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Article

# Dry powder inhalation for sildenafil citrate: formulation and performance tests

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Pulmonary arterial hypertension (PAH) is a severe disease responsible for high mortality in the affected population. Due to the limitations in treatment as severe adverse effects, high cost, we aimed to develop a simple therapeutic option for pulmonary administration, using a vasodilator sildenafil citrate (SILC) in a dry powder inhalation using lactose as carrier. A formulation of micronized SILC was prepared after 30 min wet grinding of the active with the aid of a vibration shear-mill. Solid characterization by laser diffraction and X-ray powder diffraction was assessed for active. Performance tests such as delivered-dose uniformity (DDU) and aerodynamic particle size distribution (APSD), using Dosage Unit Sampling Apparatus and Andersen Cascate Impactor, respectively, were performed. The micronization of SILC resulted in a medium size of 4.54 µm and a 50$^{th}$ percentile of 2.93 µm. For DDU, the total amount delivered ranged from 92.42 to 109.05% of the targeted dose, in agreement with the specified range (75.0-125.0%). For ASPD, a fine particle fraction of 35% was satisfactorily obtained. This formulation is simple and promising therapeutic option for the treatment of PAH with the advantage of being easily produced using less complex carrier matrix or equipment, in a short time.

**Keywords:** Pulmonary arterial hypertension. Sildenafil. Micronization. Dry powder inhalation. Delivered dose uniformity. Aerodynamic size distribution.

## INTRODUCTION

Pulmonary arterial hypertension (PAH) is a serious and progressive disease characterized by pulmonary vasoconstriction, vascular remodeling in situ, thrombosis and high mean pulmonary arterial pressure. PAH is defined as long the mean pulmonary arterial pressure is greater than 20 mmHg at rest, a pulmonary capillary wedge pressure is less than 15 mmHg, there is increased pulmonary vascular resistance and abnormal cardiac output in a normal state. As a result, there is a large right ventricular overload due to a failure of its function, leading to the patient death (Zolty, 2020; European, 2022; Brasil, 2023).

Treatment with the existing drugs does not reverse the pathophysiology and vascular remodeling characteristics of the disease but only increase the quality of life and survival of patients (Simonneau *et al*., 2019; Brasil, 2023).

Currently, dosage forms for PAH treatment can be used through continuous infusion, oral medications or inhaled carried out in a single or multidrug regimen. Several therapeutic classes of drugs used are phosphodiesterase-5 inhibitors (PDE5-I) as sildenafil citrate (Revatio®, oral); prostacyclin derivatives as iloprost (Ventavis®, inhalation), treprostinil (Tyvaso®, inhalation; Remodulin®, injectable; Orenitram® oral); prostacyclin receptor agonist as selexipag (Uptravi®, oral); prostanoids as epoprostenol (Veletri®, injection);

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endothelin receptor antagonists as oral therapy like ambrisentan (Letairis®), bosentan (Tracleer®) and macitentan (Opsumit®), and stimulators of soluble guanylate cyclase which targets the same signaling pathway as the PDE5-I, for instance, riociguat (Adempas®, oral) (Frantz, 2020; Zolty, 2020; European, 2022; FDA, 2023).

In Brazil, sildenafil citrate (SILC), ambrisentan, bosentan, iloprost and selexipag are included in the list of drugs available via the local National Health System *(Sistema Único de Saúde/*SUS) and they are very expensive, turning the access difficult. In the case of use of ambrisentan and bosentan caution should be addressed due to changes in the liver function. Among these drugs, SILC (oral) shows a considerably reduced cost compared to iloprost and bosentan or ambrisentan (Brasil, 2022; Brasil, 2023).

Problems associated with the use of oral SILC in PAH are high dose, short dosing intervals, unwanted systemic side effects and limited use in pediatric patients. Moreover, prolonged use of oral SILC causes resting hypotension and nose-bleeding, elicits painful and prolonged penile erections, and worsens pulmonary vascular occlusive disorders (Rubin, 2012; Jain *et al*., 2014; Rashid *et al*., 2017).

Orally inhaled products for pulmonary administration must meet requirements of critical quality attributes such as the total amount delivered from the device by means of delivered-dose uniformity (DDU), and the potential delivery efficacy by means of the aerodynamic particle size distribution (APSD). Abiona and colleagues (2022) discussed the challenges associated with carriers for dry powder inhalation (DPI), mainly lactose, a widely used carrier. The authors show that the conditions of manufacturing processes, as well as, physicochemical properties of lactose can greatly influence DPI formulation or performance.

Beck-Broichsitter and his group (2016) reported studies on biodegradable nanoparticle formulations containing poly(lactide-co-glycolide) polymer (PLGA) and SILC for pulmonary administration via aerosol or nebulization (Aeroneb® Pro). Sawatdee and colleagues (2013) developed nanosuspensions of SILC complexed with cyclodextrins for treating PAH by means of metered dose pressurized aerosols.

The development of DPI formulations containing SILC, alone, in complex, or with polymers using spray drying (Ghanbarzadeh *et al.,* 2016; Nguyen *et al.*, 2019; Shahin *et al*., 2019; Atipairin, Sawatdee, 2020) or supercritical $CO_2$-assisted spray drying (Restani *et al*., 2020) has also been described.

