

## **Investigation of influence factors on particle size measurement with pMDI.**

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### **Abstract**

**Laser diffraction (LD) and next generation impactor (NGI) were recommended for measurement of particle size for pressurized metered dose inhaler (pMDI) in Pharmacopoeia. Existing literature had shown that high relative humidity affects *in vitro* aerosol drug delivery of pMDI. In this study, we investigated the effect of environmental condition (temperature and humidity), flow rate and device temperature on the particle size distribution (PSD) assessment of solution pMDI (Atrovent: Ipratropium Bromide Aerosol, HFA134a) and suspension pMDI (Ventolin: Salbutamol sulfate Aerosol, HFA134a) by LD (Spraytec). We also studied the effect of temperature on the PSD assessment of pMDI by NGI. There was a decrease in particle size with higher humidity or faster flow rate, both for Ventolin and Atrovent. With the environmental temperature increase, the particle sizes of Atrovent were increase. However, the particle sizes of Ventolin were decrease at first and then increase with environmental temperature increase. In addition, with the device temperature increase, the particle sizes were decrease at first and then increase, both for Ventolin and Atrovent. For testing with NGI, mass median aerodynamic diameter (MMAD) was reduced and the fine particle fraction (FPF) increased both for Ventolin and Atrovent when comparing 25°C to 5°C. Therefore, these factors (environmental temperature and humidity, flow rate, and device temperature) had significant influence on the performance of LD or NGI, and should be controlled in the particle size measurement of pMDI.**

**Keywords:** Laser diffraction, Impactor, Particle size distribution, Pressurized metered dose inhaler.

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### **Introduction**

Pulmonary delivery is prominent way for delivering drug to respiratory tract and as a potential non-invasive administration route for the systemic and local delivery of active pharmaceutical ingredients (APIs) [1]. The advantages are rapid onset, avoidance of the first pass effect, and higher efficiency of the delivered drug [2-5]. There are three types of formulations used for pulmonary delivery: pressurized metered dose inhalers (pMDI), dry powder inhalers (DPI), and nebulizers. pMDI is released at high velocity and requires a simultaneous inhalation by the patient, and has a widely application because of carrying portability [6]. Commercial pMDI products include solution and suspension as the difference for physicochemical characteristics of APIs [7]. There are many factors to influence the therapy performance [8], such as patient's age, flow pattern, drug aerodynamic diameter [9,10]. Many studies reported that aerodynamic diameter in the range from 0.5 to 3  $\mu\text{m}$  will result in alveolar deposition; aerodynamic diameter in the range from 2  $\mu\text{m}$  to 6  $\mu\text{m}$  will result in tracheobronchial deposition; aerodynamic diameter greater than 5  $\mu\text{m}$  will result in deposition in the

upper airways. Therefore, to ensure the drug to be deposited in the lung and the range for majority of drug aerodynamic diameter must be controlled from 1 to 5  $\mu\text{m}$  [11-13]. Therefore, it's necessary to build a robust and accurate measurement of PSD for evaluation of inhaler formulation. There are many characteristic methods have been developed and mainly divided static and dynamic measurement as for the different measurement principle. The dynamic measurement is the traditional particle size analysis in the Pharmacopoeia. It utilizes multiple stages impactor with progressively decreasing cut sizes to simulate the dynamic process of aerosol in lung. Then to collect the drug from different stage using suitable method and to yield mass fractions of the drug by a validated chemical detection which is a direct assessment of API [14-18]. The representative of static measurement is laser diffraction (LD) which is a rapid and noninvasive measurement of droplet diameter. The process of measurement is that aerosol particles pass through the laser beam, and then photodetector captures the intensity and angles of light diffraction pattern [19-23]. The intensity and angles are relevant to the particle size, shape, density, and other physical properties. The data of PSD is

calculated by combined vector equations (Mie equation) with the incident wave, scattered wave and the wave inside the droplet. Therefore, this information is contributed to formulation development [24-26].

