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## PAPER

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# Characterization and comparison of *Re-Du-Ning* aerosol particles generated by different jet nebulizers

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Inhalation therapy is the first-line therapy for the treatment of respiratory diseases. *Re-Du-Ning* inhalation solution (RIS) is an aerosol derivative from the *Re-Du-Ning* injection and has been clinically used to treat respiratory diseases like pneumonia for more than twenty years in China. However, the aerosolization and inhalation performances of RIS using different nebulizers have not been characterized, which may affect the therapeutic effects of RIS on respiratory diseases. We investigated the inhalation performances of RIS using five different nebulizers utilizing Spraytec, breath simulator of BRS 2000 and NGI techniques. We tested 5 different types of jet nebulizer, using RIS and an adult breathing pattern, to determine the difference in aerosol delivery over time. The particle size distribution of RIS was monitored by a Spraytec laser particle sizer. Fine particle fraction (FPF) and mass median aerodynamic diameter (MMAD) for RIS were measured using NGI. Aerosol deposited on the filter was analysed using HPLC. Nebulization time was much longer for the Pari Boy SX (red) nebulizer than for the other nebulizers, with the minimum delivery rate (DR) and the maximum total delivered dose (TDD) and total exhalation dose (TED). Nebulization time for Pari Boy SX (blue) was the lowest, with the highest DR and the lowest TDD and TED. Furthermore, the aerodynamic particle size of RIS was much larger for the Pari blue and Pari LC Plus than other nebulizers. Pari red produced the smallest aerodynamic particle size of RIS in these five nebulizers. In addition, a good linear relationship was found between MMAD and $D_{50}$ in these five nebulizers. The results demonstrated that Pari Boy SX (red) delivered most slowly and produced the smallest aerodynamic particle size of the RIS aerosols, which may be applied to manage lower respiratory diseases. Moreover, Pari LC Plus and Pari Boy SX (blue) emitted quickly and generated larger aerodynamic particle size of RIS aerosols, which could be used to treat upper respiratory diseases. A good linear relationship between MMAD and $D_{50}$ showed Spraytec could be a reliable technique for the development, evaluation and quality control of aerosol particles of inhalation solution preparations.

## Introduction

Inhalation therapy is commonly used to deliver drugs to the lung and is accepted as the first-line therapy for the treatment of respiratory diseases,[1] such as asthma,[2] pneumonia[3] and chronic

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bronchitis.[4] A nebulizer provides a convenient method for the pulmonary delivery of a wide range of drugs, including drug doses too large for delivery by other inhaler devices and combinations of different compatible drugs, especially for the elderly and children's medication.[5,6] Notably, various nebulizers (*e.g.* conventional jet, ultrasonic and vibrating mesh nebulizers) with different properties are widely used in the clinic.[7,8] These nebulizers can produce distinct size aerosol particles, which may deposit in different locations of the respiratory system after inhalation, and result in various therapeutic effects on respiratory diseases.[9] Therefore, the choice of nebulizer device should be considered carefully and the inhalation performance of these nebulizers for each drug should be determined.

With the growing concerns regarding the adverse effects of chemical-based supplements, Traditional Chinese Medicine (TCM) has increased in popularity, especially for preventing and curing respiratory diseases.[10,11] TCM is typically administered orally or topically,[12,13] but the barrier lies in the large dosage required to

achieve effects in respiratory diseases.[14] The aerosols generated by nebulizer allow direct absorption to the bloodstream in lung alveoli, ensuring maximal penetration.[15] *Re-Du-Ning* inhalation solution (RIS) is a new preparation derivative from *Re-Du-Ning* injection, which has been clinically used to treat respiratory diseases like pneumonia for more than twenty years in China.[16] This preparation is composed of the flowers of *Lonicera japonica* Thunb, the fruits of *Gardenia jasminoides* and *Artemisia apiacea* at the ratio of 25 : 15 : 12.[17] It has been reported that these herbs are of low toxicity and have been used in China for hundreds of years to treat early febrile diseases, exogenous fever and boils.[18–21] Previous studies showed that the main compounds in the flowers of *Lonicera japonica* (*e.g.* chlorogenic acid),[22,23] the fruits of *Gardenia jasminoides* (*e.g.* geniposide)[24,25] and *Artemisia apiacea* (*e.g.* artemisinin)[26] have strong anti-inflammatory activities. And our previous work showed that RIS could suppress the secretion of inflammatory mediators through TLR4 signalling pathway in lipopolysaccharide stimulated RAW 264.7 macrophages,[27] which suggested the potential anti-inflammatory effects of RIS. However, the nebulizers of RIS are varied and the processes of aerosolization are not systemically controlled, which may negatively impact the efficiency of drug delivery.[28] In our present study, for the first time, we used five different nebulizers to generate aerosol particles of RIS. Subsequently, we determined the uniformity of delivered dose (UDD) of RIS by BRS 2000 breath simulator. Next, Spraytec was used to monitor the nebulization process and the size of aerosol droplet of RIS. Additionally, next generation impactor (NGI) was conducted to measure the aerodynamic particle size distribution. After each performance, we also compared these parameters of aerosol particles of RIS produced by five different nebulizers. This study provided guidelines to choose nebulizer for RIS in clinic.

## Material

*Re-Du-Ning* inhalation solution (RIS, batch number: 160337) was supplied by Jiangsu Kanion Pharmaceutical Corporation (Jiangsu, P. R. China). Chlorogenic acid (batch number: 110753-200413) and geniposide (batch number: 110749-200309) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P. R. China). Methanol and phosphoric acid were bought from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Distilled water (Watsons Co., Ltd, Guangzhou, P. R. China) were of HPLC grade and all other chemical reagents used were of analytical grade.

