Research article

Cichoric acid aerosol for inhalation therapy in respiratory syncytial virus

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A B S T R A C T

Cichoric acid (CA) is a caffeic acid derivative, which has significant anti respiratory syncytial virus (RSV) effect and low toxicity. However, due to the low oral bioavailability and poor intestinal absorption of CA, it is not suitable to be made into oral preparations. In this study, CA was made into metered dose inhaler (MDI), allowing the drug to target the site of action, thus achieving more effective treatment. Through preliminary experiments, the drug content and prescription composition of the preparation were determined. Clarity and stability of solution were used as indexes to screen the composition of latent solvent. Single factor and orthogonal test were used to optimize the amount of latent solvent in CA-MDI, and the optimal prescription was verified. The aerosol prepared according to the optimal formula was characterized and preliminary stability was studied. The final formula of CA-MDI was: CA 15 mg, absolute ethanol 1 g, propylene glycol 0.4 g and 1,1,1,2-tetrafluoroethane 10 g. CA-MDI was prepared with the best prescription, with the specification of 150 actuation per bottle and 75 µg per actuation. After quality inspection, three batches of inhaled aerosols showed that the main drug content per bottle was 77.91 ± 1.63 µg (n = 3), and the total number of bottles was 185 ± 3 (n = 3), all of which met the standards of China Pharmacopoeia and the proposed specifications. The preliminary stability study showed that the quality of inhaled aerosols in CA was stable and reliable.

1. Introduction

RSV is a common pathogen, which can cause respiratory tract infection in infants, the elderly and other groups with poor immunity, and is the main cause of neonatal viral pneumonia. RSV is mainly transmitted through contact, droplets, etc. [1,2]. Children with mild RSV infection will have low fever, nasal congestion, cough and other clinical manifestations, and severe cases can develop into pneumonia, bronchiolitis and asthma [3]. RSV is a single-stranded negative-strand RNA virus, which can be divided into two subtypes, A and B, of which Type A accounts for a higher proportion in epidemic period. RSV infects cells through two main immunogens, fusion protein F and surface protein G, and the imbalance of immune response caused by infection is the main factor leading to the disease. RSV natural infection produces insufficient immunity, and even if it is naturally infected many times, it can't produce lifelong immune protection. Therefore, repeated infections will occur in children and the elderly. Most hospitalized children with respiratory infection are caused by RSV infection, which seriously threatens the health and development of infants and young children [4,5]. In 2015, there were 3.2 million hospitalized patients infected with RSV in the world, resulting in the death of nearly 60,000 children younger than 5 years old, which had a huge impact on the world [6]. However, at present, there is no effective vaccine against RSV, and *anti*-RSV drugs

lack sufficient safety and adaptability. At present, drugs commonly used in clinic include leukotriene receptor antagonists, bronchodilators, glucocorticoids, antiviral drugs, etc., but some children have poor tolerance and many side effects, which need to be further improved [7].

CA is a caffeic acid derivative existing in natural plants such as Echinacea purpurea and Dandelion [8,9]. Modern pharmacological studies showed that CA had the effects of antioxidation, antibiosis and antivirus, improving immunity and lowering blood sugar. However, due to the unstable physical and chemical properties of CA, the preliminary pharmacokinetic results also show that CA has low oral bioavailability and poor intestinal absorption, which limits its application [10]. In recent years, the research on CA has focused on its pharmacological action, extraction and purification [11,12], but there are few reports about its preparation [13]. Our research group showed that [14] the *anti*-RSV effect of CA was remarkable, obviously superior to that of ribavirin and less toxic. However, the oral bioavailability of CA is low, it is easy to degrade in the stomach, and the intestinal absorption is poor. In addition, CA (structure figure 1) contains ester bonds which are easily hydrolyzed in water to produce caffeic acid and caffeic tartaric acid [15], and it is easily oxidized when exposed to light, which limits its application in medicine, food and other industries.

Inhalation preparation refers to the liquid or solid preparation in which the API is dissolved or dispersed in a suitable medium and delivered to the lungs in the form of aerosol or vapor to play a local or systemic role. Among them, metered dose inhaler (MDI), powder Aerosols for Inhalation and inhaled liquid preparation are mostly used to treat asthma or chronic obstructive pulmonary disease (COPD) [16,17]. MDI is composed of five parts: medicine, auxiliary materials, propellant, valve and pressure container. It has the characteristics of simple device, reliability, durability and portability, and has become a widely used inhalation preparation [18,19]. MDI is more complicated than traditional preparations, so three factors should be considered in drug development: prescription, patient and device.

Pulmonary drug delivery system has unique advantages for the treatment of lung diseases, which can reach the focus directly, take effect quickly and avoid the first-pass effect of the liver. According to the nature of the drug and the cost of the preparation, we chose to make CA into metered dose inhaler. In the previous research, we optimized the extraction and purification process of CA, completed the preliminary antiviral and toxicological studies in vitro and vivo, and had good medicinal properties [20]. This subject plans to develop a CA-MDI against RSV respiratory tract infection, complete the preparation and characterization and preliminary stability investigation.