Although the impactor is currently considered as “golden standard” for inhaler test, the operation is labor-intensive and comparatively slow to operate compared with LD. It is necessary to sufficiently apply LD instrument to development of inhaler. However, it is still uncertain for the control of environment condition by using LD in Pharmacopeia. Many studies investigated particle diameter changes with variation in environmental conditions. Shemirani et al. studied the effect of ambient humidity on regional delivered dose with solution and suspension MDIs by using an Alberta Idealized Throat, and found that the dose delivery of pMDI formulations are affected by environmental condition. With increasing relative humidity, the deposition in mouth-throat was increase and the lung dose fraction was decrease for both solution and suspension MDIs. Increasing temperature resulted in decreased mouth-throat deposition and increased lung deposition [27-29]. Morin et al. investigated the performance of pMDIs under a variety of temperature conditions by using an Alberta Idealized Throat and the *in vitro* lung dose was measured by gravimetric assay. They found that when ambient temperature and device temperature were decrease, the *in vitro* lung deposition was decrease [30,31]. Fink et al. found that pMDI lower respiratory tract delivery rate is 16.2% under wet condition, while 30.4% under dry condition [32,33].

As we all known, the amounts of drug deposited in lung are relevant to the aerodynamic diameter [34,35]. Therefore, the variety of lung deposition may be relevant to the variety of particle size when the environmental condition changed [36]. Presently, the environmental condition changed to affect the particle size of pMDI by using LD or NGI has not been reported [37-39]. In this study, we investigated the effect of environmental condition (temperature and humidity), flow rate and device temperature on the PSD assessment of pMDI by LD. And we also studied the effect of temperature on the PSD assessment of pMDI by NGI. The purpose of this study is to provide a reference for an accurate assessment of pMDI by either LD or NGI. We found the influence factors on particle size measurement of pMDI. Hope the Pharmacopoeia Committee of each country that will set a standard to regulate particle size measurement of pMDI.

## Materials and Methods

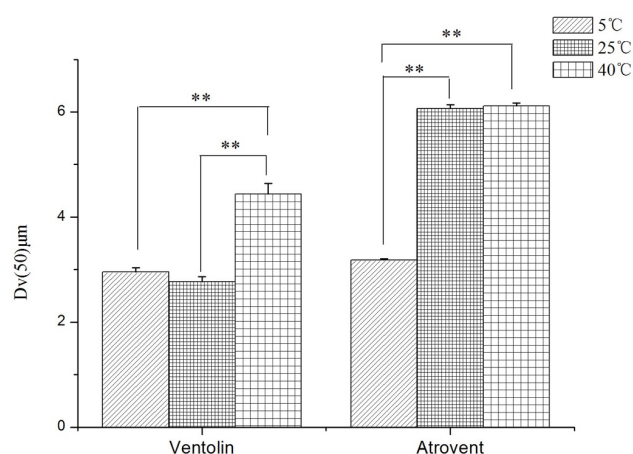
Ventolin (Salbutamol sulfate Aerosol, Propellant: HFA134a, White or almost white suspension, 100 µg\*200 press), Manufacturer: Glaxo Wellcome S.A.; Atrovent (Ipratropium Bromide Aerosol, Propellant: HFA134a, Clear transparent liquid, 20 µg\*200 press).

### Particle size and distribution measurement by LD

Two commercial pMDI particle size distribution was measured by a Spraytec LD (Malvern instruments, UK) at different

system temperatures, humidities, test flow rate and Device temperature. The LD with the inhalation cell was placed in the chamber (JinLiXin Ltd., China) which can control temperature. The chamber also has the humidity controller. System temperature variables: (5°C, 25°C, 40°C, humidity: RH 30%-50%, flow rate: 30 L/min); Humidity variables: (45%, 90%, system temperature: 25 ± 2°C, flow rate: 30 L/min). Before the experiment, in order to reach the system temperature or humidity, the system was incubated for 4 h. Flow rate variables: (0, 15, 30, 40, 50 L/min, system temperature: 25 ± 2°C, humidity: RH 30%-50%). Device temperature variables: (25°C, 40°C, 60°C, flow rate: 30 L/min, system temperature: 25 ± 2°C, humidity: RH30%-50%). pMDI device was placed in the (Shanghai YiHeng Scientific Instrument Co., Ltd.) for 4 h to an equilibrium. The pMDI was joined with an adaptor to the inlet of a USP throat which above the inhalation cell. Vacuum pump (HCP5, COPLEY Scientific, UK) was connected to the outlet of the inhalation cell.

Before the test, shake the pMDI for 5 s, and connect to the USP throat, discharge 1 immediately (Each new pMDI by firing to discard the first 5 spray). Repeat the procedure. The number of discharge was 3 times (n=3). Important parameters Dv (50) (the particle size below which 50% of the aerosol) was recorded LD Software.