Five nebulizers were involved in the study: Pari Boy SX (blue nozzle insert), Pari Boy SX (red nozzle insert), Pari Junior Boy SX, and Pari LC Plus were obtained from Pari Pharma GmbH (Starnberg, Germany). Yu Yue 403E was purchased from Jiangsu Yu Yue Medical Instrument Co., Ltd (Jiangsu, P. R. China). For each experiment, the nebulizer was filled with 2 mL of RIS.

## Methods

### Uniformity of delivered dose

The uniformity of delivered dose (UDD) has three components: delivery rate (DR), total delivered dose (TDD) and total exhalation dose (TED), which were determined as previously described.[29] Briefly, a breathing simulator (BRS 2000, Copley Scientific Limited, Nottingham, UK) mimicking an adult breathing pattern (15 breath per min, tidal volume of 500 mL, inhalation/expiration is 1 : 1) was used (see Table 1). Each nebulizer was connected to the sine pump through a filter holder (Pari Pharma GmbH, Starnberg, Germany) containing an inhalation filter (F1) and an exhalation filter (F3) using rubber adaptors specifically design to connect the nebulizer mouthpiece to the filter holder (see Fig. 1A).

The pump was switched on and the nebulizer was activated after 10 s. The nebulizer was then switched off after 60 s. The pump was also stopped after 5 s and the filter (F1) and its filter holder were removed and substituted with a new filter and holder (F2). The breath simulator and nebulizer were then restarted and lasted for 10 minutes. The RIS amount deposited on the two filters and holders, the amount remaining inside the nebulization chamber and the amount left in the glass vial were quantitatively collected using 50% ethanol, respectively. The liquids collected from each filter were transferred into 50 mL volumetric flasks and then diluted with 50% ethanol to the same volume. The amount of chlorogenic acid and geniposide of each sample were determined by HPLC analysis. Delivery rate (DR) represents the mass emitted per minute, was determined by quantifying chlorogenic acid and geniposide collected on the F1, respectively. The total delivered dose (TDD) was calculated by summing the amount of chlorogenic acid and geniposide collected on F1, F2 and their filter holders, respectively. In addition, total exhalation dose could also be obtained by calculating the drug deposited on F3. Effective spray time is the ratio of DR to TDD. Each test was carried on in triplicate.

### Real time particle size

Next, the laser diffraction-based technique consisted of a Malvern Spraytec (Malvern Instruments Ltd, Worcestershire, UK) equipped with an inhalation cell, was used to monitor the particle size diameter (PSD) of aerosols generated by the nebulizers. It consists of a Spraytec unit with a USP throat held in place by the inlet of the inhalation cell, and a high capacity vacuum pump (HCP 5, Copley Scientific, Limited, Nottingham, UK) calibrated at 15 L min$^{-1}$ was attached to the outlet of the cell (see Fig. 1B). The nebulizer was filled with 2 mL of RIS and the total aerosol collection time was 5 min. Various parameters such as $D_{10}$, $D_{50}$, $D_{90}$ and transmission were calculated by the Spraytec software (Malvern Instruments Ltd, Worcestershire, UK). $D_{10}$, $D_{50}$, and $D_{90}$ represent maximal particle size diameter

**Table 1** Four different breath profiles of the breath simulator BRS 2000

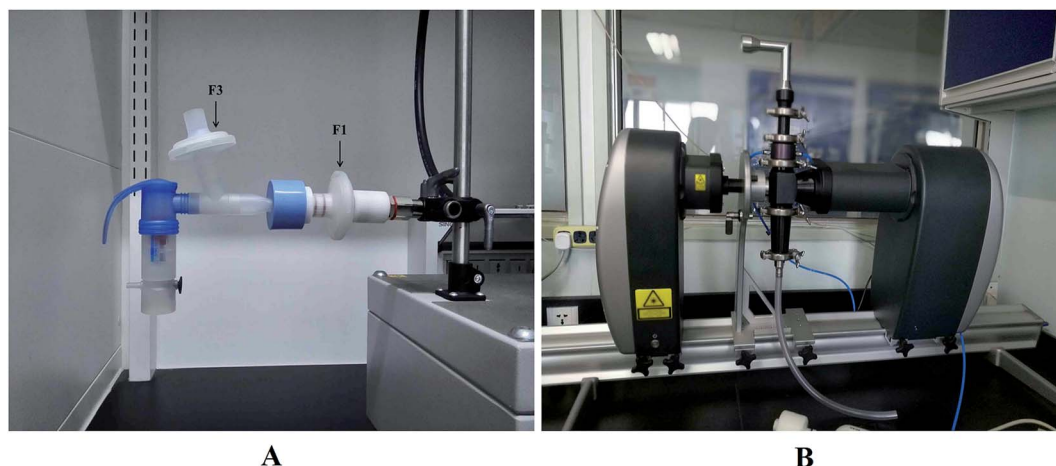| Profile | Characteristic | | | |
| --- | --- | --- | --- | --- |
| | Adult | Child | Baby | Neonate |
| Tidal volume (mL) | 500 | 155 | 50 | 25 |
| Breath frequency (cycles per min) | 15 | 25 | 30 | 40 |
| Ratio of inhalation to exhalation | 1 : 1 | 1 : 2 | 1 : 3 | 1 : 3 |

**Fig. 1** Nebulizer coupled with filters and Spraytec with inhalation cell. (A) The nebulizer of Pari Boy SX with red nozzle insert and two filter holders F1 contains an inhalation filter and F3 contains an exhalation filter. When the nebulization was run for 1 min, the filter and its filter holder (F1) were replaced by a new filter and holder (F2). The whole nebulization process was lasted for 10 min. DR was the mass emitted per minute, determined by quantifying drug deposited on F1. TDD was calculated by summing the amount of drug collected on F1, F2 and their holders. TED can also be acquired by calculating the drug amount on F3. Effective spray time is the ratio of DR to TDD. Each test was carried on in triplicate. (B) The components of Spraytec with the inhalation cell.

that includes 10%, 50%, and 90% of particles, respectively. Values presented are the average of at least 3 determinations.