Recently, Mohamed and colleagues (2021) developed SILC loaded metal-organic frameworks (SILC-MOF) nanoparticles, prepared by a sonochemical synthesis. They aimed to take profit from drug delivery porous platforms in order to increase drug load and control the release, as well as limited pharmacokinetics. A cell viability study was performed in human endothelial and pulmonary artery smooth muscle for change in respiration and cell cytotoxicity. Effects were also tested in mice-removed aorta for vasomotor response. The vasodilator response was significant and sustained compared to SILC base, however, pharmacological, toxicological and efficacy studies for the pulmonary administration need further investigation.

Based on reported formulations, significantly advanced research is foreseen for inclusion of new therapeutic approaches for PAH. Yet, they are complex or require special resources in their manufacturing or administration. Thus, the development of equally effective simple dry powder for inhalation formulations is technologically and economically important, as they can be competitively viable at more affordable costs.

The DPI is an excellent alternative dosage form for the treatment of severe lung diseases, such as PAH, provided it can carry low dose, maximize local effect, yield a faster onset of response and reduce adverse effects. Therefore, we aimed to develop a low-cost and simple solid DPI formulation containing low dose of micronized SILC in capsules, prepared by grinding the active ingredient added of lactose, as a carrier. Crystal characterization and performance tests were also conducted for quality evaluation.

## MATERIAL AND METHODS

SILC working standard and SILC active ingredient (SILC AI) (99.69% purity, batch UT2100403, 100.5% purity, batch UT2100703, respectively, Ultratech India Limited, New Mumbai, India), kindly donated by Vita Nova Institute (Hortolândia, SP, Brazil) and lactose (particle size 100 µm; Dinâmica Química Contemporânea Ltda., Diadema, SP, Brazil) were used. Hard transparent gelatinous capsules, self-locking (n. 03, 0.3 mL of internal volume; Capsugel, Rio de Janeiro, RJ, supplier Pharmacopeia Ativos Magistrais, Anápolis, GO, Brazil); acetonitrile (J.T. Baker; Phillipsburg, NJ, USA); methanol (Tedia Company, Fairfield, OH, USA); phosphoric acid (Merck, Darmstadt, Germany); triethylamine (Merck; Darmstadt, Germany); isopropanol, hexane, ethyl acetate and glycerol (LabSynth, Diadema, SP, Brazil) were used. All chemicals and reagents were of analytical or chromatographic grade.

### Micronization of sildenafil citrate

The micronization of the SILC AI was performed in a vibration shear mill (205 mm width × 155 mm height × 520 mm depth, Retsch McCrone Micronising Mill; Glen Creston, London, England). A dry or wet milling was carried at a frequency of 50 Hz in a 125 mL jar with the aid of grinding action of bits of agate (10 mm i.d.; 11 mm height) on 2.0 g of SILC. Grindings were tested in dry mode for 5, 10 min and in wet mode was tested during 5, 10 and 30 min. In the wet grinding, a wetting agent (isopropanol, 7 mL) was used by recommendation of manufacturer, for each 2.0 g of SILC. Thereafter, the obtained paste was dried (105°C, 3 h; Fanem dry oven, São Paulo, SP, Brazil). The active wet ground for 30 min was selected to compose the formulation.

### Crystal characterization

Characterization by particle size by laser diffraction (LD), scanning electronic microscopy (SEM) and X-ray powder diffraction (XRPD) was performed for SILC AI, micronized SILC samples (5, 10 min at dry mode; 5, 10, 30 min at wet grinding) and the developed inhalation formulation.

#### *Laser diffraction (LD)*

The particle size measurements were performed in triplicate, via liquid mode in a laser diffraction particle size analyzer (LS13320, Beckman Coulter, Brea, CA, USA), detection range from 0.017 to 2000 µm for SILC AI and micronized SILC samples. The samples were previously dispersed in ethyl acetate, by mechanical stirring and ultrasound for 10 min. During the analysis, the samples remained homogenized by external ultrasonic stirring coupled to the equipment. The results were expressed as diameter (µm) mean, mode, 50th percentile ($D_{50}$ or median) and 90th percentile ($D_{90}$).

#### *Scanning electron microscopy (SEM)*

A JSM-6360 LV scanning electron microscope (Jeol Ltd., Tokyo, Japan) was used for the SEM characterization of SILC AI, micronized SILC samples and the developed formulation. The samples were mounted onto metal stubs using a double-sided adhesive carbon tape and sputter-coated with a thin layer of gold (5 nm) under vacuum. The images were obtained by secondary electrons with a voltage of 15 kV and at different magnifications (×250, ×500, ×5000).

#### *X-ray powder diffraction (XRPD)*

XRPD patterns for SILC AI and micronized SILC were collected using a Rigaku Miniflex X-ray diffractometer (Rigaku Corporation, Tokyo, Japan) with a $Cu_{K\alpha}$ radiation (1.5418 Å) and scanned between 5° and 50° with 20 s degree$^{-1}$.

### Development of formulation

The formulation was developed by means of preparation of a physical manual mixture of the drug (351.2 µg SILC equivalent to 250 µg of sildenafil)

previously micronized, added of lactose (SILC to lactose millimolar ratio was $5.27 \times 10^{-4}$ to $5.46 \times 10^{-2}$) in sufficient amount to yield 20 mg of formulation. The formulation was placed in small hard gelatinous capsules.

