischer and Widdicombe, 2006).

Several studies have investigated the in vitro dissolution method for the inhaled product. In these methods, Next Generation Impactor (NGI) and Andersen Cascade Impactor (ACI) have been used as tools for collecting the aerosol particles (Davies *et al*., 2003; Son *et al*., 2010; Arora *et al*., 2010). NGI and ACI are the apparatus described in the compendial methods (United States Pharmacopeia (USP, 2009), European Pharmacopeia (EP, 2007) and British Pharmacopeia (BP, 2008) for inhaled products testing. In the NGI methods (Son *et al*., 2010; Huang et al., 2018; Lin et al., 2017), the inhaled particles collected for dissolution assessment are only present in a fraction (less than 5%) of the Fine Particle Dose (FPD). In the ACI methods, the first method (Davies *et al*., 2003) collected the particles from the induction port. These particles, however, do not represent the respirable dose (FPD), since it does not differentiate between particles delivered to the lungs and those deposited in the throat. In

another study (Arora, 2010), the dose collection was performed by using Andersen Cascade Impactor (ACI) with a collection stainless steel plate covered with polyvinylidene difluoride (PVDF) filter membranes inserted in stage four of ACI only and then, after the run was completed, the filter with the deposited drug was placed on the semi-permeable polyester supporting membrane on the Transwell® insert containing 1.4 ml dissolution media. In this study, the collected particles for dissolution assessment also represented a very small fraction of the FPD dose. Recently Tay et al., (2018) modified the ACI by removing some of the intermediate stags to test the dissolution of fine particle fraction. However, changing the intermediate stages within the truncated ACI affects the velocity within the modified ACI which change the particle cut-offs. Besides, comparing the amount collected on polytetrafluoroethylene funnel (TF) and small collection plate (sCP) using the truncated ACI with FPD collected using actual ACI doesn't ensure that it represents the actual FPD. Therefore a more robust validation method is required to ensure that all particles collected on TF and sCP have aerodynamic diameter less than about 5 μm.

On the other hand, we have adopted a different approach to FPD dose collection by modifying a Twin Stage Impinger (TSI). A similar approach was reported by Eedara et al 2019, where the dose was collected a glass plate and then was used to study the dissolution using a custom made flow perfusion cell, however they modified the pharmacopoeia method by excluding the liquid from stage 2 without validating how this would affect the FPD. Also they kept the same distance between stage 2 and the glass plate where we found in our study using this setting led to the majority of FPD dose to be deposited on the wall of stage 2 and not on the collection plate. Furthermore, Hallworth and Westmoreland reported the suitability of twin impingers for the routine quality assessment of inhaled aerosols during product development. They found that stage one of the twin impinger apparatus closely mimics the human oropharynx at the operational flow rate of 60L/min, which makes it physiologically relevant to be adapted for assessing the dissolution. The authors also found that the deposition of drug aerosol from MDI in stage two closely represented lung deposition (Hallworth & Westmoreland, 1987). In our presented method, the modified TMS for collecting the dose was validated against the pharmacopoeia method. The TSI consists of two chambers named the upper and lower stages respectively, representing the upper and lower regions of the airways. In this design, the pulmonary dose is collected in the lower stage (chamber). This technique improved the respirable particle dose collected for dissolution to 46% of FPD. Due to its simplicity and user-friendliness, the Modified Twin Stage Impinger (MTSI) saves time and labour as compared to the multistage impactors (NGI & ACI). Hence, we investigated this approach for in vitro

dissolution of the inhaled products. This work also examined the correlation between the in vitro dissolution profile and the in vivo blood c-t profile.