**Aerodynamic particle size analysis**

The NGI (Copley Scientific Limited, Nottingham, UK) was operated at 15 L min$^{-1}$ using a high capacity vacuum pump (HCP5, COPLEY Scientific, UK) and a critical flow controller (TPK2000, Copley Scientific Limited, Nottingham, UK) to measure the aerosol particle size distribution (PSD) at 5 °C.

The nebulizer was connected to the induction port mouth-piece adapter, filled with 2 mL of RIS, and the aerosol collection time was 5 min. When the aerosol finished, the nebulizer was switched off and the vacuum pump was stopped after 5 s. Drug deposition in the device, throat, all the stages and the filter was determined by HPLC analysis. For accuracy, each test was repeated three times. Results of each collector were imported to the CITDAS system (COPLEY Scientific, UK), and then the mass median aerodynamic diameter (MMAD), the fine particle fraction (FPF) and the geometric standard deviation (GSD) were calculated. Values presented are the average of at least 3 determinations.

**HPLC analysis**

The contents of chlorogenic acid and geniposide were quantitated by reverse phase HPLC according to the national drug standards of *Re-Du-Ning* injection.[17] Briefly, an LC-20a HPLC system (Shimadzu Corporation, Tokyo, Japan) consisted of a quaternary pump, a standard auto-sampler, a column oven and a diode array detector. Chromatographic separation was performed on Agilent Zorbax SB C$_{18}$ column (250 mm × 4.6 mm, 5 μm) at 30 °C. A linear gradient system consisted of A (methanol) and B (0.1% phosphoric acid water). The gradient elution profile was as follows: 0–10 min, 15–25% A; 10–30 min

25–35% A; 30–60 min, 35–50% A. The signal was monitored at 237 nm for geniposide and 324 nm for chlorogenic acid, respectively. The flow rate was maintained at 0.8 mL min$^{-1}$. Re-equilibration duration was 20 min between individual runs. All collected samples were filtered through a 0.45 μm Millipore membrane filter, and an injection volume of samples was 10 μL in each experiment.

**HPLC fingerprint analysis of RIS**

As reported in our previous work,[27] the HPLC fingerprint analysis of RIS was performed to gain chemical profile of RIS.

# Results

**HPLC fingerprint analysis of RIS**

Geniposide was used as the reference substance in the HPLC fingerprint analysis of RIS. Geniposide (peak S) and extra 13 common peaks were assessed with relative retention times and peak area ratios (see Fig. 2 and Table 2).

**Determination of chlorogenic acid and geniposide**

For quantitative consideration, the calibration curves of were established on different concentrations of nine consecutive injections. The linear concentration ranges of the calibration curves for chlorogenic acid and geniposide are 0.11–2.104 μg mL$^{-1}$ and 0.14–2.792 μg mL$^{-1}$, respectively. Moreover, regression equations calibrated for chlorogenic acid and geniposide were $y = 4 \times 10^7 x - 1301.6$ ($R^2 = 0.9998$, $n = 9$) and $y = 2 \times 10^7 x + 83.544$ ($R^2 = 0.9997$, $n = 9$), respectively. Based on the calibration curve of chlorogenic acid and geniposide, we calculated the delivery rate, total delivered dose, total exhalation dose and aerodynamic particle size distribution using the contents of
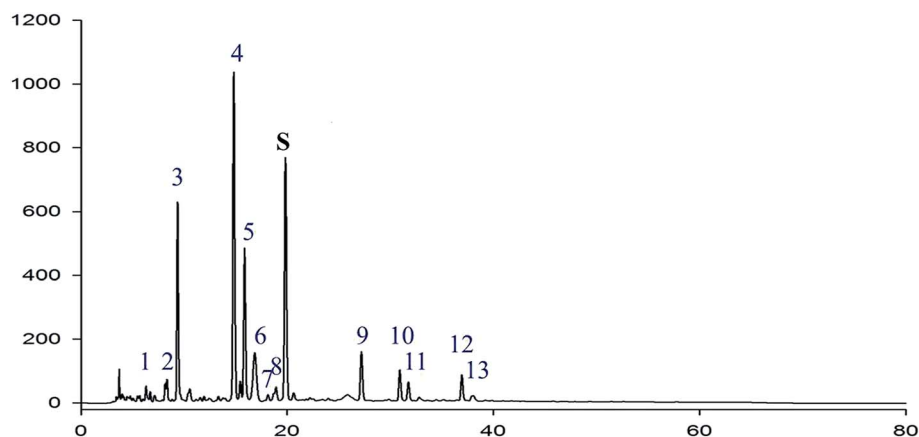
Fig. 2  HPLC fingerprint chromatogram of RIS. Peak S represented geniposide.

chlorogenic acid and geniposide in each sample, which was shown in the following results (see Fig. 3B and 5B).