## Quality control and performance tests

For the assessment of quality and performance of SILC formulation contained in capsules, the following tests were performed: weight variation, content uniformity, determination of water content by Karl Fischer titration (Metrohm, Herisau, Switzerland), high-performance liquid chromatographic-ultraviolet diode array detection (HPLC-UV/DAD, Agilent. Santa Clara, CA, USA) assay, DDU and APSD according to procedures and specifications described in the Brazilian (2019) or The United States Pharmacopeia (USP-NF, 2023). The determination of water and drug assay were performed in triplicate from a pool of 20 capsules.

A commercially available inhaler device (DPI Aerolizer® type, Fluir®, Mantecorp, Rio de Janeiro, Brazil) was used to perform APSD and DDU tests for SILC DPI capsules.

The specifications for water determination, HPLC assay and APSD tests were adapted based on the monographs of finished products like salbutamol and fluticasone propionate inhalation powder (British, 2014; USP-NF, 2023) as monographs for SILC DPI capsules dosage form were not available.

SILC, calculated as the target-delivered dose for DPI formulation, was determined using a previously validated method by HPLC-UV/DAD (Silva, 2014). Briefly, the conditions were $C_8$ column, dimensions 250×4 mm; 5 µm (LiChrospher, Merck, Darmstadt, Germany), isocratic mobile phase using acetonitrile, methanol and 1% v/v triethylamine solution pH 7.0 (45:25:30) at 1.0 mL $min^{-1}$, 30ºC and UV/DAD λ 292 nm detection.

### *Delivered-Dose Uniformity*

A dosage unit sampling apparatus (DUSA, Copley Scientific (Nottingham, UK) was used for the DDU test. The capsules were perforated with the Aerolizer® device and the delivered content was led into the DUSA by a metered flow provided, through a vacuum pump. The flow was defined as the one that produced a pressure drop of 4 kPa for a consistent period, yielding a withdrawal of 4.0 L of air from the mouthpiece of the inhaler device (USP-NF, 2023). The flow rate set for the Aerolizer® device was 65 L $min^{-1}$.

The DDU test was performed with ten capsules of SILC DPI formulation and the delivered doses were separately collected in different collection tubes, each washed with 20 mL of mobile phase. Solutions of delivered doses from the capsules were assayed by a validated HPLC-UV/DAD method (Silva, 2014).

The capsules results must comply with the DDU requirements if not less than 9 out of 10 tested doses are between 75.0-125.0% of specified target-delivered dose and none is outside the range 65.0-135.0% of the specified target dose. If the content of at most 3 doses is outside the range 75.0-125.0%, but within the range 65.0-135.0% of the target dose, the procedure must be repeated with additional 20 capsules (USP-NF, 2023). The labeled claim 351.2 µg SILC equivalent to 250 µg of sildenafil was used as the specified target-delivered dose in this work.

### *Aerodynamic Size Distribution*

The Andersen Cascade Impactor (ACI) (Copley Scientific, Nottingham, UK) was used for the APSD test. In brief, the ACI consists of an induction port in which the mouthpiece adapted to the inhaler device (Aerolizer®) is connected, and a set of sieves arranged in descending order of nozzle diameter (eight stages, from top to bottom numbered 0 to 7). Below each sieve there is a stainless steel plate in which the drug particles are deposited. Such plates, known as ACI stages, were coated with a thin layer of glycerol solution (1% v/v in hexane) deposition, as recommended in USP-NF (2023). Ten capsules were perforated by the Aerolizer® device to perform the APSD test in the ACI. The content released was separately taken by means of a vacuum-

controlled flow to the set of sieves and plates of ACI stages. The flow rate ($F_{test}$, 65 L $min^{-1}$) and the pump driving time (4 s) were defined, as described for the test DDU. The test was performed in triplicate.

Equation 1, $d_{ae,cut,Ftest} = d_{28.3}\sqrt{28.3/Ftest}$, was used to calculate the cut off diameter ($d_{ae,cut,Ftest}$) at 65 L $min^{-1}$ (Ftest), comparatively to respective nominal diameters ($d_{28.3}$) at previous stages.

To characterize the deposition performance for SILC DPI, fine particle dose (FPD, µg), fine particle fraction (FPF, %), mass median aerodynamic diameter (MMAD, µm) and the geometric standard deviation (GSD) parameters were calculated by appropriate software (Copley Inhaler Testing Data Analysis Software/CITDAS, version 3.10 Wibu, Copley Scientific, Nottingham, UK).

FPD represents the mass (µg) of particles smaller than 5 µm released per capsule, whereas the percentage of FPF is the ratio between the FPD and the total dose released from the inhaler device. MMAD is the diameter that limits the mass distribution in half (50% larger and 50% smaller particles). The GSD value indicates the size dispersion around the average, when the data are adjusted in a log-normal distribution of particle number and logarithm diameters. Formulations with GSD values greater than 1.2 indicate a polydisperse size distribution or a polymodal distribution (USP-NF, 2023; Gonda, 2004; Telko, Hickey, 2005; Andrade-Lima, Pereira, Fernandes, 2012).

## RESULTS AND DISCUSSION

### Particle Size by Laser Diffraction

SILC AI, as received, showed inappropriate particle size for use in pulmonary administration (1 to 5 µm), since the average diameter was 42.77 µm, the mode was 32.00 µm and $D_{90}$ value was equal to 86.54 µm (Table I). Therefore, it was necessary to reduce its particle size by micronization.