2. Method:

2.1 Dose collection

2.1.1 Modified Twin Stage Impinger (MTSI)

The British Pharmacopeia method for DPI performance characterisation using TSI recommends adding 7 mL and 30 mL of a solvent in the upper chamber and in the lower chambers (conical flasks), respectively. In the current work, the lower chamber of TSI (stage 2) was modified for DPI dose collection by introducing a removable stainless steel disc (5cm diameter x 2mm Thickness) into the base of the conical flask (Figure 1). The stainless steel disc was sprayed with silicone and then it was attached to stage 2 after it has been left to dry for 30 min. Afterward, MTSI was assembled with 7 mL of water placed in stage 1. The inhaler device was attached to the inlet of the MTSI. Four consecutive doses were discharged into the TSI from the inhaler at flow rate of 60 L min^{-1} for 4 s. The MTSI was then disassembled and the stainless steel disk with the collected powder on its surface was then transferred into dissolution vessels. The conical flask, the upper part and the connected tube between the upper and the lower stages were washed with the solvent and analysed using a 25 cm column packed with 5 µm C-18 (Phenomenex®). The mobile phase was methanol/acetonitrile/ acetic acid/ water (50:10:1.7:28.3) and the flow rate was 1.7 ml/min and the wave length was 242 nm (Feddah, 2000).

Validation of the modified version: The TSI and its modified version (MTSI) were tested and validated in two steps to study their suitability for inhaled product characterisations. The first step was carried out with the addition of 30 mL of the solvent at stage 2. In the second step, the sample collection disc was placed in the lower chamber (stage 2) and no solvent added. The stainless steel disc was coated with silicone for 10 minutes before the start of the run. The validation was conducted by comparing the budesonide fine particle fraction (FPF) of the delivered dose reaching the lower chamber from TSI and MTSI. Easyhaler (containing budesonide 200 micrograms/dose; two actuations) employing flow rate (60 L/min) for 5 seconds was used for this study (BP, 2018).

2.2 Dissolution study

2.2.1- Dissolution apparatus

A USP apparatus 2, (Labindia®, DS 8000) with autosampler function was used for the dissolution study. The dissolution study was performed with some modification by placing the membrane holder in the dissolution vessel (Son *et al*., 2010). The membrane holder consisted of the insert stainless steel disk, the polycarbonate (PC) membrane which retains the drug particles and allows them to diffuse on dissolution, the securing ring and the rubber o-ring which seal the disk.

The HPLC methods of budesonide and salbutamol determination in the dissolution samples were sensitive to reproducibly quantitate their two and five actuations, respectively.

2.2.2- Dissolution media

The most commonly used dissolution media for the assessment of the in vitro solubilisation of the inhalation product is SLF that contains DPPC ((Pham and Wiedmann, 2001) because of its similarity to the real lung fluid. However, Son, et al (2010) reported that SLF containing DPPC is not suitable to use with the polycarbonate (PC) membrane barriers because DPPC forms a liposomal aggregates which have a bigger size than the pore size of the PC membrane and hence their diffusion through the membrane and their wetting effect is hindered. In addition, it was also reported that the surfactant gives enough wetting effect for the lipophilic drug and it was hypothesized that using phosphate buffer saline (PBS) as a dissolution media with a membrane barrier system would be a suitable method for the quality control of this class of drugs (Son, 2010).

Three dissolution media, described below, were used in this work:

    A. Phosphate buffer (PB), 0.2 M, pH 7.4: This was prepared by dissolving potassium dihydrogen phosphate in distilled water and pH adjusted to 7.4 with 0.1M sodium hydroxide .

    B. Phosphate buffer saline (PBS): This was prepared similarly as PB to which polysorbate 80 (0.2% w/v) was added (Son et al., 2010).

    C. Gambles solution: was prepared as described by Marques *et al*., 2011.

2.2.3- Dissolution procedures

The dose(s) of the tested drug was collected on the stainless steel disk as described above (see section 2.1). The disk was removed from the Modified Twin Stage Impinger (MTSI) and covered with a pre-soaked membrane using dissolution media, and sealed by the securing O-