2. Materials and methods

2.1. Materials

High performance liquid chromatography (Technologies 1260 Series) is provided by Agilent Company (United States). One hundred thousandth scale (New Classic MC) provided by Mettler Toledo (China). The drug inhalation preparation tester (QW-1A) is provided by Tianda Tianfa Technology Co., Ltd. (Tianjin, China). One thousandth electronic balance (YP-20002) is provided by Guangzheng Medical Instrument Co., Ltd. (Shanghai, China). Internal pressure gauge (Y-60) is provided by Hongqi Instrument Co., Ltd. Silent oil-free air compressor (DW-250) is provided by Denell Energy Saving Technology Co., Ltd. The production equipment of 20 mm aerosol is provided by Jerez Electromechanical Co., Ltd. (Wuhan, China). The clarity detector (YB-2) is provided by Tianda Tianfa
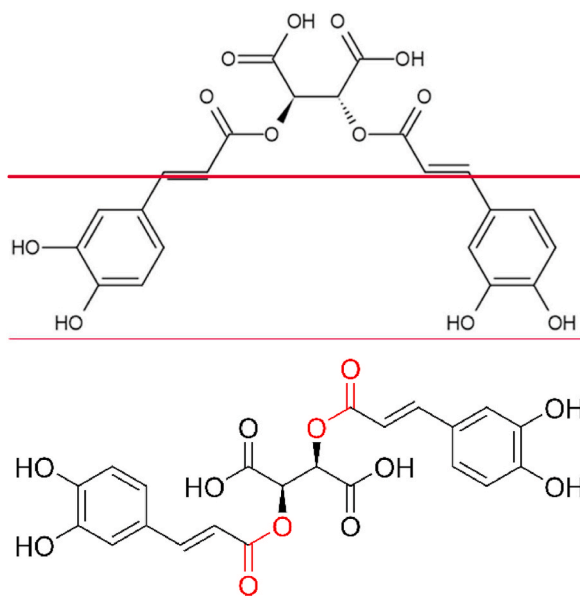

Fig. 1. Chemical structure of cichoric acid.

Technology Co., Ltd. (Tianjin, China). The reference substance of cichoric acid is provided by China Institute for Food and Drug Control. 19 mL aluminum cans of different materials are provided by Company PRESSPART.

2.2. Preparation process

Firstly, an appropriate amount of CA is accurately weighed and transferred to a 10 mL brown volumetric flask, which was dissolved by anhydrous ethanol to the scale and filtered as the stock solution. Secondly, an appropriate amount of stock solution was placed in a pressure vessel, the anhydrous ethanol, propylene glycol was added, and the propellant was pressed in the canister. Finally, the CA-MDI was weighed and the unqualified samples were removed. The preparation process of CA-MDI is as follows: 1. Aluminum cans and valves are washed, dried, and sterilized. 2. CA and co solvents are mixed, filtered, filled, sealed, and filled with propellant. 3. Quality inspection.

2.3. Quantitative method of CA

Agilent ZORBAX SB-C18 chromatographic column (4.6 mm $\times$ 250 mm, 5 μm); The mobile phase was acetonitrile -0.2% phosphoric acid solution $= 22{:}78$ (v/v); The flow rate is 1 mL/min; Column temperature: 30 °C. The detection wavelength is 326 nm, and the injection volume is 5 μL.

2.4. Selection of packaging materials

An appropriate amount of CA sample was weighed into a volumetric flask, diluted by the absolute ethanol to the scale, and the initial concentration of the solution was measured according to the requirements under "2.3 Quantitative method of CA". The liquid medicine was transferred to four different packaging materials, namely glass bottles, MDI aluminum cans, plasma coated MDI aluminum cans, and oxide film MDI aluminum cans, which were sealed and placed in a 40 °C incubator. To investigate the influence of the four containers on the concentration of CA, samples were taken for detection on the 5th and 10th days respectively.

2.5. Prescription screening

2.5.1. Selection of latent solvent

Previous experimental results showed that the maximum dose of CA was 1.5 mg/can when there was only anhydrous ethanol and propellant. Increasing the content of anhydrous ethanol can increase the drug content in each pot, but too much anhydrous ethanol will corrode the valve gasket and affect the delivery efficiency of aerosols [21,22]. The drug content in each pot is expected to reach 15 mg, and the solubility of CA is increased by adding other latent solvents. With the clarity of the solution as the index, add glycerin, PEG-400, propylene glycol, oleic acid, Tween-80, lecithin and other commonly used MDI additives. Among them, propylene glycol has a significant solubilizing effect, and will not precipitate when placed at room temperature. Therefore, anhydrous ethanol and propylene glycol were finally selected as the latent solvents of CA aerosol.

2.5.2. Selection of dosage of propellant

As the internal pressure of aerosol is generally required to be less than 0.8 MPa at 25 °C and less than 1.0 MPa at 50 °C. The internal pressure is directly related to the amount of propellant filled. 1,1,1,2-tetrafluoroethane (HFA-134a) of different weights was filled into aluminum cans, which were placed in a constant temperature water bath at the required temperature, and the aluminum cans were completely immersed in water for 30 min. The sample was taken out and shaken 6 times, and then the inlet of the pressure gauge was aligned with the valve stem. After pressing hard, the pressure reading was recorded, each pot was tested 3 times, and the results are averaged [23].