### Determination of DR, TDD and TED

Fig. 3 showed that the parameters for five different nebulizers including DR, TDD and TED were calculated by the contents of chlorogenic acid and geniposide in all samples. As shown in Fig. 3, nebulization with the Pari Boy SX with blue nozzle insert resulted in the highest DR ($0.96 \pm 0.02$ mg min$^{-1}$) and the lowest TDD ($3.17 \pm 0.20$ mg). However, nebulization with Pari Boy SX with red nozzle insert resulted in the minimal DR ($0.66 \pm 0.01$ mg min$^{-1}$) and the maximal TDD ($5.19 \pm 0.18$ mg). The DR values of other three nebulizers were similar, and the average values range from 0.76 to 0.79 mg min$^{-1}$. We also observed that nebulization with Pari LC Plus and Yuyue 403E had almost the same TDD values, and nebulization with the Pari Junior Boy SX had larger TDD value that these two nebulizers. Interestingly, the values of TED had similar trends with TDD values. Nebulization with Pari Boy SX with blue nozzle insert had the smallest TED value ($0.43 \pm 0.04$ mg) and Pari Boy SX with red nozzle insert had the largest TED value ($2.15 \pm 0.10$ mg). Values of effective nebulization time (EST) of five nebulizers were $3.30 \pm 0.24$, $8.05 \pm 0.03$, $6.16 \pm 0.22$, $4.40 \pm 0.09$, $4.71 \pm 0.38$ min, respectively. Additionally, nebulization of 2 mL RIS with these five nebulizers led to complete nebulization after 10 min. This is certainly an advantage leading to saving of medicament and therapy costs. In line with these results obtained from chlorogenic acid, the results of DR, TDD, TED and EST calculated from geniposide showed the same trend (see Fig. 3).

### Determination of real-time particle size distribution

Next, we also used Spraytec to monitor the real-time particle size distribution and transmission of RIS aerosol generated by different nebulizers in vitro. As shown in Fig. 4, we observed that the value of transmission was high before the nebulization process, indicating there were few aerosol particles generated by nebulizers. The value of transmission was remarkably decreased as the nebulization process started, showing that these nebulizers began to produce the aerosol particles. When RIS was enough to be nebulized, the values of transmission remained steady. The transmission values were then obviously increased as insufficient aerosol particles produced by these nebulizers. The transmission versus time curve was like a U-shaped curve across the whole nebulization process.

As is shown in Table 3, the average values of $D_{10}$ was from 0.81 to 1.35 μm, $D_{50}$ was from 3.08 to 4.88 μm, and $D_{90}$ was from 7.37 to 11.71 μm among these nebulizers. $D_{50}$ and $D_{90}$ involve the most aerosol particles generated by nebulizers and are the most commonly used parameters to access aerosol particle size distribution. In our study, we observed that the values of $D_{50}$ and $D_{90}$ of Pari Boy SX (blue) were the highest and the values of $D_{50}$ and $D_{90}$ of Pari Boy SX (red) were the lowest among these five nebulizers, suggesting that Pari Boy SX (blue) and Pari Boy SX (red) produce the largest and the smallest aerosol particles of RIS, respectively.

### Determination of aerodynamic particle size distribution

In addition, MMAD and FPF, the values involved in the respirable dose calculation, were determined from the aerodynamic

Table 2  Relative retention times and peak area ratios of geniposide and 13 common peaks[a]

| Peak no. | Relative retention time | Peak area ratio |
|---|---|---|
| 1 | 0.286 | 0.03 |
| 2 | 0.400 | 0.05 |
| 3 | 0.462 | 0.62 |
| 4 | 0.759 | 1.28 |
| 5 | 0.810 | 0.48 |
| 6 | 0.835 | 0.11 |
| 7 | 0.912 | 0.01 |
| 8 | 0.956 | 0.03 |
| S | 1.000 | 1.00 |
| 9 | 1.374 | 0.10 |
| 10 | 1.613 | 0.08 |
| 11 | 1.639 | 0.05 |
| 12 | 1.934 | 0.08 |
| 13 | 1.957 | 0.01 |

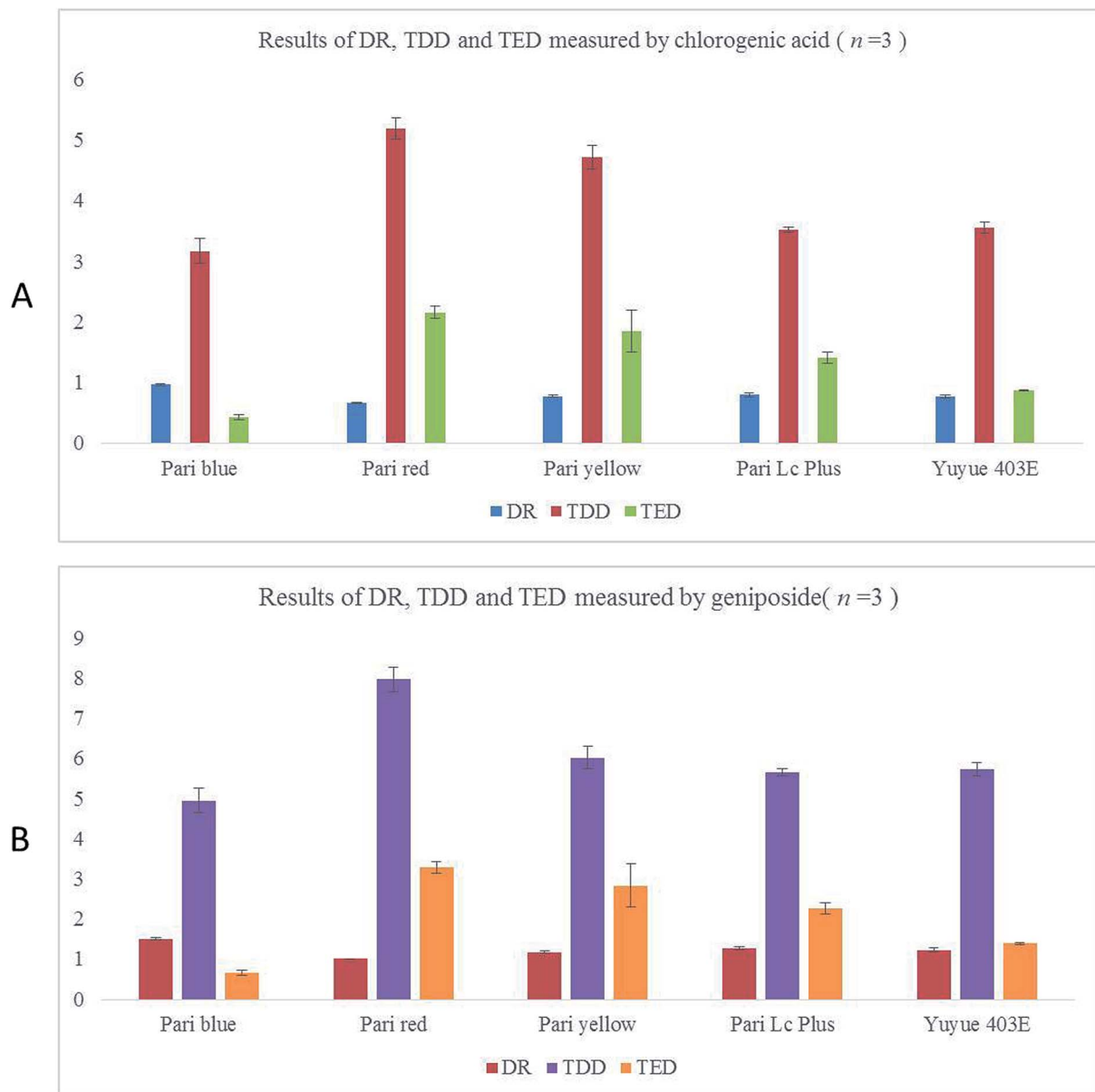[a] Peak S represented geniposide, which was selected as the reference substance.