ring (Son et al., 2010). The dissolution conditions and parameters described by Son et al. (2010) were followed. The sealed membrane holder was placed at the bottom of the dissolution vessel which contained 500 ml of the dissolution medium equilibrated to 37°C. The minimum operational volume with USP apparatus 2 is 500 ml as the hydrodynamics becomes very unstable for dissolution testing below this volume (Crist GB, 2009). The paddle speed set at 75 rpm. It was reported by Son et al (2010) that this speed enhances the diffusion of the dissolved particles of the drug from the powder bed into the media as well as it is vital to maintain the consistency in the dissolution media . The autosampler was programmed to draw 5 mL of the sample solution from the dissolution vessel at time intervals of 5, 15, 30, 60, 90 and 120 min. and replaced with fresh medium from the reservoir. The samples were syringe filtered (0.45 mm) and, after discarding initial 2 mL, transferred to the HPLC vials for analysis.

The experiments were performed in triplicate in each dissolution medium.

2.2.4- Dissolution profile comparison

The dissolution profiles were assessed by applying model independent method by using ratio test procedure and pair wise comparison such as difference (f_1) and similarity (f_2) factor (Polli e al., 1997). The comparison factors simplify dissolution profile data into one number (Duan et al., 2011) which should be between 0-100 (Costa et al., 2001). It is argued that a similarity factor (f_2) closer to 100 suggests higher similarity, while the difference factor (f_1) approaching zero indicates higher dissimilarity (Costa et al., 2001).

The similarity factor is calculated from Equation 1 given below (Costa et al., 2001):

$$f_2 = 50 \times log \left\{ \left[1 + (\frac{1}{n}) \sum_{t=1}^{n} |R - T|^2 \right]^{-0.5} \times 100 \right\}$$

Equation 1: Similarity factor (f_2)

Where n is the number of the dissolution samples, R and T are the mean percentage of drug release for the reference and the test profile, respectively, at each time interval.

2.2.5- Conversion of dissolution profile to plasma concentration (c-t) profile

Dissolution measures the rate at which the drug could form a solution after it is released from the dosage form. It is usually performed to predict the dissolution of the drug in the GI tract and more recently to estimate dissolution of the drug in the airways.

1 The convolution method that was reported by Qureshi (2010) to study the IVIVC for diltiazem
2 products has been adapted in this work. The convolution method utilises the data obtained from
3 the dissolution to estimate the blood drug levels using pharmacokinetic parameters of the tested
4 product which could be obtained from the literature. The authors of the presented work assume
5 that this approach has the potential to be extrapolated for the study of IVIVC of inhaled product
6 as dissolution is the property of the product and it is unlikely to change the pharmacokinetics
7 parameters of the drug (Qureshi (2010).

8 Qureshi (2010) described the method to transform in vitro dissolution profile to in vivo plasma
9 concentration profile. Firstly, the dissolution percentage of drug released is converted to the
10 amount of drug for each sampling time point. The amount of drug at each time point reflects
11 on the diffusion and release of the drug through the membrane of the collection disc. This
12 amount is multiplied by the bioavailability factor of the drugs to obtain the amount available
13 in the blood. Finally, the drug concentration for each time point is calculated by dividing the
14 amount in the blood by the distribution volume. Subsequently, the amount of the drug
15 eliminated from the blood is estimated using its reported elimination rate (Derendorf *et al*.,
16 1998). The total bioavailable drug is obtained by adding up all the drug amounts for each time
17 point.

18 The c-t profile is drawn by plotting the time (h) on the x-axis and the concentration (ng/L) on
19 the y-axis (Qureshi, 2010).

20

21 **3. Results and Discussion**

22 **3.1- Method Validation with Modified Twin Stage Impinger (MTSI)**

23 The performance of the MTSI was validated to ensure that the alteration and the absence of the
24 30 mL solvent in the lower chamber had not affected the particle deposition. The FPD of
25 budesonide was compared between TSI and MTSI using the BP (2018) method.

26

27 Figure 2 shows the mean (n=5) deposited mass of two budesonide actuations in each part of
28 TSI and MTSI. The relative standard deviation (RSD) of the mass obtained for five runs for
29 the throat, lower and upper chambers was $< 0.5\%$.