A certain weight of drug liquid was added into the aerosol aluminum pot, and after the valve was installed, the propellant of different weight was injected into the aluminum canisters and shaken evenly. According to the method of total spraying amount under Item 0113 of Part IV of the Chinese Pharmacopoeia (2020 Edition), the total spraying amount of aerosol containing different propellant were investigated. The aluminum canisters filled with liquid and propellant were taken out and weighed accurately, and then they were sprayed into the containers with proper amount of absorbing liquid in the fume hood continuously until they are sprayed out. Finally, these aluminum canisters were wiped dry, weighed accurately, and the ejection weight rate of each bottle was calculated.

Table 1
Clarity and stability score of CA aerosol.

Character	Score
Completely clear and transparent	6
More clear and transparent	5
Emulsion	4
milky white	3
layered	2
Precipitation	1

HFA-134a was filled 10 g in aluminum canisters to investigate the change of internal pressure of the propellant during the use of MDI. Take every 10 actuation as a unit for measurement, and conduct 3 groups in parallel.

2.5.3. Selection of propylene glycol dosage

The amount of CA was 15 mg, anhydrous ethanol 1.2 g, and HFA-134a 10 g. The dosage of different propylene glycol was studied. The liquid medicine was filled in aluminum canisters and high-pressure glass containers respectively by pressure filling method, and each prescription was in three groups in parallel. After being placed in a dark place at room temperature for 24 h, send it to the transparency tester to observe the property, score it according to the performance scoring table (Table 1), and test the dose of fine particles in the prescription.

2.5.4. Selection of anhydrous ethanol dosage

The prescription consists of CA 15 mg, propylene glycol 0.4 g, HFA-134a 10 g and different doses of absolute ethanol. Other operations are the same as "2.5.3 Selection of propylene glycol dosage".

2.5.5. Orthogonal experiment

With the amount of anhydrous ethanol (A), propylene glycol (B), HFA-134a (C) as the investigation factors, Fine particle fraction (FPF)as the investigation index, the experiment was conducted according to L9 (3^4) orthogonal table, and the factor level table is shown in Table 2.

2.5.6. Validation test

Three batches of validation tests were conducted according to the optimized prescription. The main drug content and fine particle dosage of each actuation of sample were checked and the number of press shots is 20.

2.6. Evaluation of the prepared CA-MDI formulations

2.6.1. Total number of discharges per canister

It was done by counting the number of priming discharges at intervals of not less than 5 s until the canister was empty. According to the method of the Chinese Pharmacopoeia (2020 Edition), the experiment was done three times for every formulation and the average number was recorded.

2.6.2. The emitted dose drug content

The CA-MDI aerosol is fully shaken, and the nozzle is sprayed for 5 times. The nozzle is cleaned with methanol. After it is fully dried, inverted into a 100 mL beaker and added 40 mL methanol. The nozzle is immersed under the liquid level of the absorption solution (at least 25 mm). The nozzle is sprayed for 10 times (note that the actuation interval is 5 s and the nozzle is slowly shaken). Take out the test sample, wash the inside and outside of the socket with methanol, combine the absorption solution, transfer it quantitatively to a 50 mL volumetric flask and dilute it to the scale, and determine it according to the method under the content determination item. The average drug content per press is obtained by dividing the result by the number of sampling sprays.

2.6.3. Fine particle fraction

It was done by twin-stage impactor (TSITSI). The specific operations are as follows: 30 mL methanol receiving solution is placed in the lower conical flask, and 7 mL methanol receiving solution is placed in the upper conical flask. The sample is fully shaken, sprayed for 5 times, and pressed for 20 times (note that the actuation interval is 5 s and the sample is slowly shaken). Clean the specified parts with methanol, combine the washing solution and the receiving solution in the lower conical flask, and put them into a 50 mL volumetric flask. Dilute them to the scale with methanol, shake them fully, filter them, accurately measure an appropriate amount of the continued filtrate, and dilute them with methanol to produce a solution containing about 10 μg per 1 mL.

2.6.4. Leakage rate determination

It was determined by selecting 12 canisters. To check any leak from the containers, they were allowed to stand in an upright position at temperature of $25 \pm 2\,°C$ for 24 h. After equilibration, each canister was weighed (W1) in g with reporting the date and the time to the nearest half hour. In our experiment canisters were left for 72 h. After a period of 72 h, the canisters were reweighed separately and the weight was recorded (W2) in g with reporting the date and time to the nearest half hour. The weight of canister and valve W3 = 7.52 g, and the annual leakage rate is calculated according to the following formula [24].

Table 2
Factor level table.