The results for SILC particle size characterization before and after micronization (during 5, 10 and 30 min by dry or wet grinding) by using laser diffraction are shown in Table I. The values of $D_{50}$ and $D_{90}$ correspond to the diameter for which 50% and 90% of particles are smaller than the stated diameter, respectively.

The micronization by dry grinding during either 5 or 10 min resulted in particle diameters only 2 to 3-fold smaller than those of the SILC AI, as received. $D_{50}$ and $D_{90}$ values ranged from 8.75 to 32.87 µm, however, they were still larger than the desired values (Table I). No significant changes resulted when the micronization time was increased from 5 to 10 min.

The micronization by wet grinding using isopropanol (7 mL per each 2.0 g of SILC) was more efficient to reduce the particle size of SILC AI compared to the dry grinding. Particles reached diameters of about 5 to 7-fold smaller than the original diameter during grinding for either 5 or 10 min. Diameters up to 10-fold smaller were registered when micronization time reached 30 min, as shown in Table I.

The dispersion of the results (triplicate) was expressed in relative standard deviation (%RSD). The results were satisfactory for all samples tested according to USP-NF (2023), as long as they are less than 20% for central diameters, such as $D_{50}$ and medium diameter; and up to 30% for not central diameters, such as the 10th ($D_{10}$) or the 90th ($D_{90}$) percentile. In the dry and wet grinding modes, during 5 min, 50% of particles were above 5 µm. Nevertheless, micronized samples by wet grinding during 10 and 30 min showed 50% of particles smaller than 5 µm, the cut-off size value for an inhalation formulation. Additionally, the wet micronization of SILC during 30 min showed even more satisfactory results for $D_{50}$ and $D_{90}$ values (2.93 and 9.08 µm) compared to those (4.70 and 13.65 µm) by wet grinding for 10 min, respectively.

Therefore, micronized SILC by wet grinding, during 30 min, was selected for the preparation of SILC DPI formulation. Although $D_{90}$ value (9.08 µm) of the selected sample was greater than 5 µm, this result only, does not hinder the feasibility of the formulation, since the size distribution is not the sole

predictive of the lung deposition profile that a set of particles may show. In addition to the particle size features, other physicochemical and rheological properties of the materials were evaluated, such as the aerodynamic diameter ($d_{ae}$). Yet, particles with the same physical diameter may have different values of $d_{ae}$ and, consequently, show different aerodynamic behavior before an inspiratory flow (Telko, Hickey, 2005).

## Scanning Electronic Microscopy (SEM)

Figure 1 A-C shows the morphology of the particles indicated by the photomicrographs of A) non-micronized SILC AI, and SILC DPI formulation containing the physical mixture of the micronized active substance (wet grinding, 30 min) plus lactose (100 µm particle size) in different magnification, B) ×500, scale bar 100 µm and C) ×5000, scale bar 10 µm). The non-micronized drug particles appear as crystals in the form of sheaths or needle clusters with various lengths and sizes. The drug crystal structures are seen on the photomicrographs of SILC DPI formulation, however, in less proportion. Moreover, the small crystals of SILC are adhered to the surfaces of lactose particles, the majority as irregular shaped crystals.

These results are consistent with the literature reports about the role of lactose in DPI formulations, as a drug carrier to the respiratory tract. As SILC drug has physically adhered to the surface of the carrier during formulation, the process of powder aerosolization is subjected to forces that break the weak interaction forces between the drug/carrier. Furthermore, as the particle size of the carrier is about 20-fold (100 µm) that of the drug, its particles are deposited in the oral cavity, while the smaller drug particles follow their flow to the lower airways (Islam, Cleary, 2012).

## X-Ray Powder Diffraction

The XRPD patterns for SILC AI and the micronized drug using different modes and time are shown in Figure 2. XRPD analysis was performed to evaluate possible