30

31 The effect of the distance (x) between the filter and the surface of the lower chamber base
32 (inserted disk) on the collected drug amount on the inserted disk was also studied (Figure 3).
33 The several levels studied included: 0.2 cm, 1 cm, 2 cm and >2 cm (Figure 4A, 4B) using two
34 actuations of budesonide Easyhaler.

1   The results revealed that the high level at 2 cm (Figure 4) led to reduced amount collected on

2   the disk, while increasing deposition on the flask wall. Level >2 cm including 3 and 4 cm

3   resulted in even more reduction of the drug amount collected on the disk. This is because the

4   position of the filter at this distance was too far from the disk; therefore, the delivered amount

5   spread on the flask wall. On the other hand, the 0.2 cm filter height led to the highest drug

6   amount on the disk, because the position of the filter was close enough to the inserted disk

7   surface, which minimised loss of the drug particles to the flask wall. Additionally, the 0.2cm

8   filter height gives enough distance to allow twin impinger disassembling without disrupting

9   the amount of drug on the inserted disk. Therefore, based on this study the distance (x) 0.2 cm

10   was used during the dose collection.

11

12   **3.2- Dissolution study**

13   The dissolution of the drug through the membrane was measured by quantifying the amount of

14   drug diffused from the membrane on the stainless steel disc into the dissolution medium. This

15   diffusion is controlled and affected by the membrane pore size, tortuosity and thickness. The

16   polycarbonate membranes used in this study were of 6 µm uniform thickness, having uniform

17   and precise non-tortuous air-bubble free 0.05 µm pores, possessing uniform cylindrical

18   swelling-resistant channels, thereby facilitating smooth diffusion and un-restricted dissolution.

19   They do not create air bubbles and they are composed of homogenous 0.05 µm non-tortuous

20   cylindrical pores on their surface (Son *et al*., 2009; Marre *et al*., 2001).

21

22   **Budesonide dissolution profile**

23   The dissolution profile of budesonide (BD) was studied in three media: Gambles solution, 0.2M

24   phosphate buffer (PB) and phosphate buffer saline (PBS). The results show that a large

25   proportion of budesonide was released in these media (Figure 5, Table 1). This shows the

26   advantage of the modified Twin Impinger design as it allows for conducting the dissolution

27   study using the minimum number of doses (only 2 doses were actuated from the inhalers) and

28   hence forming a thin powder bed on the disk. This correlates with the fact that the thickness of

29   the powder on the disk has a significant effect on the powder wetting inside the membrane

30   holder and hence on the drug release, especially for the lipophilic drug (Son *et al*., 2010),

31   Besides, the thick powder bed has more agglomerated drug particles, which leads to surface

32   area reduction, that is an important factor for the wettability and solubility of poorly soluble

33   drugs (May, 2013). The solubility of BD in SLF was previously measured using the shake-

34   flask method (Jouyban, 2010) and it was 14.74 µg/ml while its reported solubility in PB and

PBS was 17 µg/ml and 53 µg/ml respectively (May, 2013). The dissolution curve shows that the cumulative release percentage of budesonide in the PBS after 2 hours was $95.76 \pm 1.72\%$ and the residual drug amount on the membrane holder was 2.55 µg (Figure 5 A, Table 1). As for the dissolution profile of budesonide obtained using Gambles solution, these values were $80.78 \pm 3.6\%$ and 16.6 µg (Figure 5 C, Table 1). Further, the budesonide dissolution profile in PB produced $78.88 \pm 3.99\%$ and 14.4 µg (Figure 5B, Table 1). In addition, it can be noticed that the dissolution profiles for budesonide in SLF, PBS and PB followed similar trend of the reported budesonide solubility in these media.