Level	Factor		
	Ethano l (A)/g	Propylene glycol (B)/g	HFA-134a(C)/g
1	1	0.4	10
2	1.2	0.6	11
3	1.4	0.8	12

$$\text{leakage rate peer year} = \frac{365 \times 24 \times (W1 - W2)}{72 \times (W1 - W3)} \times 100\%$$

2.6.5. Uniformity of the delivered dose drug content

It was done by drug inhalation preparation teste (QW-1A). According to the provisions of the device manual, fully shake the sample before measurement, insert the suction device into the suction nozzle adapter, press once, extract air for 5 s, and remove the suction device. Repeat the above process to collect the minimum recommended clinical dose in the product manual. Clean the filter paper and the inside of the collection tube with methanol, combine the cleaning solution and dilute it into a 5 mL volumetric flask. The first, second, and third press (initial three doses), the 75th, 76th, 77th, 78th press (middle), and the 148, 149, 150th press (rear) of the canister delivery dose were measured respectively, totaling 10 doses. The delivery dose between canisters was measured by selecting 10 canisters. Among them, 3 cans were selected to determine the first and last press respectively, and 4 cans to determine the middle press. The other operations is the same as the dose delivered in the canisters.

2.6.6. Spray mode and spray form

Spray form refers to the shape and size of spray clouds, while spray mode refers to the size and character of spray patterns sprayed on paper. MDI's spray form and spray mode reflect the performance of valves and actuators. If there is a deviation between spray mode and spray form, it indicates that the uniformity of delivery dose is low and the ability to reach the predetermined part of the lungs is poor [25].

2.6.6.1. Spray mode.
The pressing device is calibrated according to the operating procedures, adjust the position of each component of the instrument, read the background, and adjust the camera parameters. Each batch of products produced before, during and after the production of a canister is randomly selected. Press 5 times, with an interval of 5 s, and press once for measurement. Set the threshold so that the image contains 95% spray.

2.6.6.2. Spray form.
Calibrate the pressing device according to the operating procedures. The parameters of the pressing device and the sample collection are the same as "2.6.6.1 spray Mode" in this chapter. The shape of spray from the driver nozzle to 6 cm of the laser beam was detected.

2.7. Preliminary stability investigation

2.7.1. Test of influencing factors
The stability study was carried out according to the guideline for the stability of 9001 raw materials and preparations in the Chinese Pharmacopoeia (2020). Because the CA-MDI was packed in opaque aluminum canister, the light had little effect on it, so the influence factor test was only conducted at high temperature and high humidity. In addition, the influence of three different placement directions on the CA-MDI was investigated.

2.7.1.1. High humidity.
CA-MDI were stored at 25 °C and relative humidity (92.5% ± 5%). Sampling time point is 0, 5, 10 days.

2.7.1.2. High temperature.
CA-MDI was put into a 40 °C incubator and sampled for inspection on the 0, 5 and 10 days.

2.7.1.3. Different placement directions.
CA-MDI was placed in a 40 °C incubator in three different directions (upward, Downward and horizontal) and sampled for inspection on the 5th and 10th days.

2.7.2. Accelerated test
It was determined by selecting 3 batches of CA-MDI (21CAQWJ01, 21CAQWJ02, 21CAQWJ03)). According to the Guiding Principles for the Study of the Stability of Traditional Chinese Medicine and Natural Medicine, the aerosol can be kept for 6 months at a temperature of (30 ± 2) °C and a relative humidity of (65 ± 5)%. Samples were taken out for detected at the end of month 0, 1, 2, 3 and 6 respectively. Finally, we will use April 1, 2021 as the sampling point for the 0th month.

2.7.3. Long term test
It was determined by selecting 3 batches of CA-MDI (21CAQWJ01, 21CAQWJ02, 21CAQWJ03)). According to the Guiding Principles for the Study of the Stability of Traditional Chinese Medicine and Natural Medicine, the aerosol can be kept at a temperature of (25 ± 2) °C and a relative humidity of (60 ± 10)%. Samples were taken out for detected at the end of month 0, 1, 2, 3 and 6 respectively. Finally, we will use April 1, 2021 as the sampling point for the 0th month.

2.8. Statistical analysis

Statistical data were analyzed by one-way analysis of variance (ANOVA) and $p < 0.05$ was considered to be significant with 95% confidence intervals.

3. Results and discussion

3.1. Selection of packaging materials

Results as shown in Table 3, there was no significant difference in the effect of four container materials on the concentration of CA ($P > 0.05$). Considering the cost and safety factors, the high pressure resistant MDI aluminum can was finally selected as the inner packing material of CA-MDI, which is convenient for transportation and storage. The valve is intended to use HFA-134a special valve.

3.2. Prescription screening

3.2.1. Selection of dosage of propellant

The aerosol canister has a capacity of 19 mL. The aerosol generally requires 100–200 presses per canister, and the aerosol is expected to reach 150 presses. It can be seen from Table 4 that the filling volume of 10 g per bottle can reach 150 presses/canister, and the maximum dosage of propellant is 12 g. When the filling volume of each bottle is more than 10 g, the emitted shot weight rate is more than 90% (as shown in Table 5). The maximum filling volume of 12 g is not much different from that of 10 g, considering safety and ensuring the emitted shot weight. The dosage of projectile agent is 10 g.