Fig. 3 Uniformity delivery dose results of five different nebulizers determined by HPLC. (A) Results measured by chlorogenic acid ($n = 3$). (B) Results measured by geniposide ($n = 3$). DR, TDD and TED represented delivery rate, total delivered dose and total exhalation dose, respectively.

particle size distribution obtained from the NGI. As shown in Fig. 5 and Table 4, the highest values of MMAD among these nebulizers were obtained with Pari LC Plus and Pari blue. However, Pari LC Plus and Pari blue presented the lowest values of FPF with 44.7% and 45.7%, respectively. The Pari yellow and Yuyue 403E produced aerodynamic droplet size at 4.21 and 4.58 μm, respectively. The values of FPF in these two nebulizers were 58.8% and 52.6%, respectively. Meanwhile, it was found that the lowest aerodynamic particle size (3.12 μm) of RIS was generated by Pari red with the highest FPF value (76.0), showing that the highest percentages of particles produced by Pari red are below 5 μm. Additionally, similar trend was observed in the results of NGI measured by geniposide (see Fig. 5 and Table 5).

### Correlation analysis of MMAD and $D_{50}$

In order to evaluate the Spraytec as a reliable measuring instrument for characterizing particle size for RIS, the MMAD measured by NGI were compared with and plotted against the $D_{50}$ measured by Spraytec. As shown from Fig. 6, a linear relationship existed between the MMAD and the $D_{50}$ when calculated with the regression. The regression equations for chlorogenic acid and geniposide were $y = 0.7645x + 0.6418$ ($r = 0.9606$, $n = 15$) and $y = 0.7918x + 0.5731$ ($r = 0.9645$, $n = 15$), respectively, indicating a good correlation ($R^2$ of 0.9606 and 0.9645, respectively) between the $D_{50}$ and MMAD (see Fig. 6).
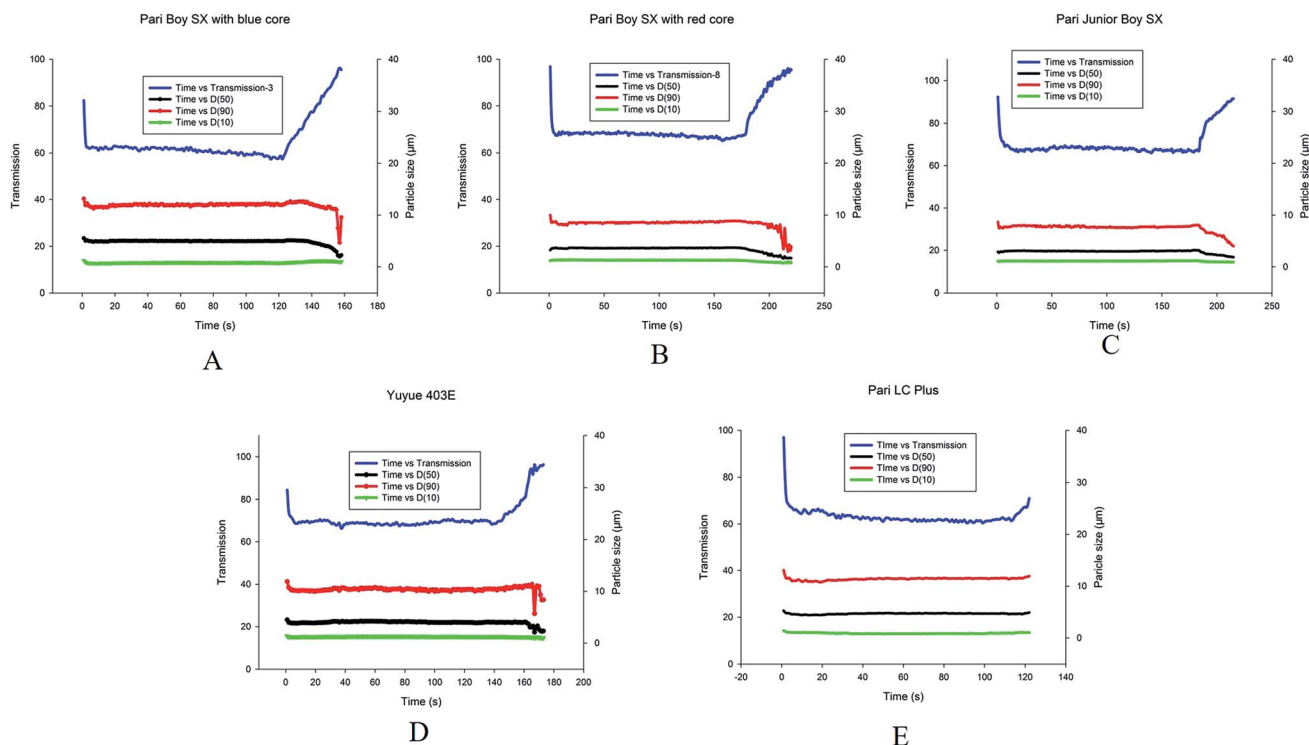
**Fig. 4** The Spraytec monitors the result of 5 different jet nebulizers. The inlet gas velocity was calibrated at 15 L min$^{-1}$. $D_{10}$, $D_{50}$, $D_{90}$ and transmission were calculated automatically in the Spraytec software. $D_{10}$, $D_{50}$, and $D_{90}$ represent maximal particle size diameter that includes 10%, 50%, and 90% of particles, respectively. (A) Pari Boy SX with blue nozzle insert; (B) Pari Boy SX with red nozzle insert; (C) Pari Junior Boy SX; (D) Pari LC Plus; (E) Yuyue 403E. Values given are the mean ± SD at least 3 determinations.