**Figure 1.** DV(50) of Ventolin and Atrovent test by LD at three system temperatures; \* $p < 0.05$ ; \*\* $p < 0.01$ .

### Particle size characterization by NGI

The pMDI was joined with an adaptor to the inlet of the NGI (COPLEY Scientific, UK). The NGI was operated at 30 L/min by the vacuum pump. Before the test, shake the pMDI for 5 s, and connect to the USP throat, discharge 1 immediately (Each new pMDI by firing to discard the first 5 spray). Repeat the procedure. (Ventolin: n=10; Atrovent: n=30 (To reach the HPLC quantitative line). The samples were collected by ultrapure water (Mili-QA10, USA) and assayed to quantify Ventolin and Atrovent deposition in the USP throat and each NGI collection cups. Quantitative analysis was performed by HPLC. NGI important parameters: MMAD (Mass median aerodynamic diameter), GSD (Geometric standard deviation)

and FPF (Fine particle fraction) were calculated by CITDAS software (COPLEY Scientific, UK).

**Chromatographic conditions**

The sample collected from NGI was analyzed by HPLC (LC-20A, Shimadzu corporation, Japan). Ventolin: Chromatographic column: XDB-C18 (Agilent, 150 × 4.6 mm, 5 μm); Mobile phase: 0.08 mol/L. sodium dihydrogen phosphate solution (pH 3.10 ± 0.05) and methanol (85:15, v/v); UV detection wavelength: 276 nm; Flow rate: 1.0 mL/min; Injection volume: 20 μL; Column temperature: 40°C; Injection time: 5 min.

**Atrovent:** Chromatographic column: XDB-C18 (Agilent, 150\*4.6 mm, 5 μm); Mobile phase: 0.012 mol/L Sodium 1-heptanesulfonate solution (pH 3.20 ± 0.05) and acetonitrile (78:22, v/v); UV detection wavelength: 210 nm; Flow rate: 1.0 mL/min; Injection volume: 20 μL; Column temperature: 40°C; Injection time: 15 min [40].

**Statistical analysis**

Using the software (SPSS 20.0, SPSS Inc., Chicago,USA) to get analysis of significant results.

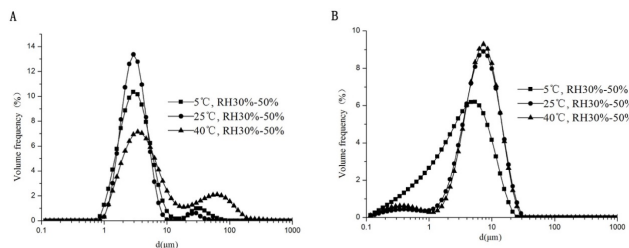


Figure 2. The frequency distribution of particle volumes of Ventolin and Atrovent test by LD at three temperatures; A: Ventolin; B: Atrovent.

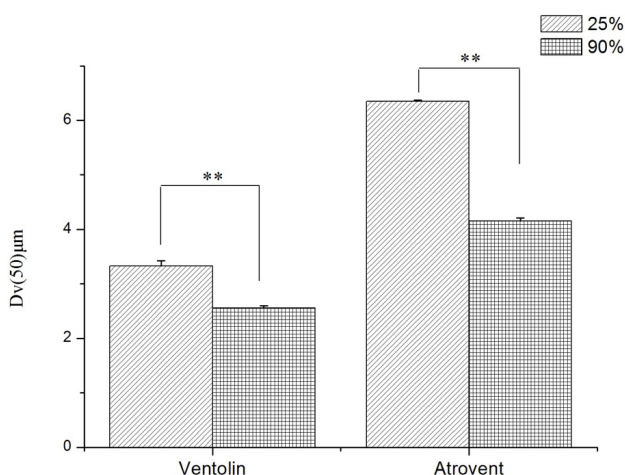


Figure 3. DV(50) of Ventolin and Atrovent test by LD at two humidities; \*p<0.05; \*\*p<0.01.

**Results**

**Effects of the system temperature (LD)**

As shown in Figure 1, Ventolin: Dv(50) tested at 40°C increased by 37.57% and 33.47%, respectively, compared with at 25°C and 5°C. It was a highly dominant difference, but no significant difference between at 25°C and 5°C. Atrovent: Dv(50) tested at 40°C and 25°C increased by 47.99% and 47.59%, respectively, compared with at 5°C. It was a highly dominant difference, but no significant difference between at 25°C and 40°C.