The change of the internal pressure of the propellant during the use of MDI is shown in Table 6. When the cumulative number of times reaches 170, the droplets can no longer be seen during the spray, and the pressure is very small. According to Table 6, it is known that the internal pressure of the propellant in MDI is basically constant during the use of the propellant for a limited number of times.

3.2.2. Selection of propylene glycol dosage

The results are shown in Table 7. The results showed that the clarity of the solution was obviously improved with the increase of the amount of propylene glycol. However, excessive dosage of propylene glycol will lead to delamination. The solution can keep clear and transparent without precipitation when the dosage of propylene glycol is 0.4–1.2 g, and the FPF decreases with the increase of the amount of propylene glycol, and when the dosage is more than 1 g, the FPF decreases obviously, so the amount of propylene glycol is 0.4 g.

3.2.3. Selection of anhydrous ethanol dosage

The results are shown in Table 8. The results showed that the lowest dosage of anhydrous ethanol was 1 g, and the FPF value decreased gradually with the increase of the amount of anhydrous ethanol, so the amount of anhydrous ethanol was 1 g.

3.2.4. Orthogonal experiment

The orthogonal table and visual analysis table are shown in Table 9, and the variance analysis table is shown in Table 10. The results showed that the order of influence of the three factors on FPF was propylene glycol dosage > absolute ethanol dosage > HFA-134a dosage, and the best condition was A1B1C3. It can be seen from the analysis of variance that the amount of propylene glycol has a significant impact on FPF ($p < 0.01$). 1 g and 1.2 g of absolute ethanol have little impact on the results, while the amount of HFA-134a has the least impact. Considering the economic cost and safety, the best experimental condition is A1B1C1. The final prescription composition: anhydrous ethanol 1 g, propylene glycol 0.4 g, and HFA-134a10 g.

3.2.5. Validation experiment

The results are shown in Table 11. The results showed that the FPF of the three batches of samples was 28.68%–33.98%, with an average value of $31.36 \pm 2.65\%$, which was in line with the FPF limit specified in the pharmacopoeia. Therefore, the final formulation of CA-MDI was determined as HFA-134a 10 g, anhydrous ethanol 1 g, propylene glycol 0.4 g.

3.3. Characterization

3.3.1. Quality inspection

It can be seen from Table 12 that the total pressing times of each canister of the tested sample are more than 150. CA-MDI contains 77.90 μg of the emitted dose drug content per press, the average dosage of FPF per press is 30.45%, the annual leakage rate is 0.51%, and the average delivery dose was 70.96 μg. In short, all indicators meet the previous design requirements.

Table 3
Results of investigation on the effect of different material containers on the content of CA ($\bar{x} \pm s$, n = 3).

o	Days		
	0 D	5 D	10 D
	Concentration (mg/mL)		
Glass	68.27 ± 3.54	67.49 ± 0.17	62.90 ± 0.26
MDI aluminum		64.06 ± 3.70	63.37 ± 3.55
plasma coated Aluminum		67.06 ± 5.01	61.51 ± 6.71
Oxide film aluminum		67.15 ± 1.94	65.59 ± 2.20

Table 4
Relationship between the amount of propellant added and internal pressure.

number	Propellant (g)	Internal pressure 25 °C (MPa)	Internal pressure 50 °C (MPa)	Total number
1	6	0.77	0.78	101
2	8	0.78	0.80	143
3	10	0.77	0.79	168
4	11	0.78	0.82	194
5	12	0.79	0.82	225
6	12.2	0.82		

Table 5
The rate of emitted shot weight by Propellant with different weight.

Propellant (g)	Emitted shot weight rate (%)
10	92.3
11	92.85
12	94.49

Table 6
Results of internal pressure change of propellant during use. ($\overline{x}$ ±s, n = 3).

number	Propellant (g)	internal pressure (MPa)	Cumulative times
1	10.13 ± 0.1	0.78 ± 0.0	10
2	9.56 ± 0.07	0.77 ± 0.02	20
3	9.02 ± 0.05	0.77 ± 0.02	30
4	8.47 ± 0.06	0.77 ± 0.02	40
5	7.91 ± 0.07	0.75 ± 0.01	50
6	7.33 ± 0.07	0.74 ± 0.02	60
7	6.69 ± 0.19	0.73 ± 0.03	70
8	6.10 ± 0.17	0.72 ± 0.01	80
9	5.59 ± 0.28	0.73 ± 0.02	90
10	5.06 ± 0.34	0.73 ± 0.02	100
11	4.66 ± 0.43	0.75 ± 0.03	110
12	3.96 ± 0.41	0.73 ± 0.05	120
13	3.41 ± 0.44	0.75 ± 0.03	130
14	2.87 ± 0.46	0.77 ± 0.03	140
15	2.39 ± 0.51	0.76 ± 0.02	150
16	1.95 ± 0.51	0.73 ± 0.02	160
17	1.56 ± 0.44	0.50 ± 0.25	170
18	1.35 ± 0.26	0.37 ± 0.59	180

Table 7
Effect of different propylene glycol dosage on aerosol properties ($\overline{x}$ ±s, n = 3).