## Discussion

In recent years, the focus of development in nebulizer technology has been geared towards making devices to be more patient-friendly.[30] In terms of providing more efficient delivery, breath-enhanced nebulizer (such as Pari Boy SX, an attached compressor produces compressed gas) can deliver a more stable and consistent aerosol to the lungs when directed to the medication holding chamber through a narrow hole, wasting less medication.[31,32] However, different types of breath-enhanced nebulizers are designed to have different drug delivery efficiencies and produce a variety of particle sizes, which may interact with a range of user-specific factors, including drug formulation and breathing pattern, to affect the delivered dose and deposited locations.[33] In our present study, we used five classic breath-enhanced nebulizers to produce aerosol of RIS and compared parameters of aerosol particles measured by chlorogenic acid and geniposide, respectively. We found that these nebulizers delivered different doses of RIS and generated aerosol particle with various sizes, which may partially impact therapeutic effect of RIS on respiratory diseases.

BRS 2000 breath simulator is increasingly used in testing *in vitro* dose uniformity and aerodynamic particle size distribution data and covers a range of breathing patterns from neonate through to neonate, infant, child and adult.[34] In our study, we chose an adult breathing pattern and determined the DR, TDD, and TED of aerosol of RIS produced by five nebulizers. The results indicated that the Pari Boy SX with red nozzle insert

emitted aerosol of RIS most slowly, and the nebulization dosage and the loss of RIS were the largest among the five nebulizers. The lower flowrate of Pari red means lower air velocity, thus with the smaller hole in the holding chamber. Therefore, the emission of drug particles is more efficient and smaller particles are delivered from the device. In contrast with Pari red, Pari blue emitted aerosol of RIS most quickly, but the amount of aerosol emitted was the fewest among these five nebulizers, suggesting that larger particles were produced by Pari blue.

Particle size is a key parameter in defining the deposition pattern and bio-availability of drug material delivered to the respiratory system using nebulizers.[35–37] The optimum particle size for deposition into the lung periphery and the lower airways

**Table 3** Results of $D_{10}$, $D_{50}$, $D_{90}$ of five different nebulizers ($n = 3$)[a]

| Nebulizer | $D_{10}$ (μm) | $D_{50}$ (μm) | $D_{90}$ (μm) |
|---|---|---|---|
| Pari blue | 0.81 ± 0.06 | 4.88 ± 0.07 | 11.71 ± 0.20 |
| Pari red | 1.30 ± 0.17 | 3.08 ± 0.29 | 7.37 ± 0.86 |
| Pari yellow | 1.35 ± 0.09 | 3.49 ± 0.06 | 8.13 ± 0.26 |
| Pari LC Plus | 1.27 ± 0.32 | 4.67 ± 0.02 | 11.00 ± 0.28 |
| Yuyue 403E | 1.30 ± 0.02 | 4.18 ± 0.12 | 10.69 ± 0.33 |