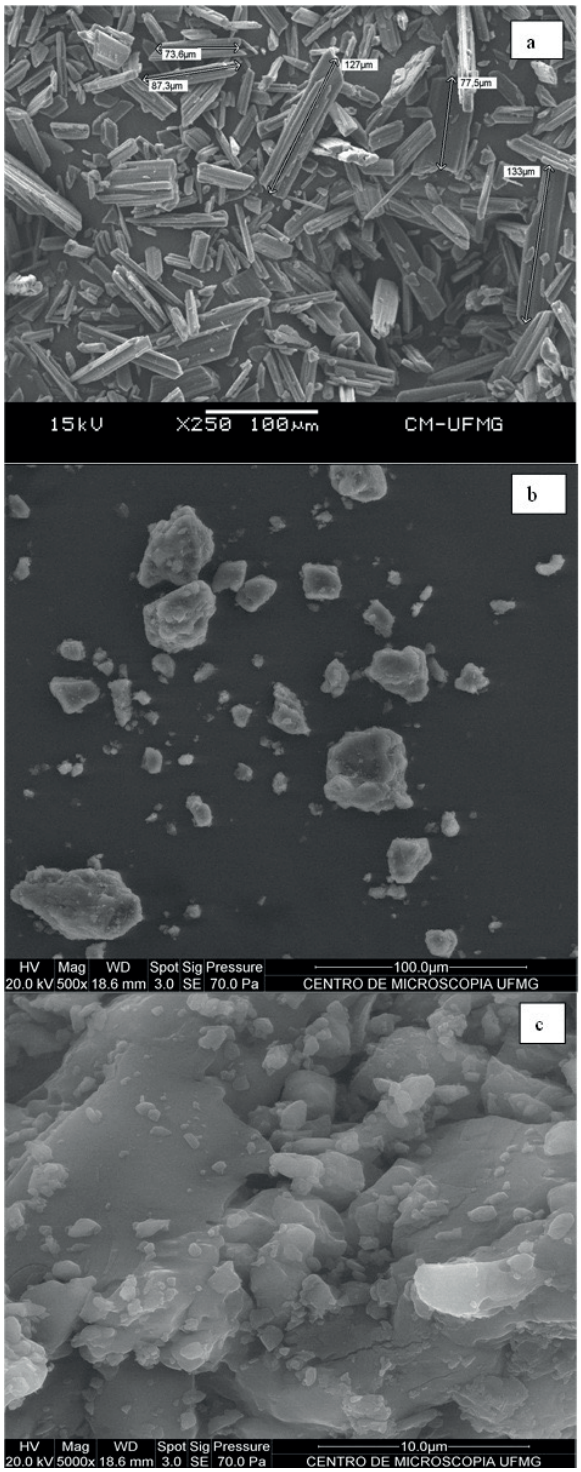


**FIGURE 1 -** Photomicrographs of A) sildenafil citrate (SILC) active ingredient not micronized (×250, scale bar 100 µm), and DPI formulation containing SILC micronized (wet grinding, 30 min) plus lactose, B) ×500, scale bar 100 µm and C) ×5000, scale bar 10 µm, obtained by scanning electron microscopy.

changes in the crystal structure profile of SILC particles due to the size reduction effects after the micronization process. It is possible to observe that in the blue profile of XRPD crystalline SILC AI form there are several diffraction peaks, characterized by defined reflections. Two peaks are more intense (8.15º; 14.40º), two other peaks are of medium intensity (10.30º; 19.80°) and the remaining peaks are of low intensity. Those peaks confirmed the presence of SILC in crystalline form, as observed in the SEM images (Figure 1). It can be seen that there is overlapping of the peaks, with small displacements of peaks due to the stress caused by the grinding process, in Figure 2. The preferential orientation of the most intense peaks is observed to the detriment of the peaks with lower intensity (and masked) in the raw material. In the milled samples, the effect of preferential orientation is not observed in the same way, under the same measurement conditions (sample holder area and exposure time). The considerable difference in intensity (see insert) of the largest peaks in the raw material is due to the stress caused by milling, which increases the randomness of the exposed crystalline planes, reducing the effect of preferential orientation in the preparation of the milled samples.

The XRPD pattern of SILC AI (blue line) and SILC micronized by dry mode (red line) or wet grinding (5 min, black; 30 min, pink) in different time intervals did not significantly change. The overlapping of XRPD patterns of the micronized drug shows the same characteristic peaks (8.15°; 10.30°; 14.40° and 19.80°) compared to SILC drug, but with less intensity. Therefore, there was no change either between the crystal structures of the drug during the dry or wet grinding in different time intervals (5; 30 min), as evidenced in insert around 13 to 16°.

## Development of formulation for inhalation

Micronized SILC used in the preparation of DPI formulation was selected based on the distribution of its particle size, as discussed in the *Particle Size by Laser Diffraction* section. The physical mixture was prepared in lactose (100 µm) carrier since it is the most widely excipient used in DPI formulations. Its particle size is very close to that used for inhalation, available

**TABLE I -** Particle size analysis results for sildenafil citrate (SILC) before and after the micronization obtained by laser diffraction characterization (n = 3)

| Sample | | Parameter (µm) (% RSD) [a] | | | |
|---|---|---|---|---|---|
| | | Average diameter | Mode | $D_{50}$ [b] | $D_{90}$ [b] |
| **SILC active ingredient** | | **42.77 (10.53)** | **32.00 (10.10)** | **33.77 (12.08)** | **86.54 (10.48)** |
| SILC micronization | | | | | |
| Dry grinding | 5 min | 12.17 (11.46) | 19.17 (5.30) | 8.75 (6.79) | 26.45 (8.48) |
| | 10 min | 16.74 (7.75) | 19.82 (9.34) | 13.41 (6.35) | 32.87 (10.58) |
| Wet grinding | 5 min | 7.69 (10.50) | 5.90 (9.32) | 5.67 (12.24) | 17.05 (10.73) |
| | 10 min | 6.56 (9.90) | 5.36 (0.00) | 4.70 (3.47) | 13.65 (11.10) |
| | 30 min | 4.54 (5.82) | 3.07 (9.32) | 2.93 (5.75) | 9.08 (12.43) |

[a]Relative standard deviation. [b]$D_{50}$ or $D_{90}$, 50th percentile ($D_{50}$ or median) and 90th percentile, respectively.