The similarity factor (f_2) between the phosphate buffer saline (PBS) and phosphate buffer (PB) was calculated using Gambles solution (SLF) as a reference. SLF does not contain surfactant and it is usually used as a model for the lung fluid (Stopford *et al*., 2003; Colombo *et al*., 2008). The f_2 values were 47.64 and 69.74 for PBS and PB, respectively, which shows that the phosphate buffer (PB) can be used as an alternative dissolution media to SLF for testing budesonide dissolution. Unlike SLF, phosphate buffer saline (PBS) contains 0.2% (w/v) polysorbate 80 (surfactant) which enhances drug saturation solubility and the wettability of the hydrophobic drug, thereby preventing the aggregation and enhancing the dissolution rate (Riley *et al*., 2012; Wiedmann *et al*., 2000; Son *et al*., 2009). Figure 5 (D) shows that the PBS dissolution media gave the highest dissolution profile as compared to the SLF and PB which is likely due to the presence of surfactant (polysorbate 80) in the PBS composition. However, the fact that PBS f_2 value is less than 50 suggests that PBS cannot be used as alternative dissolution media to SLF for testing budesonide dissolution profile.

It is worth noting that the lowest budesonide in vitro dissolution profile was in the SLF (Gambles solution). Interestingly, SLF and SLF containing dipalmitoylphosphatidylcholine (DPPC) are commonly used as dissolution media for the assessment of the in vitro solubilisation of the inhalation products. Nevertheless, as indicated in the literature, using SLF containing DPPC with the polycarbonate membrane barriers is not practical (Son *et al*., 2010). On the other hand, PBS gives enough wetting for the lipophilic drug (budesonide) due to presence of the surfactant (polysorbate 80) and its compatibility with a membrane barrier system. The results of current work, therefore, indicate that PBS could be an appropriate dissolution medium that can serve as a quality control test for hydrophobic drugs.

Salbutamol dissolution profile

1 Phosphate buffer saline (PBS) was excluded from the dissolution study for salbutamol sulphate

2 (SS) since it showed the lower similarity factor (f_2) value (47.64) compared to SLF (Gambles

3 solution) for budesonide. The cumulative release percentages after one hour for the dissolution

4 profile using PB and SLF were 81.39% and 81.26%, respectively (Table 2). This implies that

5 most of the SS delivered dose deposited on the disk dissolved in the first hour. This high

6 dissolution is due to high solubility of salbutamol sulphate which was 11.8 in SLF and 14.5

7 mg/ml in PB. The dissolution profile is heavily dependent on the solubility of the drug in the

8 dissolution medium. The higher the solubility, the faster the dissolution. Also, release profiles

9 vary depending on the dissolution pattern of the drug product in different media.

10

11 Figure 6 shows that approximately 80% of SS was released within the first 5 minutes of

12 dissolution and the rate of dissolution in the two media decreased with time. Further, the trends

13 of both profiles were similar. Additionally, the respective dissolution profiles were more

14 independent of the dissolution in the aqueous media and on drug loading and reflected the high

15 solubility of SS in water (Son *et al.*, 2010).

16

17 The similarity factor (f_2) between SLF (used as reference) and PB was 81.35. This high f_2 value

18 indicates that PB is a suitable substitute to simulate lung fluids for in vitro dissolution

19 assessment of hydrophilic drugs since it is simple and consists of fewer ingredients. Besides,

20 PB preparation is easier and faster as compared to SLF. These results are consistent with

21 budesonide dissolution profile in that the similarity factor (f_2) was 69.74 (>50) for SLF and PB.

22 **3.3 <u>In-vitro In-vivo Correlation</u>**

23 The c-t profile prediction has gained increasing importance in drug development and

24 pharmaceutical product evaluation (Qureshi, 2010). A dissolution profile can be

25 mathematically converted to a blood concentration-time profile (c-t profile) by convolution

26 (Sakore and Chakraborty, 2011) using the in vivo pharmacokinetic parameters reported in the

27 literature (Qureshi, 2010). The pharmacokinetic parameters include bioavailability factor,

28 volume of distribution, elimination half-life ($t_{1/2}$) and the elimination rate constant (K_e)

29 (Derendorf *et al.*, 1998). For the current work these pharmacokinetic parameters for budesonide

30 and salbutamol were respectively obtained from Derendorf *et al.* (1998) and Jiang *et al.* (2016).