[a] $D_{10}$, $D_{50}$, and $D_{90}$ were calculated automatically by the Spraytec software. The inlet gas velocity was calibrated at 15 L min$^{-1}$. $D_{10}$, $D_{50}$, and $D_{90}$ represent maximal particle size diameter that includes 10%, 50%, and 90% of particles, respectively. Values given are the mean ± SD at least 3 determinations.
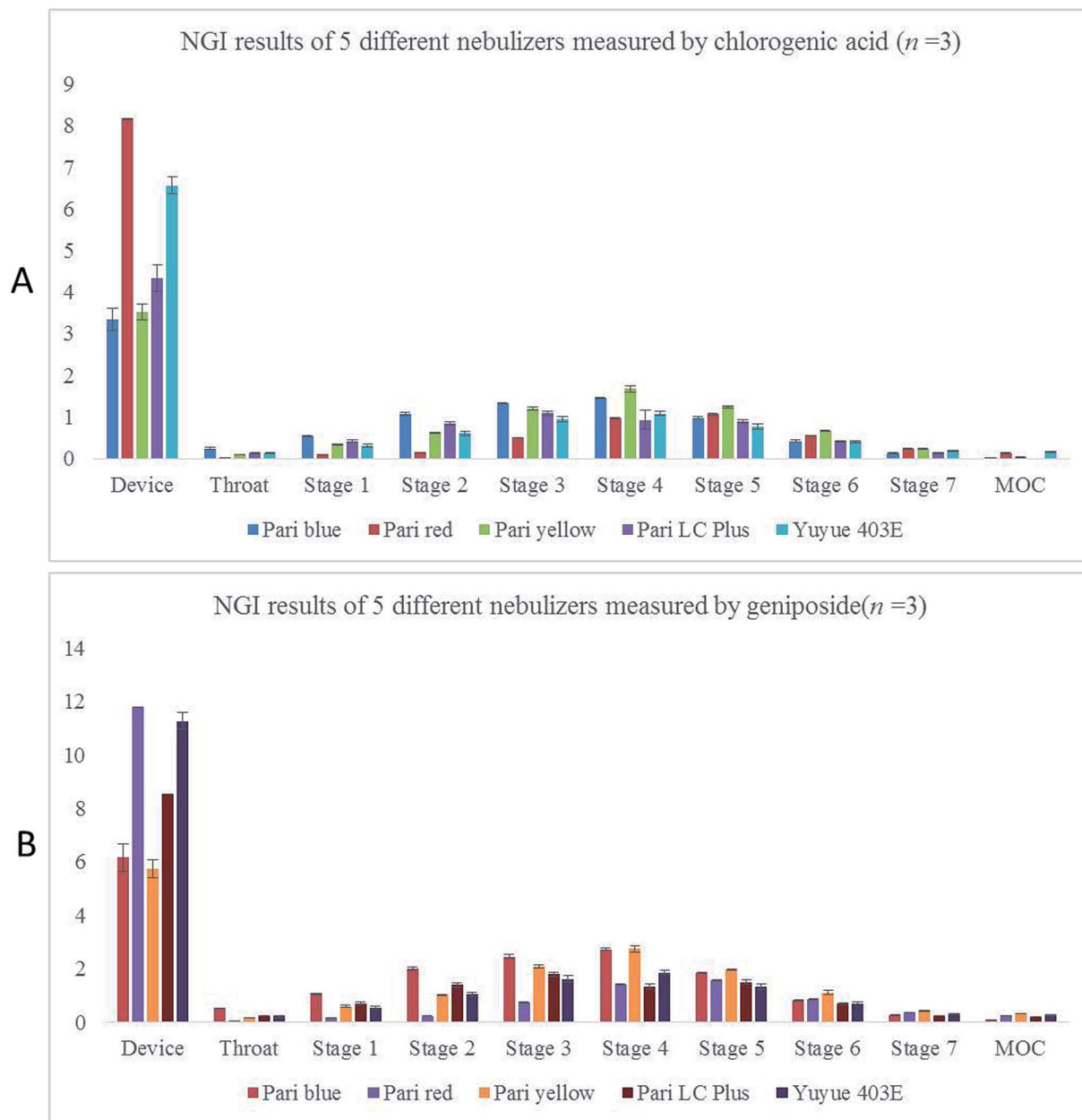
Fig. 5   NGI results of five different nebulizers determined by HPLC. (A) NGI results measured by chlorogenic acid ($n = 3$). (B) NGI results measured by geniposide ($n = 3$).

should be between 1 and 5 μm, whereas, particles with size between 5 and 10 μm deposit mostly in the conducting airways.[38–40] Currently, many types of apparatuses have been widely employed for aerosol particle size characterization, such as NGI and Spraytec.[41,42] The NGI is a high-performance cascade

impactor for classifying aerosol particle into size fractions for testing inhaled drug delivery devices such as nebulizers.[43] Moreover, Spraytec is one of the most popular laser diffraction devices specifically designed to address the unique requirements of aerosol and spray droplet characterization and

Table 4   Aerodynamic particle size distribution (APSD) results of 5 different nebulizers measured by chlorogenic acid ($n = 3$)

| Index | Pari blue | Pari red | Pari yellow | Pari LC Plus | Yuyue 403E |
|---|---|---|---|---|---|
| MMAD (μm) | $5.33 \pm 0.04$ | $3.12 \pm 0.00$ | $4.21 \pm 0.06$ | $5.38 \pm 0.13$ | $4.58 \pm 0.13$ |
| FPF (%) | $44.7 \pm 0.46$ | $76.0 \pm 0.14$ | $58.8 \pm 0.69$ | $45.7 \pm 2.52$ | $52.6 \pm 1.46$ |
| GSD | $2.10 \pm 0.01$ | $1.94 \pm 0.01$ | $2.84 \pm 0.54$ | $2.08 \pm 0.08$ | $2.15 \pm 0.09$ |

**Table 5**  APSD results of 5 different jet nebulizers calculated by geniposide ($n = 3$)[a]

| APSD index | Pari blue | Pari red | Pari yellow | Pari LC Plus | Yuyue 403E |
|---|---|---|---|---|---|
| MMAD (μm) | $5.25 \pm 0.13$ | $3.09 \pm 0.01$ | $4.10 \pm 0.04$ | $5.23 \pm 0.13$ | $4.61 \pm 0.15$ |
| FPF (%) | $45.5 \pm 0.13$ | $75.8 \pm 0.09$ | $60.0 \pm 0.51$ | $47.1 \pm 2.45$ | $52.8 \pm 1.73$ |
| GSD | $2.16 \pm 0.07$ | $1.97 \pm 0.00$ | $2.08 \pm 0.08$ | $2.11 \pm 0.05$ | $2.15 \pm 0.07$ |