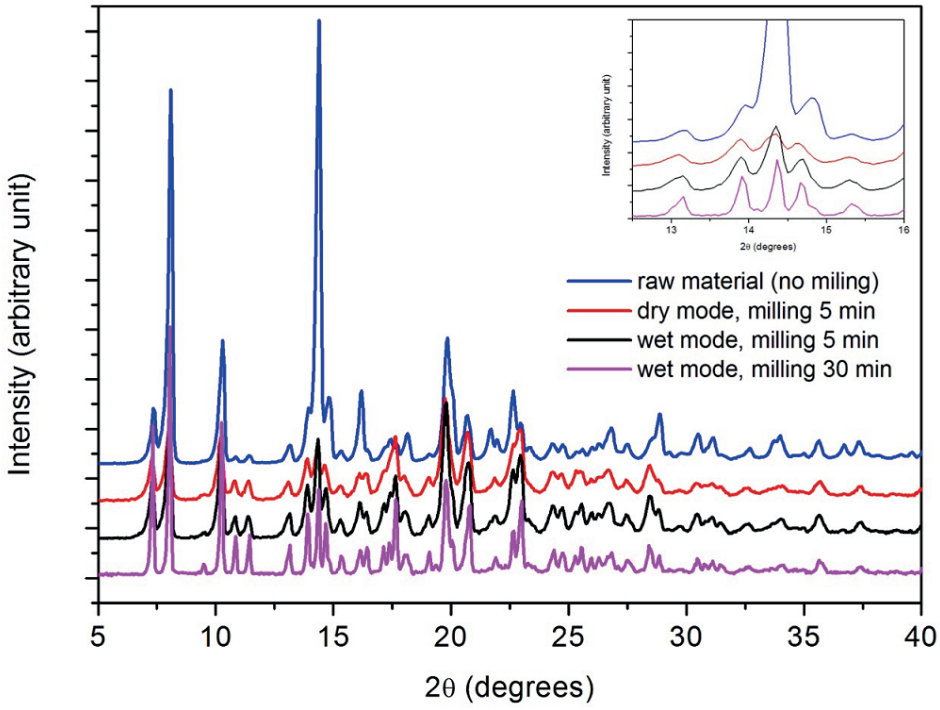


**FIGURE 2 -** X-ray powder diffraction overlay pattern for sildenafil citrate (SILC, blue line) and SILC micronized by dry mode (red line) or wet grinding (5 min, black; and pink, 30 min) lines.

in the market. Moreover, lactose has advantages of being a low-cost carrier, a toxicity profile and safety investigated and well-established, in addition to a good flowability (Steckel, Bolzen, 2004).

The dose of sildenafil used (250 µg, equivalent to 351.2 µg of SILC) was designed based on reports that describe that it can generally be reduced of 40 to 100 times relative to the oral dose (Sawatdee, Phetmung, Srichana, 2013; Sawatdee *et al.*, 2014). Thus, as the treatment protocols for PAH recommend initial oral dose around 25 mg of sildenafil, gradually increasing to 100 mg, a 100-fold reduction of the lowest dose (25 mg) was selected for the development of SILC DPI formulation (López-Guarch *et al*., 2004; Chockalingam *et al*., 2005; Garg, Sharma, Sinha, 2007; American, 2009; Galiè *et al*., 2010).

To our knowledge, there are no commercially available formulations for pulmonary administration containing SILC for the treatment of PAH, despite some in vivo studies show the effectiveness of SILC inhalation in liquid forms, aerosol or nebulization as monotherapy (Aubin *et al*., 2008; Rashid *et al*., 2017).

## Quality Control and performance tests

In summary, minimal quality control tests for SILC DPI developed formulation in capsules are presented in Table II.

The results for weight variation meet the requirements if the maximum deviation does not exceed ±10.0% of the average weight (for capsules with an average weight lower than 300 mg) and %RSD does not exceed 4%. For the content uniformity test, the acceptance value (AV) found was 11.83, which does not exceed the specification of less than 15 (Brasil, 2012; Brazilian, 2019).

The assay range 80.0 to 120.0% of the labeled amount was adopted as content specification, based on monographs of similar products such as beclomethasone, sodium cromoglycate, budesonide,

**TABLE II -** Results of quality control tests (weight variation, content uniformity, water content and assay) for capsules containing sildenafil citrate (SILC) dry powder for inhalation

| Parameter | Test | | | |
|---|---|---|---|---|
| | **Weight variation (n=10)** | **Content uniformity (n=10)** | **Water content (n=3)** | **Assay (n=3)** |
| Average | 59.60 mg [b] | 104.78% | 5.35% | 102.35% |
| % Range of deviation (minimum-maximum) | 0.058-1.90 [c] | 99.11-109.21 [e] | 5.31-5.39 | 102.15-102.49 [e] |
| % RSD [a] | 1.39 | 3.40 | 0.75 | 0.18% |
| Acceptance value | NA [d] | 11.83 | NA [d] | NA [d] |

[a]Relative standard deviation, RSD. [b]Powder weight plus empty capsule (38.91 mg, n=20). [c]Deviation in relation to weight average. [d]Not applicable. [e]Percentage of the labeled amount.

fenoterol, salbutamol or fluticasone capsules inhalation powder (British, 2014; USP-NF 2023). The determination of water content is very important, since it can influence the aerodynamic behavior of the particles and consequently, the lung deposition. The moisture content must be within the range 4.5 to 5.5% w/w for DPI formulations containing lactose as carrier, Furthermore, the water content range (5.31-5.39% w/w) obtained is, similarly, within the specification limit of the monograph salbutamol DPI product, described in British (2014). Hence, the capsules containing SILC in a DPI formulation met the adapted requirements for quality control evaluation.