31

32 Table 3 and Figures 7 and 8 show the predicted C_{max} and t_{max} measured from the predicted c-t

33 profile. The table also compares the corresponding in vivo values.

34

The comparison of the predicted c-t dissolution profiles (Figures 7 and 8) with the in vivo c-t profile of the inhaled budesonide (Derendorf *et al.*, 1998) and salbutamol (Jiang *et al.*, 2016), clearly show their similar release characteristics and indicate the in vivo predictive effectiveness of the in vitro dissolution method.

The t_{max} values obtained from the predicted profile for both budesonide and salbutamol using PB and SLF dissolution media resembled the actual in vivo values (Table 3). However, the t_{max} for budesonide using the PBS dissolution medium was about one hour, which is higher than the in vivo t_{max} range. This could be due the presence of the polysorbate 80 surfactant, which is different from the real lung surfactant (DPPC).

Further, the C_{max} values were different and reflected the difference in the initial budesonide dose used for in vivo (400 µg) and in vitro (200 µg) studies. In one of the clinical studies, Kaiser (1999) found that C_{max} was linearly related to the budesonide dose and noted that the C_{max} was increased by a factor of ~2 as the dose was doubled. In the present study, the in vitro predicted C_{max} for the 200 µg dose was 0.21-0.24 nmol/L. Hence, the in vitro predicted C_{max} for the 400 µg dose would be 0.42-0.48 nmol/L. The variability between predicted C_{max} values from the real in vivo values may also be due to the fact that 46% of the FPD that had deposited on the disk only (not all the FPD) was used for the dissolution study. However, in the real in vivo plasma study, the FPD that would reach the lung might thereby lead to a higher C_{max} value. Increasing the in vitro FPD collection efficiency could therefore enhance the predictive effectiveness of in vitro dissolution for in vivo studies.

4. Conclusion

We have conducted the dissolution on more than 46% of the fine-particle dose (FPD). This design is simple, easy and fast for testing dissolution of inhaled products. We believe that there is a potential for this design to be used in quality control and for evaluating the manufacturability of inhaled products by studying the in vitro/in vivo correlation (IVIVC) of inhaled particles.

Our proposed IVIVC model is a suitable and practical procedure to obtain blood drug c-t profiles from dissolution studies. The current suggested model predicted the PK parameters' values fairly accurately, making the design more cost- and time-effective.

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Figure captions

Figure 1. A schematic diagram showing MTSI (A) The modified TSI, (B) The lower chamber flask fitted with the insert disk, (C) The modified flask, the inserted stainless steel disk and the securing ring, (D) [*]Stainless steel disk with 5 actuations of the drug.
[*]The inserted stainless steel disk provides an airtight seal simply by push on at the bottom of the lower chamber flask

Figure 2. Particles deposition of budesonide Easyhaler using the conventional Twin Impinger (lower chamber contains 30 ml methanol) and using modified version of Twin Impinger (lower chamber dry without 30 ml methanol).
Upper dry: modified Twin Impinger (lower chamber dry without 30 ml methanol).
Upper with: conventional Twin Impinger (lower chamber contains 30 ml methanol)

Figure 1. A schematic diagram of Twin Impinger lower chamber.

Figure 4. A comparison the amount of budesonide collected in the Twin Impinger lower chamber parts (tube, flask and the inserted disk) (A) at 0.2cm and 1cm filter height. (B) at 0.2cm and 2cm.

Figure 5 . (A) The dissolution profile of BD in PBS. (B) The dissolution profile of BD in PB.(C) dissolution profile of BD in SLF1. (D) Comparison of BD dissolution profile of three dissolution media, PBS, PB and SLF.
All values are mean (SD), n=3

Figure 6. Comparison of the dissolution profile of SS in PB and SLF

Figure 7. The predicted C-t profiles for BD in three dissolution media (phosphate buffer (PB), phosphate buffer saline (PBS) and simulating lung fluid (SLF).

Figure 8. Predicted C-t profiles derived from dissolution profiles of SS in PB and SLF