Propylene glycol (g)	Character	Score	FPF (%)
0.2	Granular precipitation suspension	1	No
0.4	Clarification and transparency	6	26.20 ± 1.4
0.6	Clarification and transparency	6	23.27 ± 1.6
0.8	Clarification and transparency	6	24.82 ± 2.9
1.0	Clarification and transparency	6	17.68 ± 1.1
1.2	Clarification and transparency	6	17.24 ± 1.3
1.4	More clarified	5	No
1.6	Stratification	2	No

Table 8
Effect of different anhydrous ethanol dosage on aerosol properties. ($\overline{x}$ ±s, n = 3).

anhydrous ethanol (g)	Character	Score	FPF (%)
0.8	milky white	3	No
1.0	Clarification and transparency	6	28.88 ± 3.7
1.2	Clarification and transparency	6	26.20 ± 1.4
1.4	Clarification and transparency	6	22.84 ± 1.9

Table 9

Orthogonal design table.

number	A	B	C	D	Result
1	1	1	1	1	29.43
2	1	2	2	2	28.04
3	1	3	3	3	23.65
4	2	1	2	3	29.58
5	2	2	3	1	27.39
6	2	3	1	2	19.37
7	3	1	3	2	28.94
8	3	2	1	3	24.94
9	3	3	2	1	19.42
Mean 1	27.040	29.317	24.580	25.413	
Mean 2	25.447	26.790	25.680	25.450	
Mean 3	24.433	20.813	26.660	26.057	
Range	2.607	8.504	2.08	0.644	

Table 10

Analysis of variance.

factor	Sum of Squares of Deviations	freedom	mean square	F	Significance
Anhydrous ethanol	10.360	2	5.180	13.227	$P < 0.1$
Propylene glycol	114.411	2	57.206	146.069	$P < 0.01$
HFA-134a	6.497	2	3.248	8.294	
D (error)	0.783	2	0.392		

Table 11

Sampling results of prescription validation of CA-MDI.

Number	The emitted dose drug content (µg)	FPD (µg)	FPF (%)	Mean (%)
01	76.79	26.09	33.98	31.36 ± 2.65
02	81.43	23.36	28.68	
03	81.39	25.58	31.43	

3.3.2. Spray mode

The image is shown in Fig. 2. The ellipticity and elliptical area can be known from the image. The specific experimental results are shown in Table 13. Table 13 shows that the ellipticity of CA-MDI spray mode (3 cm) is 1.02641–1.12044, the elliptical area is 4.15348–5.02544 cm^2, the average elliptical rate is 1.06614, the elliptical area is 4.73019 cm^2, the elliptical rate of CA-MDI spray mode (6 cm) is 1.0257–1.07423, the elliptical area is 9.38956–11.4967 cm^2, the average elliptical rate is 1.05268, and the elliptical area is 10.44718 cm^2. The ovality of the spray mode of CA-MDI was 96%–106% of the average ovality, and the ovality area was 85%–111% of the average ovality. The result shows that the delivery uniformity is high.

3.3.3. Spray form

Detect the shape of spray at 6 cm from the driver nozzle to the laser beam. The width and angle of spray can be seen from figure 3. Specific data are shown in Table 14. The results of spray geometry of CA-MDI showed that the width was 2.50292–2.86686 cm, the angle was 17.2968–18.825, the average width was 2.58964 cm, and the angle was 18.1496. The spray of CA-MDI has a short spray shape length, about 5.5 cm, its width is 96%–111% of the average value, and its angle is 95%–104% of the average value.

3.4. Preliminary stability investigation
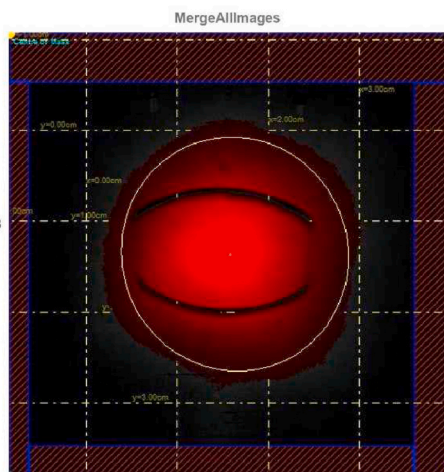
3.4.1. High humidity

The results are shown in Table 15. The experimental results showed that after 10 days of storage at 25 °C and RH92.5% ± 5%, the

Table 12

Quality inspection results of CA-MDI.

index Batch No	21CAQWJ01	21CAQWJ02	21CAQWJ03	Mean
Total number	184 ± 2	188 ± 7	184 ± 1	185 ± 3
emitted dose drug content (µg)	77.53 ± 1.37	76.5 ± 2.74	79.69 ± 0.67	77.91 ± 1.63
FPD (µg)	32.48 ± 6.64	30.10 ± 4.23	35.97 ± 1.87	32.5 ± 3.07
Delivered dose (µg)	68.22 ± 4.82	69.99 ± 6.36	74.39 ± 6.07	70.8 ± 3.18
Leakage rate (%)	0.64	0.46	0.44	0.51

Fig. 2. Spray mode image of CA-MDI.