## Delivered-Dose Uniformity

The results of DDU performance test for SILC DPI capsules are shown in Table III. The targeted labeled value (LV), 351.2 µg of SILC, was considered for the calculations of drug mass percent released from the device. The results for the DDU test for SILC DPI capsules showed values in the range 92.42 to 109.05% LV. Specifications are met if not more than one unit outside the range of 75.0 to 125.0% LV is found, according to the USP-NF, 2023, general method <601> *Aerosols, nasal sprays, metered dose inhalers, and dry powder inhalers.* Therefore, the results for the capsules prepared to contain micronized SILC met the DDU test requirements. This test is important to assure that the patients inhale the recommended dose in the dosage form.

## Aerodynamic Size Distribution

In the performance of APSD test using the ACI, the released content of ten capsules from the inhaler device and subsequently, the mass deposited in each stage were determined. However, the $d_{ae,cut}$ values are usually defined according to the nominal flow 28.3 L $min^{-1}$ for products of metered-dose inhalers (MDI) type. They assume the values 9.0; 5.8; 4.7; 3.3; 2.1; 1.1; 0.7 and 0.4 µm for stages 0 to 7, respectively (USP-NF, 2023).

For DPI calculation of the cut off diameters, Equation 1 yields the formula $d_{ae,\,cut,Ftest} = 9\ \sqrt{(28.3/65)}$, thus, the value 5.94 for the stage 0 (dae,cut = 9 µm), calculated employing the high flow. Therefore, the nominal flow 65 L $min^{-1}$ used for DPI yielded new calculated values 3.83; 3.10; 2.18; 1.39; 0.73; 0.46 and 0.26 µm for the 1st to the 7th stage, respectively, as shown in Table IV.

In addition to the size distribution, good laboratory

**TABLE III -** Dosage unit uniformity results for determination of sildenafil citrate (SILC) release from capsules for oral inhalation

| Dosage form units | Parameters | |
|---|---|---|
| | **Delivered mass (µg)** | **Target-delivered dose [a] (%)** |
| 1 | 325.95 | 92.81 |
| 2 | 335.46 | 95.52 |
| 3 | 324.58 | 92.42 |
| 4 | 342.90 | 97.64 |
| 5 | 328.25 | 93.46 |
| 6 | 395.78 | 109.05 |
| 7 | 337.45 | 96.08 |
| 8 | 334.50 | 95.25 |
| 9 | 337.13 | 95.99 |
| 10 | 331.43 | 94.37 |
| Average (% RSD) [b] | 338.06 | 96.26 (4.96) |

[a]The labeled value of 351.2 µg SILC corresponds to 250 µg of sildenafil, regarded as the target-delivered dose. [b]Relative standard deviation, RSD.

practices dictate that a mass balance evaluation must be performed in order to confirm that the amount of the drug discharged from the inhaler, is within an acceptable range. The test is valid if the mass balance is not less than 75.0% and not more than 125.0% of the average mass determined during testing for DDU. Although this is not a performance test for the inhaler dosage form, it helps to ensure that the mass balance (120.44%), found for the SILC DPI capsules, is within the range recommended by USP-NF (2023), thus, the results are valid.

A variation of %RSD values greater than 10% in the drug mass deposited is verified in stage 5 on, with a more pronounced increase in stages 6 and 7. This variation can be explained by the fact that particles with aerodynamic diameter less than 0.5 µm, collected in stages 6 and 7, move through the airways by Brownian diffusion. Therefore, their deposition, as well as, their movement happens randomly. When administered in vivo, these particles shall largely be exhaled (Telko, Hickey, 2005; Scheuch *et al.,* 2006; Beck-Broichsitter *et al*., 2016). According to USP-NF (2023), the stages 6 and 7 may be omitted for tests performed with flows higher than 60 L $min^{-1}$, as in this case.

A greater amount of drug deposit is observed in the initial stages, mouthpiece, induction port and stage 0 due to the presence of particles larger than 5 µm, which would be deposited by inertial impaction, especially in vivo, in the oropharyngeal region. These results were expected, according to reports in the literature and DPI

**TABLE IV** - Aerodynamic particle size distribution average results for 10 capsules for oral inhalation containing 351.2 µg of sildenafil citrate (n = 3)

| ACI stages ($d_{ae,cut}$, µm) | Calculated $d_{ae,cut}$ values (µm) | Deposited mass µg/capsule (% RSD) [a] | % Deposited mass [b] |
|---|---|---|---|
| Mouthpiece | - | 51.79 (5.05) | 12.71 |
| Induction port | - | 114.00 (2.89) | 28.00 |
| 0 | 5.94 | 103.45 (3.91) | 25.42 |
| 1 | 3.83 | 39.23 (3.92) | 9.64 |
| 2 | 3.10 | 30.71 (4.56) | 7.54 |
| 3 | 2.18 | 34.83 (6.55) | 8.55 |
| 4 | 1.30 | 22.00 (7.79) | 5.40 |
| 5 | 0.73 | 5.57 (15.29) | 1.36 |
| 6 | 0.46 | 2.72 (34.23) | 0.66 |
| 7 | 0.26 | 2.86 (19.77) | 0.70 |
| Total mass (%RSD [a]) | - | 407.15 (4.08) | - |
| % Mass balance [c] | - | 120.44 | - |

[a]Relative standard deviation, RSD. [b]Calculated relative to the total mass deposited from the device. [c]Calculated relative to the average mass, obtained in the DDU test, being 338.06 µg of sildenafil citrate.

formulations, commercially available in the market. Particles deposited in this region can be swallowed and may show systemic action, thereby, a suitable and important orientation is that the patient should take measures to avoid this inconvenience, by washing the mouth after using such a medicine (Scheuch *et al.*, 2006).