As shown in Figure 2, Ventolin: there were no 0.1-1 μm small particles at 3 temperatures of 5°C, 25°C and 40°C. And there was a phenomenon that the maximum peak increases first and then decreases with increasing temperature. Atrovent: there were 0.1-1 μm small particles at 3 temperatures. And the maximum peak curve shift right with increasing temperature.

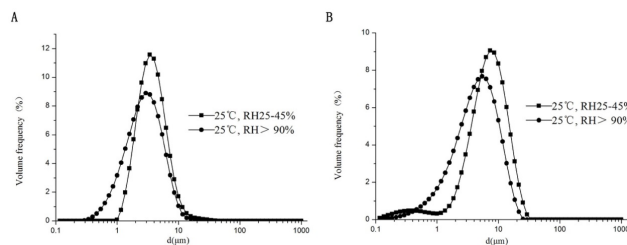


Figure 4. The frequency distribution of particle volumes of Ventolin and Atrovent test by LD at two humidities; A: Ventolin; B: Atrovent.

**Effects of the system temperature (NGI)**

As shown in Table 1, FPF and MMAD tested between 5°C and 25°C have a highly dominant difference. MMAD tested at 5°C increased by 38.14% (Ventolin), 73.75% (Atrovent), respectively, compared with at 25°C; FPF reduced 36.13% (Ventolin), 36.22% (Atrovent).

**Effects of the system humidity (LD)**

As shown in Figure 3, Dv(50) tested at RH 25 ± 1.3% increased by 23.29% (Ventolin) and 34.58% (Atrovent), respectively, compared with RH>90%. Both were a highly dominant difference.

As shown in Figure 4, Ventolin: there was 0.1-1 μm small particles at RH>90%. The maximum peak curve shift right with reducing humidity, and the 0.1-1 μm small particles disappeared at RH25 ± 1.3%. Atrovent: there were 0.1-1 μm small particles at 2 humidifies. And the maximum peak curve shift right with reducing humidity.

**Effects of the flow rate (LD)**

As shown in Figure 5, Ventolin: Dv(50) tested at 0 L/min increased by 47.51%, 38.25% and 41.48%, respectively, compared with at 40 L/min, 30 L/min and 15 L/min. It was a highly dominant difference, but no significant difference between at 40 L/min, 30 L/min and 15 L/min. Atrovent:

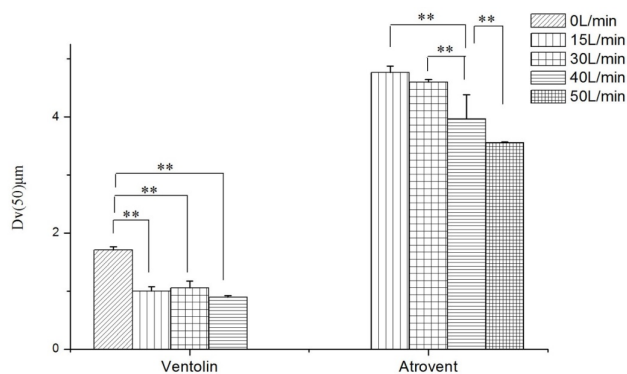
Dv(50) tested at 40 L/min decreased by 16.84% and 13.80%, respectively, compared with at 15L/min and 30L/min. It was a highly dominant difference. Dv(50) tested at 50 L/min

decreased 11.56%, compared with at 40 L/min (highly dominant difference), no significant difference between at 30 L/min and 15 L/min. 0 L/min cannot trigger the measurement.

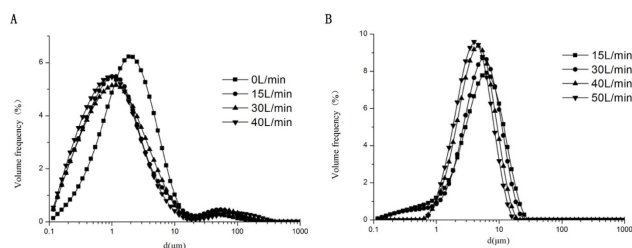
**Table 1.** The value of MMAD and FPF tested by NGI at 5C and 25C.

Drug	5°C, MMAD (µm)	25°C, MMAD (µm)	5°C, FPF (%)	25°C, FPF (%)
Ventolin	4.869 ± 0.064	3.012 ± 0.017*	17.82 ± 1.47	27.901 ± 0.945**
Atrovent	3.387 ± 0.090	0.889 ± 0.001*	24.411 ± 1.270	38.275 ± 2.112**

Note: \*p<0.05; \*\*p<0.01.