Table 13
Ovality and Ovality Area of CA-MDI spray Mode (6 cm, 3 cm).

position	6 cm		3 cm	
Serial No	Ratio	Area (cm2)	Ratio	Area (cm2)
21CAQWJ01-1	1.03591	9.92964	1.04229	5.02544
21CAQWJ01-2	1.04921	10.34680	1.04960	5.01950
21CAQWJ01-3	1.03575	10.79560	1.05968	4.39727
21CAQWJ02-1	1.03165	10.48880	1.05626	4.49449
21CAQWJ02-2	1.09620	11.49670	1.09384	5.02078
21CAQWJ02-3	1.02570	10.67880	1.04151	4.73361
21CAQWJ03-1	1.07423	10.53670	1.12044	4.87175
21CAQWJ03-2	1.06921	10.36110	1.02641	4.15348
21CAQWJ03-3	1.05622	9.38956	1.10521	4.85543

measured values of each index had little change compared with the initial values ($p > 0.05$), so the high humidity environment had little effect on CA-MDI.

3.4.2. High temperature test

The results are shown in Table 16. The results showed that the CA content of CA-MDI decreased significantly after 10 days of storage at 40 °C, with a statistically significant difference ($p < 0.05$), indicating that the CA-MDI was unstable under high temperature.

3.4.3. Influence of different placement orientations

The results are shown in Table 17. The experiment shows that at the 95% confidence level, there is no significant difference between the three placement orientations, indicating that placement orientations have no effect on the stability of CA-MDI in a short period of time.

3.4.4. Accelerated test

The test results are shown in Table 18. It can be seen from the results that after six months of accelerated test, all the test indexes of three batches of CA-MDI meet the quality standards, indicating that they are stable under six months of accelerated test. Among them, the main drug content and delivery dose have significant changes in April and May ($p < 0.05$), and there is no significant difference from May to October ($p > 0.05$), indicating that the drug content changes mainly in the first month of the beginning, and then tends to
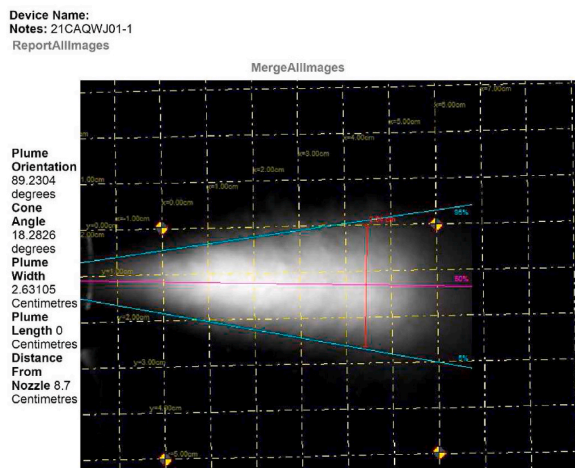
Fig. 3. Morphological image of CA-MDI spray (21CAQWJ01-1).

Table 14
Test results of spray Morphology of CA-MDI.

Serial No	Width (cm)	Angle (degrees)
21CAQWJ01-1	2.63105	18.2826
21CAQWJ01-2	2.86686	18.8250
21CAQWJ01-3	2.54891	18.1459
21CAQWJ02-1	2.71156	18.6331
21CAQWJ02-2	2.57396	18.0471
21CAQWJ02-3	2.38253	17.2968
21CAQWJ03-1	2.51403	19.7495
21CAQWJ03-2	2.57494	18.2596
21CAQWJ03-3	2.50292	18.1071

Table 15
Test results of factors affecting high humidity of ca-MDI ($\bar{x} \pm s$).

Index	0 d	5 d	10 d
drug content (µg, n = 3)	82.79 ± 5.03	84.88 ± 6.81	79.68 ± 9.01
FPD (µg, n = 3)	31.26 ± 6.16	28.27 ± 7.71	29.02 ± 8.89
Leakage rate (%, n = 12)	0	0.0070 ± 0.003	0.0072 ± 0.002
Delivered dose (µg, n = 10)	71.11 ± 6.28	72.11 ± 7.32	72.44 ± 4.78

Table 16
Test results of factors affecting high temperature of CA-MDI ($\bar{x} \pm s$).

index	0 d	5 d	10 d
drug content (µg, n = 3)	77.53 ± 1.37	73.65 ± 3.13	69.79 ± 1.04
FPD (µg, n = 3)	30.09 ± 4.23	27.15 ± 5.64	26.75 ± 0.20
Leakage rate (%, n = 12)	0	0.013 ± 0.002	0.016 ± 0.001
Delivered dose (µg, n = 10)	69.99 ± 6.36	66.16 ± 4.07	70.46 ± 5.46

Table 17
Effects of different positions of ca-MDI.

Index	0 d	5 d			10 d		
Position		upward	Downward	horizontal	upward	Downward	horizontal
drug content (µg)	74.62	66.51	66.86	69.01	69.26	67.98	71.05
FPD (µg)	24.19	17.81	20.70	27.26	27.98	21.09	23.00
Leakage rate (%)	0	0.011	0.017	0.016	0.023	0.022	0.023
Related substances (%)	0.91	1.06	0.97	1.39	1.08	1.45	1.4

be stable. The content of related substances (total impurities) in October significantly increased compared to April ($p < 0.05$), which may be related to changes in certain components in the formulation, but further research is needed on the specific components.