In fluticasone DPI monograph, described in USP-NF (2023), the specified range of deposited drug from the mouthpiece to stage 0 is 56-84%. Likewise, the total mass deposition percentage of SILC DPI found in the mouthpiece to stage 0 regions was 66.13% (Table IV) indicates that the developed formulation meets the similar recommendations.

Nguyen and colleagues (2019) reported a carrier-free dry powder inhaler (DPI) formulation with sildenafil free base and SILC, using a spray drying. technique and L-leucine as a dispersibility enhancer. Together with solids characterization, an excellent aerodynamic performance emitted dose (89.39%) and FPF (80.08%) resulted, however, after a multiparameter factorial, complex design evaluation. A Calu-3 (human-originated airway epithelial) cell model and its suitability for DPI was also tested to evaluate permeation.

The FPD and FPF values found were 127.36 µg and 34.57%, respectively. The FPF value found is in agreement with values of a wide range deposition, reported for DPI formulations available on the market. Such wide range values are due to the fact that pulmonary deposition depends on many factors

such as the patient inspiratory flow, the type of inhaler device used, as well as the $d_{ae}$ value of the particles. A wide range of FPF results described in the literature is such as 6 to 14%; 15 to 30% (Smith, Parry-Billings, 2003) and 12 to 40% for (Islam, Gladki, 2008). Thus, it is important to consider such deposition range in the development of dose planning for inhaled formulations (Pereira, 2007; Sawatdee, Phetmung, Srichana, 2013; Sawatdee *et al*., 2014).

The MMAD values obtained (4.90 µm) in the APSD test were adequate for inhaling formulations. nevertheless, they were larger than the average size and $D_{50}$ obtained by the laser diffraction technique (4.54 µm and 2.93 µm, respectively; Table I). Therefore, the $d_{ae}$ value is the most appropriate parameter for measuring the particle size of DPI because it associates to the aerodynamic behavior, what is related to the size, density and shape of particles (Gonda, 2004; Andrade-Lima, Pereira, Fernandes, 2012).

Rashid and coworkers (2017) developed a porous PLGA polymer based inhalable microparticles of SILC by a water-in-oil-in-water (w/o/w) double emulsion solvent evaporation method for pulmonary absorption studies. This formulation showed an optimal MMAD of 4.68 ± 0.12 µm, a value close to that obtained in this work.

The APSD for SILC DPI formulation showed a polydisperse value due to the high GSD value of 2.14, greater than 1.2. Values from 0.9 to 1.2 are usually found for monodispersions. Most DPI products are polydisperse, what implies the presence of larger particles to be deposited, mainly in the oropharyngeal portion (Telko, Hickey, 2005; USP-NF, 2023). These results explain the highest percentage of deposited drug found in the initial portions of the ACI (mouthpiece to stage 0), as shown in Table IV.

Shahin and colleagues (2019) developed inhalable spray-dried SILC loaded hydrogel microparticles as carrier for sustained pulmonary delivery. The results for aerodynamic properties evaluated by ACI included FPF ranging between 24 and 30%, MMAD of 4.6 to 4.8 µm and GSD, from 1.5 to 1.7. These results are close to those obtained in the present work, however the proposed formulation is simpler and less expensive. Those authors conducted cell viability studies, nevertheless, the results indicate the need for further evaluation of cytotoxicity of the formulations.

In the work conducted by Ghanbarzadeh and collaborators (2016), SILC carrier free DPI formulations were prepared with different ratios of water and organic solvents such as methanol and dimethylformamide, by spray drying technique. The results ranged from 2 to 70% (FPF), 2 to 3 µm (MMAD) and 2 to 11 (GSD), using the next generation impactor. The high variability of the results indicate that parameters such the ratio and type of solvent, besides the drying conditions used impact the powder aerodynamic properties. Thus, a careful toxicity study is needed due to presence of solvent residues, despite a higher FPF ratio obtained.

The present study shows a simple DPI SILC formulation by micronized SILC mixed with lactose. Together with its satisfactory performance and quality control tests it is a potential therapeutic alternative for oral form of sildenafil, at a reduced cost and dose. The authors agree with Abiona *et al.* (2022) who emphasize that the design of experiments is important for an optimization of critical quality control attributes, such as the desired particle size, shape and other performance features for DPI, so that there can be a better understanding of surface energetics and particles interactions.

## CONCLUSIONS

A simple formulation (containing 351.2 µg of micronized SILC, wet grinded for 30 min and added of lactose, as carrier) was developed and met requirements for quality control and performance tests for DPI dosage form. The results for FPF (34.57%) and MMAD (4.90 µm) were comparatively satisfactory. These values may be used as recommendations for SILC DPI, since no specifications were found in monographs for such tests in DPI dosage forms.

The developed DPI formulation offers a choice for reduced cost for PAH patients who urgently necessitate

more effective and cost-accessible medications. It may be a therapeutic option for the treatment of PAH with the advantage of being easily produced. Further investigation must be performed for *in vivo* studies in order to confirm the advantages and to validate a similar an upscaled proposed inhalation formulation.

Due to difficulties to accessing medication, it is wise to search for new therapeutic options, primarily aiming a reduction in dosage and side effects, an increase of action in the lungs, and a better cost/benefit ratio.

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## DECLARATION OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All data are available within the article or in its supplementary materials.

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