**Figure 5.** DV(50) of Ventolin and Atrovent test by LD at different flow rate; \*p<0.05; \*\*p<0.01.



**Figure 6.** The frequency distribution of particle volumes of Ventolin and Atrovent test by LD at different flow rate; A: Ventolin; B: Atrovent.

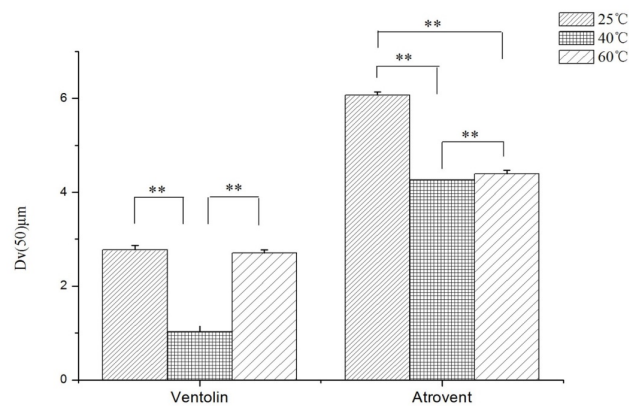
As shown in Figure 6, Ventolin: there were 0.1-1 µm small particles at 4 flow rates of 0 L/min, 15 L/min, 30 L/min and 40 L/min. And the ratio of 0.1-1 µm small particles increased with increasing flow rate, the maximum peak curve shift left with increasing flow rate. Atrovent: there were 0.1-1 µm small particles at 2 flow rates of 15 L/min, 30 L/min. There were no 0.1-1 µm small particles at 2 flow rates of 40 L/min and 50 L/min. And the maximum peak curve shift left with increasing flow rate.

#### Effects of the device temperature (LD)

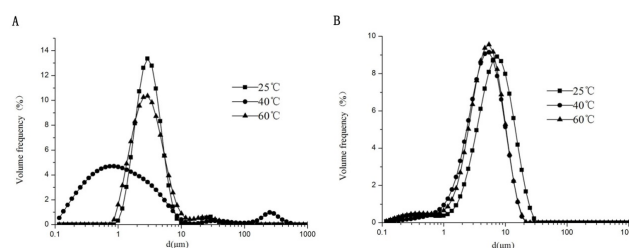
As shown in Figure 7, Ventolin: Dv(50) tested at 40°C increased by 63.10% and 62.25%, respectively, compared with at 25°C and 60°C. It was a highly dominant difference, but no significant difference between at 25°C and 60°C. Atrovent: Dv(50) tested at 25°C increased by 29.76% and 27.62%, respectively, compared with at 40°C and 60°C (highly

dominant difference). Dv(50) tested at 60°C increased 2.96%, compared with at 40°C.

As shown in Figure 8, Ventolin: there was 0.1-1 µm small particles at the temperatures of 25°C, but no at 25°C and 60°C. And the maximum peak curve shift left with increasing temperature, but shift right when the temperature increased to 60°C. Atrovent: there were 0.1-1 µm small particles at 3 temperatures of 25°C, 40°C and 60°C. And the maximum peak curve shift left with increasing temperature, but shift right when the temperature increased to 60°C.



**Figure 7.** DV(50) of Ventolin and Atrovent test by LD at three device temperatures; \*p<0.05; \*\*p<0.01.



**Figure 8.** The frequency distribution of particle volumes of ventolin and atrovent test by LD at three device temperatures; A: Ventolin; B: Atrovent.

#### Discussion

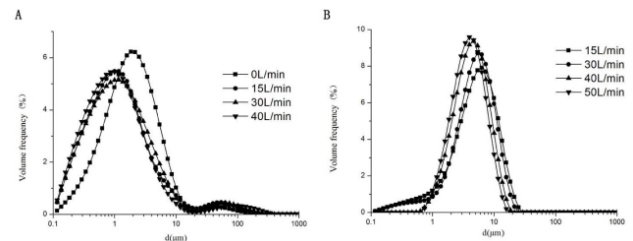
At present, it is not clear how to control the environmental condition (temperature and humidity), flow rate, and device temperature when measured pMDI particle sizes by LD (Spraytec). Although it is recommended that NGI must be

precooled at 5°C for at least 90 min to reduce the bias and variability caused by heat transfer-related evaporation when measured nebulizer particle size by NGI. However, the control of test condition is uncertain for pMDI by using NGI [30,41]. As for our results, these factors (environmental temperature and humidity, flow rate, and device temperature) had significant influence on the performance of LD or NGI, and should be controlled in the particle size measurement of pMDI.