3.4.5. Long term test

The results are shown in Table 19. It can be seen from the results that the three batches of CA-MDI were stored under long-term test conditions, and all detection indicators met the quality standards. The main drug content and delivery dose did not change significantly ($p > 0.05$), indicating that the preparation process and storage conditions of this product were reliable.

4. Conclusion

In this paper, the preparation, characterization and stability of CA-MDI were studied. A HPLC method for the determination of CA was established and the methodology was investigated. The results showed that the method was stable, reliable and specific. The dosage of the preparation was determined and characterized through laboratory research. A preliminary study was conducted on the stability of CA-MDI, including influencing factor test, accelerated test and long-term test. High humidity and different placement directions had no significant impact on the stability of CA-MDI, while high temperature had significant impact. Under the conditions of 6-month accelerated test and long-term test, three batches of CA-MDI met the quality standards, which shows the preparation process and storage conditions of the product are reliable. However, under the accelerated test conditions, the drug content and other indicators in CA-MDI changed, and the specific reasons need to be further studied.

5. Discussion

The lung is the target organ of many respiratory viruses, such as influenza, RSV and SARS-CoV-2, which is mainly transmitted by inhalation. Due to the fact that the human lungs only occupy a relatively small part of the body, treating respiratory diseases through oral or intravenous administration may not be very effective and may require a systemic dose, leading to side effects. Generally speaking, inhaled drugs can be quickly and non-invasive delivered to the respiratory tract, where they may have beneficial therapeutic effects. Their dosage leads to low exposure to the rest of the body to minimize systemic adverse reactions. Therefore, inhalation is the preferred route of administration for the treatment of respiratory diseases [26].

The research group conducted a preliminary study on the anti RSV effect of CA in vitro. The results showed that CA had an inhibitory effect on RSV induced cytopathic changes, had no inhibitory effect on RSV adsorption and penetration into cells, and had an inhibitory effect on RSV genome replication and proliferation [20]. Furthermore, the preparation process for extracting and purifying CA from Echinacea purpurea was optimized [27]. On this paper, this study prepared CA-MDI, which can not only stabilize the preservation of chicory acid, but also directly reach the lesion through inhalation administration. Further pharmacodynamic evaluation and safety of CA-MDI will be conducted in the later stage.

Table 18
Accelerated test results of CA-MDI (Batch Nov21CAQWJ01/21CAQWJ02/21CAQWJ03).

Index	0 month (April)	1 month (May)	2 month (June)	3 month (July)	6 month (October)
Character *	Yellowish clear liquid	Yellowish clear liquid	Yellowish clear liquid	Yellowish clear liquid	Yellowish clear liquid
Identify *	Qualified	Qualified	Qualified	Qualified	Qualified
Total number	184/188/183	186/188/182	170/171/172	174/171/173	167/173/169
Drug content (μg/press)	77.53/76.50/79.69	71.54/69.69/66.54	70.96/67.40/67.98	66.78/65.61/71.74	65.85/66.40/70.67
FPD (μg/press)	32.87/30.10/35.99	31.95/22.48/27.35	27.91/28.38/33.07	25.56/22.50/25.31	36.27/39.42/31.86
Delivered dose (μg)	68.22/69.99/74.39	64.42/63.10/61.41	60.94/58.82/62.20	64.21/61.36/65.24	64.17/61.78/64.20
Related substances (%)	0.5/0.8/0.5	0.86/1.41/1.09	1.36/1.72/1.69	2.29/2.54/2.05	5.15/4.30/4.91
Leakage rate (%)	0	0.26/0.13/0.12	0.27/0.30/0.29	0.21/0.23/0.21	0.51/0.53/0.52
Microbial limit *	Qualified	Qualified	Qualified	Qualified	Qualified

Note: * means that the test results of three batches are the same.

Table 19
Long term experiment of CA-MD (21CAQWJ01/21CAQWJ02/21CAQWJ03).

Index	0 month (April)	3 month (July)	6 month (October)
Character *	Yellowish clear liquid	Yellowish clear liquid	Yellowish clear liquid
Identify *	Qualified	Qualified	Qualified
Total number	184/188/183	170/172/172	164/174/167
Drug content (μg/press)	77.53/76.50/79.69	72.50/73.68/78.94	73.95/71.90/76.27
FPD (μg/press)	32.87/30.10/35.99	28.97/28.81/28.93	28.37/17.60/19.60
Delivered dose (μg)	68.22/69.99/74.39	62.69/62.45/63.72	66.70/67.44/67.21
Related substances (%)	0.5/0.8/0.5	0.92/1.69/1.30	2.85/3.25/1.30
Leakage rate (%)	0	0.12/0.19/0.16	0.10/0.12/0.11
Microbial limit *	Qualified	Qualified	Qualified

Note: * means that the test results of three batches are the same.

Declaration of competing interest

The authors declare no conflict of interest.

Ethical approval statement

NA.

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