[a] MMAD represents mass median aerodynamic diameter, FPF means fine particle fraction and GSD is short for geometric standard deviation. Values given are the mean $\pm$ SD at least 3 determinations.
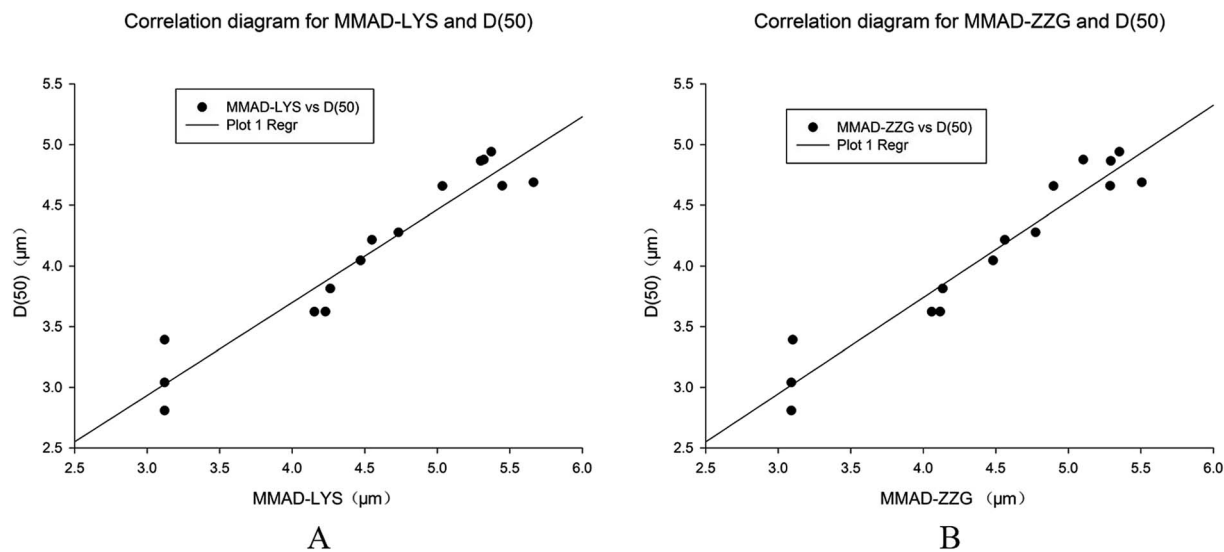


**Fig. 6**  Correlation analysis of $D_{50}$ and MMAD. (A) shows the correlation of $D_{50}$ and MMAD measured by chlorogenic acid; (B) shows the correlation of $D_{50}$ and MMAD measured by geniposide.

provides real-time and accurate droplet measurement.[44–46] Consistent with the results of DR, TDD, and TED, we observed that Pari Boy SX (red) produced the lowest aerosol particle size of RIS among these five nebulizers, suggested by the values of $D_{50}$ and MMAD. We also found that the largest FPF in Pari Boy SX (red) among these five nebulizers. It has been reported that the higher FPF translates into lower and upper airway doses and higher deposited doses in the lungs.[47,48] These results indicated that the small particles generated by Pari Boy SX (red) deposition within the respiratory system is dominated by sedimentation and diffusion within the deep lung. Furthermore, the values of $D_{50}$ and MMAD of Pari LC Plus and Pari blue were larger than other three nebulizers with lower FPF values, indicating that the aerosol particles of RIS emitted by these two nebulizers may mainly deposit by impaction in the oropharynx and bronchi, prior to reaching the lungs.

In addition, we also found that a good linear relationship between MMAD and $D_{50}$ in these five nebulizers. The existence of this linear relationship demonstrated that the results of the Spraytec are useful for size evaluation of aerosol particles of RIS, suggesting that the Spraytec could quickly provide a very good evaluation of the aerodynamic behaviour of the particles of RIS produced by these five nebulizers.

# Conclusion

Collectively, our study characterized and compared the parameters of five different nebulizers for RIS, including DR, TDD, TED, MMAD, and $D_{50}$, by BRS 2000 breath simulator, Spraytec, and NGI *in vitro*. The results showed that Pari Boy SX with red nozzle insert emitted most slowly and produce the smallest aerodynamic particle size of RIS, which may be applied to manage lower respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and asthma. Moreover, Pari LC Plus and Pari Boy SX with blue nozzle insert delivered quickly and larger aerodynamic particle size of RIS, which may deposit in upper or mid airways better, and could be used to treat upper respiratory diseases. Moreover, there is a good linear correlation between MMAD and $D_{50}$, suggesting that Spraytec can be a useful tool for rapid screening of the most suitable device in the early stage of nebulizer study. This study provides guidelines for the nebulizer choice of RIS in managing respiratory diseases.

# Abbreviations

| | |
|---|---|
| RIS | *Re-Du-Ning* inhalation solution |
| NGI | Next generation impactor |

| PSD | Particle size distribution |
| FPF | Fine particle fraction |
| MMAD | Mass median aerodynamic diameter |
| HPLC | High performance liquid chromatography |
| DR | Delivery rate |
| TDD | Total delivered dose |
| TED | Total exhalation dose |
| $D_{10}$, $D_{50}$ and $D_{90}$ | The maximal particle size diameter that includes 10%, 50% and 90% of particles, respectively |
| TCM | Traditional Chinese Medicine |
| UDD | The uniformity of delivered dose |
| GSD | Geometric standard deviation |
| COPD | Chronic obstructive pulmonary disease |

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## Authors' contributions

Conceptualization, Gan Luo; Investigation, Cui Li, Yi Zhang, Yonghua Tao and Gan Luo; Methodology and software, Xiaoyan Gao; Formal analysis, Chanjuan Yu and Xinyue Liu; Funding, Yi Zhang and Gan Luo; Writing-origin draft, Gan Luo and Yi Zhang; Writing-review & editing, Brian Chi-Yan Cheng.

## Conflicts of interest

The authors declare that they have no competing interests.

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