As described in Figure 5, there are large influences on the droplet particles with temperature increased. Although the extent is difference for suspension and solution formulations. As for the excipients content are different for formulations. Small particles (0.1-1  $\mu\text{m}$ ) were all not detected at three temperatures for Ventolin. However, those small particles were detected for Atrovent at three temperatures. In addition, as to our study, the results of pMDI particle sizes are different when measured at different temperature. With the increasing of temperature, the particle sizes of Atrovent are increasing. Interestingly, the particle sizes of Ventolin are decreased when the temperature are increased from 5°C to 25°C. And with the temperature increasing to 40°C, the particle sizes are increased. The differences may be related to the composition of formulation. Ventolin is suspension and aerosol contained drug particles and propellant. So, a single actuation may produce one particle in several droplets. Propellant only droplets evaporated rapidly and leaving the residual particles to condensation. As regulated formulation manufacturing process, the drug particles should be micronized at a certain size and most of those are larger than 1  $\mu\text{m}$ . Conversely, Atrovent is solution formulation and may be yielded one particle for every actuated droplet. Therefore, small particles may be detected for Atrovent and particle sizes were temperature-dependent. It corresponds to the results. But for NGI, the MMAD was reduced and the FPF increased both for Ventolin and Atrovent when comparing 25°C to 5°C. This phenomenon is attributed that during the testing, evaporation may be caused by heat transfer from the NGI to the droplet cloud. So, it was recommended that NGI must be precooled at 5°C for at least 90 min when measured the nebulised aerosol droplet size. Therefore, it is necessary to regulate the environmental condition when measured pMDI particle size by NGI [30,42,43].

As showed in Figure 7, with the increasing of related humidity, the particle sizes were decreased. The phenomenon may be attributed to that aerosol droplets are easily evaporated at low humidity ( $\text{RH}_{25} \pm 1.3\%$ ). However, the influence for Atrovent is stronger than Ventolin. The humidity decrease from  $\text{RH} > 90\%$  to  $\text{RH}_{25} \pm 1.3\%$ , the increase in  $D_v(50)$  on average is 30.36% (Ventolin) and 52.86% (Atrovent). At the low humidity, the small droplets are ease to vapor, causing an apparent shift to larger diameter of the PSD. So, the outcome is that measured particle sizes are increase as the humidity decrease. As described in Figure 9, the operation flow rate also had an influence on particle size of pMDI. As for the results, the particle sizes were decrease with the flow rate increase. It corresponds to Feddah's research. The reason is that there may be a highly turbulent field when the high-velocity pMDI plume

encounters slower inhalation air flow. In addition, the faster inhalation air flow is, the shorter droplet evaporation time. Apart from that, the increasing of air flow may decrease the density of droplet and to stimulate evaporation [44]. So, it may result in decrease of particle size. There was a flow-dependent behavior for Atrovent (solution formulation). And that influence was weak for Ventolin (suspension formulation). It should relate to the difference of formulation composition, such as actuator orifice, valve design and expipient [45].



**Figure 9.** The frequency distribution of particle volumes of Ventolin and Atrovent test by LD at different flow rate. A: Ventolin; B: Atrovent.

As we all known, the production of pMDI aerosol particles mainly rely on pressure of propellant evaporation. Therefore, the particle sizes depend on the pressure of propellant, diameter of actuator orifice and formulation composition. The pressure of propellant is related to temperature, actuator orifice and formulation composition, but the orifice and composition cannot be changed for a certain pMDI product [42]. As to results, with the device temperature increase, the particle sizes were decrease at first from 25°C to 40°C and then increase from 40°C to 60°C, both for ventolin and atrovent. The change of Atrovent is weaker than Ventolin. When the temperature increase from 25°C to 40°C, the decrease in  $D_v(50)$  on average is 63.10% (Ventolin) and 29.76% (Atrovent). Then temperature increase from 40°C to 60°C, the increase in  $D_v(50)$  on average is 164.97% (Ventolin) and 3.05% (Atrovent). These differences may be attributed that propellant composition. Except for the HFA134a, the excipients of Atrovent were also included water and ethanol. But excipient of Ventolin was only HFA134a [27]. Related study had been reported that ethanol may reduce overall formulation vapor pressure and increasing evaporation time because of much lower vapor pressure [46].

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